mated as closely as possible around the lead-off wires. A clean dressing is applied and the animal is allowed to recover. Healthy animals generally do not require special post-operative care and rarely do they attempt to scratch at the wires. The animals remain docile and tractable and no difficulty has been experienced in soldering the lead-off wires to the EEG apparatus. When the animals have just been fed and are confined in a small, dark, warm, sound-proofed box, they drop off to sleep readily and continuous recordings can be taken over a period of hours. Examination of the brain of an animal with electrodes implanted for five months showed only negligible gliosis immediately around the electrodes and a slight herniation of the cortex through the slit in the dura. Accordingly, it is believed that by this method electrodes may be implanted in a normal brain and remain permanently serviceable.

Sample observations. The records shown in Fig. 3 were obtained from a cat on the seventh post-operative day. The animal was prepared as described above, and in addition 4 insulated, ball-tipped silver wires were inserted into small trephine holes to form the 4 cortical electrodes. These records are presented to demonstrate the feasibility of the method; a full account of studies on subcortical activity in normal wakefulness and sleep will be published at a later date.

Acknowledgment is made to Drs. H. W. Magoun, R. S. Snider, and D. B. Lindsley for laboratory facilities and guidance.

Received January 8, 1951. P.S.E.B.M., 1951, v76.

Temporary Accumulation of Eosinophilic Leucocytes in Spleen of Mice Following Administration of Cortisone. (18477)

DAVID M. SPAIN AND WILLIAM THALHIMER.

From the Division of Pathology, Grasslands Hospital, and the Department of Laboratories and Research of Westchester County, Valhalla, N. Y.

Allen(1) reported the occurrence of conspicuous numbers of eosinophilic leucocytes in the spleen in cases of sudden death caused by trauma and coronary occlusion. It was also noted that in the spleens of individuals who survived several hours the number of eosinophiles were progressively less as the period of survival increased. The explanation of this phenomenon was not known at that time. It was speculated, however, that it might be due to either histamine or acetylcholine release. Thorn(2) later described a test to ascertain the efficiency of the adrenal cortex by the disappearance of eosinophilic leucocytes from the circulating blood upon adrenal cortical stimulation by ACTH or adrenaline, or by injection of cortisone. It now appears most likely that the phenomenon described by Allen can be explained best by adrenal cortical stimulation during periods of stress and it would appear, from the observations in this report, that the eosinophiles upon disappearing from the circulating blood are stored temporarily within the spleen. In order to test this hypothesis the following investigation was undertaken:

Materials and methods. 24 male Swiss albino mice weighing 25 g each were divided into 2 groups of 12. One group was injected subcutaneously with 2.5 mg of cortisone acetate (Cortone acetate, Merck) and the other group served as controls. Eight hours post injection the animals were sacrificed by sharp blows on the back of the necks. This resulted in instantaneous death. All animals were necropsied; the spleen and thymus glands were fixed in formalin and in Zenker's solutions, were prepared for histologic examina-

1. Allen, A. C., *Arch. Path.*, 1944, v37, 20.

2. Thorn, G. W., Forsham, P. H., Prunty, F. T., Garnet, M. D., and Hills, A. G., *J.A.M.A.*, 1948, v137, 1005.

TABLE I. Comparison of the Average Number of Eosinophilic Leucocytes in the Spleen Per Oil Immersion Field in the Cortisone Treated Mice That Were Sacrificed 8 Hours Post Injection and in the Untreated Controls.

Mouse No.	1	2	3	4	5	6	7	8	9	10	11	12	Avg
Cortisone treated mice	1.7	1.9	.2	3.0	1.7	2.3	1.5	.8	1.0	1.2	.8	1.5	1.47
Control mice	.2	.2	.3	.1	.05	.2	.1	.3	.1	.2	.05	.2	0.16

tion, and stained with H & E and Giemsa stains. The spleens and the thymus glands of both groups of animals were examined under oil immersion magnification (× 950) and the eosinophilic leucocytes in each field were counted. Forty such fields were counted in each spleen with the edge of each field represented by the capsule of the spleen. Although the eosinophiles were present throughout the spleen, there was a tendency for more to collect near the capsule than in the splenic pulp elsewhere. The results of these counts are given in Table I. In addition six other similar mice were treated with 2.5 mg of cortisone and two were sacrificed 2, 4 and 6 hours post injection. These were studied as above.

Results. Examination of Table I indicates that there is a significant difference in the number of eosinophiles in the two groups studied. On the average there were 9 times as many eosinophilic leucocytes in the spleens of the cortisone acetate treated animals as in the untreated controls. It is also interesting that in one instance (Mouse No. 3) the cortisone treated animal did not show any increase in splenic eosinophilic leucocytes. In this animal it was noted that the usual reduction in size of the spleen that follows cortisone treatment did not occur. In 2 mice circulating eosinophile counts and in 3 mice the smears of peripheral blood from the tail veins were studied for eosinophilic leucocytes prior to injection with cortisone acetate and prior to sacrifice. These confirmed the fact that a marked drop occurred in the circulating eosinophilic leucocytes at the end of 8 hours in the cortisone acetate treated mice. The other treated mice that were sacrificed 2, 4 and 6 hours post injection did not reveal any significant increase in splenic eosinophilic leucocytes. In these latter animals the only changes noted was the progressive reduction in the sizes of the spleens and the disintegration of the lymphocytes that is typical of the alarm reaction. The thymus glands did not reveal any eosinophilia.

If it is postulated that in these experiments all, or the majority, of the circulating eosinophilic leucocytes are temporarily stored in the spleen it is important to calculate whether the spleen is large enough to store all the eosinophiles and whether the number of eosinophiles as seen in each oil immersion field could account for the number that disappeared from the circulating blood.

Weight of mouse = 25 g. Blood vol. 1/10 of body weight, 2.5 g = 2.5 cc; normal W.B.C. count 8,000 per cu mm; eosinophiles = 2% = 160 per cu mm.

Eosinophiles in total blood vol. = 160,000 x 2.5 = 400,000. Dimension of single eosinophile = 12 μ diameter. 1 square mm (1000 μ square) 12 μ thick would accommodate 83 x 83 eosinophiles = 6,889. 1 cubic mm would accommodate 6,889 x 83 = 571,787 eosinophiles. Or more than the total number of circulating eosinophiles. Size of normal mouse spleen is 20 x 5 x 3 mm = 300 cu mm. If *all* the circulating eosinophiles were stored in the spleen, *each cubic mm* would contain 1/300th of the total number of eosinophiles or 400,000 divided by 300 = 1333.

Since a cubic millimeter will accommodate 571,787 cells the size of eosinophiles it is necessary to calculate what percentage of 571,787 is 1333 eosinophiles, which is 0.233%. In one oil immersion field of sections of mouse spleen there are about 900 cells of all types which are approximately the same size as the eosinophiles. One per cent of 900 cells is 9 cells and 0.233% is slightly more than 2 eosinophiles per oil immersion field.

The spleens of the cortisone treated mice sacrificed at the end of about 8 hours were found to be about half normal in size. If only

one-half of the circulating eosinophiles were stored in these spleens the number of eosinophiles per oil immersion field would remain the same. Thus on the basis of these calculations, although approximate, it appears reasonable that the number of eosinophilic leucocytes seen in the spleen could account for the number disappearing from the circulating blood. Further investigations are being conducted in order to determine the fate of the eosinophilic leucocytes following adrenal cortical stimulation in splenectomized animals.

Summary. In a group of Swiss albino mice injected with cortisone acetate it was found that 8 hours post injection the spleens contained a significant increase in the number of eosinophilic leucocytes. It is postulated that this may be the locale of the eosinophilic leucocytes during their disappearance from the circulating blood following adrenal cortical stimulation. The number of eosinophilic leucocytes found in the spleen could account for the number that disappeared from the circulating blood following a single injection of cortisone acetate.

Received January 8, 1951. P.S.E.B.M., 1951, v76.

Ascorbic Acid Concentration in the Adrenals of Lymphosarcoma-Bearing Mice.* (18478)

ELIJAH ADAMS† AND ABRAHAM WHITE.‡

From the Department of Physiological Chemistry, School of Medicine, University of California Medical Center, Los Angeles.

Savard(1) has described recently a progressive reduction in ascorbic acid concentration in the adrenals of female Carworth Farm mice during the growth of a transplanted tumor (sarcoma 180). The lymphocyte is known to be an end cell of pituitary-adrenal cortical secretions, and the possibility that the products of lymphocyte destruction may exert a suppressive influence on this endocrine system has been noted in a recent publication(2). It seemed of interest, therefore, to investigate adrenal ascorbic acid levels in mice bearing a rapidly growing lymphosarcoma. The present report describes these observations as well as incidental observations concerned with a sex difference in the responsiveness of adrenal ascorbic acid to the administration of histamine and adrenocorticotrophic hormone (ACTH).

Methods. Male and female mice of the CBA strain (Strong) 8 to 10 weeks of age were used. Male mice were inoculated subcutaneously with a small fragment of lymphosarcoma(3) and were autopsied at varying intervals thereafter. Histamine acid phosphate (Abbott) was administered intraperitoneally as a solution in normal saline in doses ranging from 0.25 to 5.0 mg of histamine base per 100 g body weight. One mg of ACTH (Armour) was administered intraperitoneally as a fresh solution in distilled water. All intraperitoneal injections were given in a volume not exceeding 0.5 ml and all mice were sacrificed 1 hour following the administration of either substance. The determination of ascorbic acid in the adrenals of fasted mice was made at the end of a 48-hour period during which mice were maintained in individual metabolism cages with free access to water and without food. Im-

* This investigation was aided by a grant from the Division of Grants and Fellowships, National Institutes of Health, U. S. Public Health Service.

† Postdoctoral Fellow, American Cancer Society. Present address: University of Utah School of Medicine, Salt Lake City, Utah.

‡ Present address: Chemical Specialties Co., Inc., New York, N. Y.

1. Savard, K., *Science,* 1948, v108, 381.

2. Roberts, S., and White, A., *J. Biol. Chem.,* 1949, v178, 151.

3. Gardner, W. U., Dougherty, T. F., and Williams, W. L., *Cancer Research,* 1944, v4, 73.