high as 50 mg/kg given intravenously does not alter the pressor response to small doses of epinephrine.

*Summary and conclusions.* The subcutaneous injection of epinephrine solution containing sodium bisulfite as an antioxidant produced a more rapid and higher rise in systolic blood pressure than did the same amount of epinephrine in a solution containing sodium hypophosphite as a preservative. Small doses of rutin given intravenously immediately before the epinephrine did not significantly alter the "bisulfite effect" nor did the rutin influence the effect of epinephrine.

Received March 28, 1951. P.S.E.B.M., 1951, v77.

## Leukocyte Response to Stress in Normal and Adrenalectomized Rats Pretreated with Ascorbic Acid. (18714)

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Ascorbic acid treatment to animals exposed to stress has been observed to exert certain protective actions which are related to adrenal cortical function(1-3). This ability of ascorbic acid has been established by survival studies, and by studies on the functional status of the cortex. It was demonstrated that ascorbic acid pretreatment to intact rats prevented the eosinopenia following epinephrin stress; this was associated with a prevention of the depletion of adrenal steroids(3).

This paper reports results which indicate that there may exist an ascorbic acid-eosinophil alliance independent of the adrenal.

*Materials and methods.* Adult female Osborne-Mendel rats were used in this study. Twelve sham-adrenalectomized rats (7-8 days post-operative) were divided into 2 groups. Group I animals were pretreated with ascorbic acid (Vitamin C injectable, sodium ascorbate, Roche), 87.5 mg/100/g in two intra-peritoneal injections; Group II rats received pretreatment with saline solution. Two groups of adrenalectomized rats, (7-8 days post-operative) were given respectively, pretreatment with ascorbic acid, (Group III), and pretreatment with saline (Group IV). About 6-8 hours after the last pretreatment injection, all animals were given single subcutaneous injections of epinephrin at the rate of 0.03 mg/100 g. Total and differential leukocyte counts (Randolph's method)(4) of the tail blood were made of all animals in the following instances: Count (a) before pretreatment, Count (b) 3 hours after the injection of epinephrin, and Count (c) 24 hours after epinephrin. The mean differences of Counts (b), and (c) from Count (a) were calculated and are presented along with the standard errors in Table I. Counts made previous to the experiment indicated that the operation of sham-adrenalectomy did not disturb the leukocyte picture, but adrenalectomy led to a significant leukopenia, lymphopenia, and eosinopenia. Adrenalectomy did not however, affect the polymorphonuclear count.

*Results.* Both groups of sham-adrenalectomized rats (I and II) exhibited a significant decrease of lymphocytes 3 hours after the injection of epinephrin and returned to prestress levels 24 hours after the stress. The injection of epinephrin to adrenalectomized rats (III and IV) failed to alter the number of circulating lymphocytes. The expected rise in polymorphonuclear leukocytes (neu-

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2. Dugal, L. P., and Leblanc, J., *Rev. canad. de biol.*, 1949, v8, 440.

3. Bacchus, H., and Toompas, C. A., *Science*, 1951, v113, 269, 367.

4. Randolph, T. G., *J. Allergy*, 1944, v15, 89.

TABLE I. Leukocyte Response to Stress.

| | Type of rat | Mean Differences (M.D.) from control levels.* Sham-adrenalectomized‡ | | | | Adrenalectomized‡ | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. rats | 6 | | 6 | | 6 | | 6 | |
| | Pretreatment† | Saline | | Ascorbic acid | | Saline | | Ascorbic acid | |
| | Hr after epinephrin | 3 (a-b)* | 24 (a-c) | 3 (a-b) | 24 (a-c) | 3 (a-b) | 24 (a-c) | 3 (a-b) | 24 (a-c) |
| Change of the leukocytes | Leukocytes 1000/mm³ ± S.E. | +2.5 2.3 | −.8 .5 | −.5 1.4 | −1.5 1.8 | +5.4§ .9 | −.5 1.0 | +8.6§ 3.5 | −.2 .9 |
| | Lymphocytes 1000/mm³ ± S.E. | −2.5§ .9 | −.5 .3 | −3.3§ 1.3 | −.6 1.1 | +.1 .5 | +.2 1.1 | +6.9 3.6 | +.5 1.1 |
| | Polymorphs 1000/mm³ ± S.E. | +4.9§ 1.3 | −.3 .2 | 3.4§ .9 | −.8 .3 | +5.3§ .7 | −.9 .5 | +3.5§ 1.1 | −.6 .3 |
| | Eosinophils per mm³ ± S.E. | −176§ 24 | +22 36 | +242§ 88 | +132 66 | +484§ 95 | +110 45 | +176 97 | +506§ 96 |

* Count (a) was made just prior to pretreatment; count (b), 3 hr following epinephrin; count (c), 24 hr after epinephrin. (a-b) = count (a) compared to count (b).
† Pretreatment with saline or with ascorbic acid 7 to 8 days after operation.
‡ 7-8 days post-operative.
§ Mean difference is significant.

trophils) occurred in sham-adrenalectomized rats 3 hours after, and returned to pre-stress levels 24 hours after, the epinephrin injection. A significant rise in the neutrophil count was observed in all adrenalectomized rats (III and IV) 3 hours after the stress, these returned to pre-stress levels by 24 hours following the injection.

*The eosinophil response.* The expected eosinopenia 3 hours after stress was observed in Group II rats but was absent in animals pretreated with ascorbic acid (Group I). There was a significant rise in the number of circulating eosinophils in the Group I rats; this rise of eosinophils was similar to the rise observed in adrenalectomzed rats 3 hours after epinephrin, and was observed in a previous study in intact rats(3). In the rats which were adrenalectomized and supplied with ascorbic acid (Group III), the rise of eosinophils was delayed to 24 hours after the injection of epinephrin; it is noted that within this same period the levels in Group II and Group IV rats returned to prestress levels.

*Discussion.* The data presented in Table I show the response of the blood leukocytes to stress under a variety of conditions. Since the response was of importance, only the mean differences and the standard errors are presented in the table, the absolute numbers are not presented. The data suggest that ascorbic acid may exert an effect on the leukocyte picture even in the absence of the adrenal. It is noteworthy that the eosinophil response of the ascorbic acid pretreated sham-adrenalectomized rat is similar to that of the adrenalectomized rat exposed to stress. This observation is in consonance with observations that the vitamin prevented the adrenal hypertrophy following stress, and the depletion of steroids characteristic of stress(2,3). It is also apparent that ascorbic acid pretreatment to adrenalectomized rats altered the response of the eosinophils to epinephrin stressor. Instead of the rise observed in this study in pretreated animals, and in other studies(5), at the end of 3 hours after epinephrin, there is a delay of the response. Whether this delay is of any significance remains to be determined but it is very evident from these data that ascorbic acid modified the response of the eosinophils in the absence of the adrenal gland. It is well known that the leukocytes are concerned with the transportation of ascorbic acid(6). Should the action of the vitamin observed in this study involve any action on the transportation of ascorbic acid, then it becomes necessary to assume that the eosinophils and not the other elements of the leukocytes are probably involved in these changes. It has been suggested that ascorbic acid is related to the production and utilization of

5. Dury, A., *Am. J. Physiol.*, 1950, v160, 75.
6. Heinemann, M., *J. Clin. Invest.*, 1941, v20, 467.

adrenal cortical hormones(7,8), but it is not clear whether these possible functions of ascorbic acid are related to its alliance with the eosinophils.

*Summary.* The action of ascorbic acid on the leukocyte response of normal (sham-adrenalectomized) and adrenalectomized rats was investigated. The data indicate that, of all the leukocytes studied, only the eosinophils are related to the action of ascorbic acid. The vitamin alters the eosinopenia of stress in intact rats, and delays the eosinophilia characteristic of stress in adrenalectomized rats.

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The author acknowledges the helpful interest of Doctor C. E. Leese.

Received April 18, 1951. P.S.E.B.M., 1951, v77.

## A Continuously Recording Scanning Device for Radio-Chromatographic Analysis of Labelled Antigen-Antibody Reactions.*† (18715)

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This communication describes an application of paper chromatography to the study of interactions of antigen with antibody and a method of continuous recording to indicate the distribution of radio-isotopically labelled components. Castaneda(1) has described some reactions of Brucella antigens with their antisera on paper chromatograms using hematoxylin stained material to indicate movement or fixation of the antigen by antisera. By this means he developed a qualitative test for the presence of brucella antibodies in human sera.

Using bovine serum albumin isotopically labelled with $I^{131}$ by the method of Knox and Endicott(2) we have attempted some quantitative measurements of antibody titre to this antigen. Also it has been possible to observe the chromatographic behavior of soluble complexes formed by antigen antibody interactions in the region of antigen excess.

*Chromatographic methods.* The technic described by Franklin and Quastel(3) for the chromatography of protein mixtures was adapted to the chromatography of varying volumes of $I^{131}$ labelled bovine serum albumin with a standard volume of antiserum using aqueous buffer and salt solutions of suitable pH and ionic strength. The capillary ascent technic of Williams and Kirby(4) using Whatman No. 1 paper strips ¾ inch wide has been used throughout. The antiserum spots usually 10 or 20 $mm^3$ were placed with calibrated hemoglobin pipettes at marked starting points 2 inches above the lower end of the paper strip. The desired volume of $I^{131}$ labelled antigen solution was superimposed on the antiserum spot by delivery with a Linderstrom-Lang-Holter microburette. The volume usually ranged from 1-20 $mm^3$. In order to achieve more uniform distribution of the antigen over the antiserum spot a small drop of isotonic saline solution was placed near the tip of the microburette and allowed to wash down the tip of the burette to the paper. There the drop of saline solution

* The technical assistance of Miss Martha C. Havens is gratefully acknowledged.

† The authors are indebted to Dr. William C. Knox for providing the standard precipitin curve on the antiserum tested.

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2. Knox, W. C., and Endicott, F. C., *J. Immunol.*, 1950, v65, 523.

3. Franklin, A. E. and Quastel, J. H., *Science*, 1949, v110, 447.

4. Williams, R. J. and Kirby, H., *Science*, 1948, v107, 481.