Splenic Arteriovenous Differences in Blood Cells During the Hematologic Reaction to Adrenal Cortical Stimulation

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STIMULATION of the normal adrenal cortex by pituitary adrenocorticotropic hormone (ACTH), by epinephrine or by a variety of stressful situations consistently results in eosinopenia.[1-5] This effect is dependent on an increased output of 11,17-oxysteroids by the adrenal cortex.[2, 3] In the adrenalectomized animal, on the other hand, the administration of epinephrine or the application of mild stress usually results in eosinophilia.[3, 4, 6] Neither the mechanism of adrenal-mediated eosinopenia nor of the adrenalectomy eosinophilia has been elucidated. The role of the spleen in both phenomena has been studied. Splenectomy does not alter the degree of eosinopenia induced by ACTH in man,[1] by mild stress in the mouse[3] and by epinephrine in the rat.[4, 6] However, Dury[6] found that splenectomy abolished post-ephinephrine eosinophilia in adrenalectomized rats, while Speirs and Meyer[3] obtained the apparently conflicting evidence that mild stress resulted in eosinophilia in splenectomized-adrenalectomized mice as well as in adrenalectomized animals.

The present experiments investigated further the relation of the spleen to the circulating eosinophil level by means of the study of splenic arteriovenous eosinophil differences in the intact dog after the administration of ACTH or epinephrine. This method was applied to determine whether the spleen of the intact animal entraps eosinophils in significant quantity, a function which other tissues could assume after splenectomy, or whether, on the contrary, the spleen actually puts out eosinophils during the period when circulating eosinophil levels are declining.

In addition, A-V differences of neutrophils, lymphocytes and erythrocytes (hematocrit) were studied to determine whether the spleen acts upon other blood cells in the same manner as upon eosinophils during the period of developing eosinopenia.

Methods

Procedure

Six male and 4 female mongrel dogs were studied. The animals were anesthetized by slow intravenous injection of 30 mg./Kg. of sodium pentobarbital. The spleen was exposed through a left rectus incision and was gently placed in warm saline pads on the abdominal wall. The splenic vein was dissected free and a 19 gauge needle inserted into the vein or one

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of its two main splenic tributaries. The exposed femoral artery was similarly punctured. The puncture sites were sealed with collodion. Clotting was prevented by the attachment to each needle of a tuberculin syringe containing heparin (10 mg./cc.), 0.05 cc. of which was infused after each sampling of blood. "Control" blood samples* were obtained simultaneously from the femoral artery and the splenic vein as soon as the needles were placed and again approximately fifteen minutes later after the spleen had resumed its initial size (moderate shrinkage occurred during handling). Samples were obtained at fifteen to thirty minute intervals after epinephrine or ACTH was given. At each sampling, the first ½ to 1 cc. of blood was discarded and the next 2 cc. transferred to a bottle containing dried balanced oxalate for hematologic studies.

Total eosinophils were evaluated by the method of Randolph,[7] with the following modifications: (1) Two pipets were filled for each count. Each pipet was used to load the two sides of one Levy-Fuchs-Rosenthal 0.2 mm. deep hemocytometer. The total number of eosinophils counted on all four sides multiplied by 1.56 determined the eosinophils per cu. mm. (2) The diluting solution contained 25 mg. of phloxine and 50 cc. of propylene glycol brought to a volume of 100 cc. with water. White blood counts were made in duplicate. Total neutrophils and lymphocytes were estimated from the WBC multiplied by percentages obtained from 200 cell differential counts on Wright's stained films. Hematocrits were determined by the method of Wintrobe.[8]

In 7 experiments epinephrine hydrochloride was injected intra-arterially over a two minute period in a dose of 0.2 mg./Kg. In 2 dogs ACTH (Armour Lot H-3706) was injected intra-muscularly in a dose equivalent to 24 mg. of Armour Standard LA-1-A adrenocorticotropin. Injections were made after the spleen had remained unhandled for at least fifteen minutes. In 1 animal, no injection was made. The splenic width at a constant point was measured every fifteen minutes throughout the experiment.

Analysis of Results

The standard deviation about any chamber count is a function of the square root of the count.[9, 10] Therefore, where a wide range of counts is encountered in the same experiment, the successive A-V differences may be made comparable by dividing each by the square root of its arterial count. When A-V eosinophil and neutrophil† differences were thus standardized, a mean standard A-V difference and the standard error of that mean difference was calculated for each experiment. Significance and probable significance were reported for P-values of less than 0.01 and less than 0.02, respectively.

The A-V differences of eosinophils and neutrophils to which this treatment was applied included those obtained from the time the arterial eosinophil level first dropped below its control level until a minimum was reached or the experiment terminated. Thus, the period of acute splenic discharge induced by epinephrine was excluded. Hematocrit A-V differences were considered for the same period and in the same way except that no standardization was required.

Eosinophil/neutrophil (E/N) and eosinophil/hematocrit (E/H) ratios were calculated for each arterial and venous count. The A-V difference in the ratio

* The true control eosinophil count was not important in the present study. Actually, in 4 instances in which data was available, the eosinophil count in antecubital vein blood drawn prior to any procedure was essentially the same as the first "control" samples from the femoral artery and splenic vein.

† The neutrophil count is the product of the WBC times the differential percentage. The standard error is a function of both component errors and these errors in turn vary as the square root of the mean and the square root of the differential percentage, respectively. Thus, as an approximation the square root of the neutrophil count may be used as the divisor for standardization.

measured the comparative direction and degree of the splenic effect on the two types of cells. The significance of these A-V differences was evaluated by the t-test.

All 10 experiments were treated together since no essential differences were noted between the hematologic results after epinephrine, ACTH or operation alone and since the effect of operation was common to all.

TABLE 1.—*Effects of Various Procedures on Arterial Eosinophil Counts and on Splenic Arteriovenous Eosinophil Differences*

Exp. No.	Arterial Control Cells per cu. mm.	Per Cent Change %	Duration of Decline Min.	No. Blood Samples during Decline	During Decline	
					Mean A-V Difference Cells/cu. mm.	Mean Standardized A-V Difference ± S.E. Mean Difference*
Operation + Epinephrine 0.2 mg./Kg. I. A.						
1	1036	−78	70	4	+40	+1.19 ± 3.47
2	1022	−61	150	6	−181	−9.60 ± 2.31 (S)
3	111	−70	135	6	−12	−1.77 ± 1.46
4	2531	−73	150	8	−21	−0.80 ± 1.55
5	584	−62	165	7	−150	−9.38 ± 5.86
6	477	−73	250	8	−105	−6.80 ± 1.60 (S)
7	986	−63	270	9	−148	−6.09 ± 1.17 (S)
Operation + ACTH 24 mg. I. M.						
8	1359	−82	300	13	−151	−6.48 ± 0.96 (S)
9	731	−87	150	7	−4.7	−0.68 ± 0.76
Operation Alone						
10	203	−96	390	7	−2.0	−0.53 ± 0.53
Mean	866	−74	203	7.5	−74	−4.09 ± 1.26 (S)

* Mean standardized A-V Difference = $\frac{\Sigma \frac{A - V}{\sqrt{A}}}{N}$ S = P < 0.01

RESULTS

In all 10 animals, the arterial eosinophil count declined markedly from the "control" values (average 866 cells per cu. mm.; range 111 to 2,531 cells per cu. mm.) to levels 61 to 96 per cent lower (average 74.5 per cent). The arterial neutrophil count rose from a "control" average of 8,880 cells per cu.mm. (range 3,620 to 21,200 cells per cu. mm.) to counts 21 to 367 per cent above this "control" value (average 160.2 per cent). Arterial lymphocyte counts varied widely but tended to decline over the course of the reaction. Arterial hematocrits did not change consistently.

Average splenic A-V differences immediately after splenic venepuncture were insignificantly positive for eosinophils (+27 ± 43 cells per cu. mm.) and hematocrit (+2.8 ± 1.3 volumes per cent), and significantly positive for neutrophils

(+1,210 ± 414 cells per cu. mm.; $P < 0.02$). Venous E/N and E/H ratios were not significantly different from arterial ratios at this time.

In 9 out of 10 animals, the average splenic A-V eosinophil difference during the period of developing eosinopenia was negative (table 1). In 4 experiments, the mean standardized A-V difference was significantly negative by t-test. When the

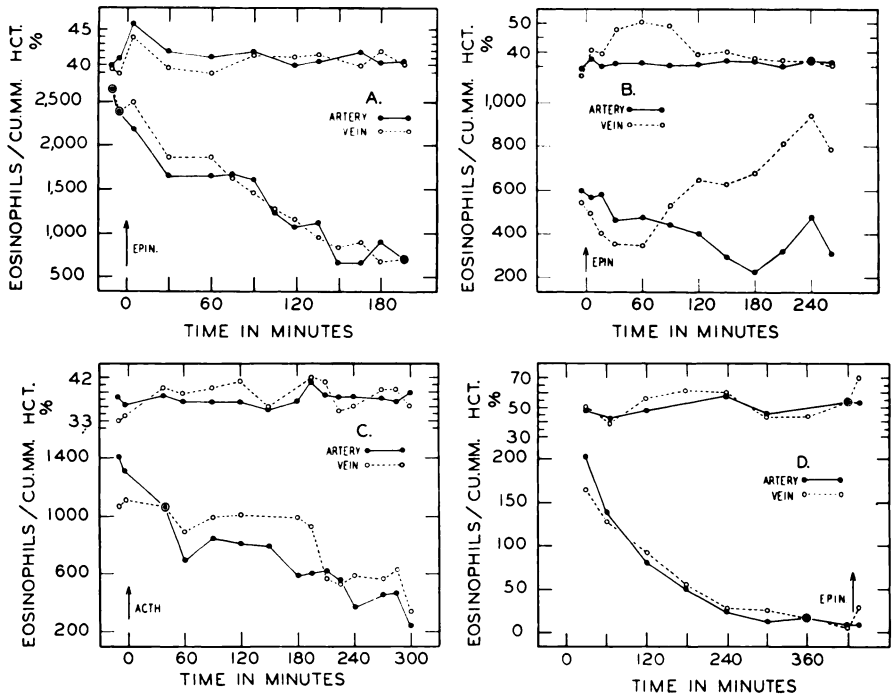

FIG. 1.—Eosinophil counts and hematocrits in femoral artery (indicated by solid lines and black circles) and splenic vein (indicated by broken lines and open circles) after epinephrine (0.2 mg./Kg.), ACTH (24 mg.) and operation alone.

A. Experiment 4. No significant A-V differences occurred. Note the lack of correlation in time between A-V differences for eosinophils and for hematocrit.

B. Experiment 5. A large negative A-V eosinophil difference occurred during the last two and a half hours of this experiment. Note the negative correlation in time between A-V differences for eosinophils and for hematocrit.

C. Experiment 8. A significantly negative A-V difference for eosinophils occurred. The ratio of eosinophils to hematocrit in the vein was significantly greater than that in the artery.

D. Experiment 10. No significant A-V differences occurred. Note the small but definite rise in venous eosinophil count and the larger rise in hematocrit after the administration of epinephrine at the termination of the experiment.

mean standardized A-V differences for all 10 experiments were averaged, this overall mean value was significantly negative ($t = -3.25$, $P < 0.01$). The time course of development of A-V differences was variable (fig. 1, A-D).

The mean splenic A-V difference for neutrophils was positive in 6 out of 9 experiments during the eosinophil decline, but in no case significantly so (table 2). The average of these mean standardized A-V differences for all 9 experiments was also positive, but was not a real difference ($t = +1.97$, $P > 0.05$). There

was a consistent difference between the splenic effect on neutrophils and on eosinophils. In all 9 experiments the mean A-V difference in eosinophil/neutrophil ratio was negative. The overall mean A-V difference in ratio for 9 experiments was significantly negative at the 2 per cent probability level ($t = -3.18$).

Arteriovenous hematocrit differences were positive in 4 experiments and negative in 4 (table 3). Only in Experiment 7 was the mean A-V difference significantly negative. None of the positive deviations were significant. The overall mean A-V difference for 8 experiments was insignificant ($t = -0.42$, $P > 0.6$).

TABLE 2.—*Effect of Various Procedures on Arterial Neutrophil Counts and on Splenic Arteriovenous Neutrophil Differences*

Exp. No.	Arterial Control Cells per cu. mm.	Per Cent Change %	During Eosinophil Decline		
			Mean A-V Difference Cells per cu. mm.	Mean Standardized A-V Difference ± S.E. Mean Difference*	E/N Ratio. Mean A-V Difference. ±S.E. Mean Difference. Eosinophils per 100 Neutrophils
Operation + Epinephrine 0.2 mg./Kg. I. A.					
1	9,420	+21	+3030	+30.6 ± 11.4	−1.6 ± 1.5
2	6,570	+267	—	—	—
3	21,200	+121	+450	+1.6 ± 11.2	−0.033 ± 0.037
4	11,840	+21	−43	−1.1 ± 7.0	−0.13 ± 0.94
5	5,640	+252	+960	+8.6 ± 6.6	−1.3 ± 0.90
6	8,940	+128	+1490	+11.6 ± 5.5	−0.76 ± 0.17 (S)
7	5,600	+178	+1170	+9.5 ± 6.4	−2.1 ± 0.72
Operation + ACTH 24 mg. I.M.					
8	9,280	+122	−25	−0.4 ± 7.9	−1.9 ± 0.86
9	6,700	+125	+1740	+13.1 ± 11.2	−0.24 ± 0.25
Operation Alone					
10	3,620	+367	−560	−7.7 ± 11.6	−0.003 ± 0.14
Mean	8,881	+160	−912	+7.31 ± 3.71	−0.91 ± 0.28 (PS)

*Mean standardized A-V difference = $\frac{\sum^{A-V}\sqrt{A}}{N}$ S = P < 0.01 PS = P < 0.02

In the 6 experiments in which eosinophil/hematocrit ratios could be calculated, the mean A-V difference in this ratio was negative, in 3 cases significantly so. The overall mean A-V difference in ratio for the 6 experiments was significantly negative at the 2 per cent probability level ($t = -3.86$).

Values for lymphocyte counts were too unreliable to allow satisfactory analysis of their A-V differences.

Marked splenic contraction occurred while epinephrine was being given. Splenic transverse diameter returned to or near normal within fifteen minutes. In all 10 experiments a gradual reduction in splenic size occurred during the period for which hematologic values are reported. This was reflected by an average decrease in transverse diameter of 10.8 per cent (range, 2 to 20 per cent).

Discussion

The absence of a significantly positive A-V eosinophil difference in any experiment excludes the possibility of a net uptake of eosinophils by the intact spleen during the development of adrenal-mediated eosinopenia. The present work thereby confirms the conclusion drawn previously from studies on splenectomized animals that the spleen does not contribute to the development of such eosinopenia.

TABLE 3.—*Effects of Various Procedures on Arterial Hematocrit and on Splenic Arteriovenous Hematocrit Differences*

Exp. No.	Arterial Control Vol. RBC per 100 cc. Blood	Per Cent Change %	During Eosinophil Decline	
			Mean A-V Difference ±S.E. Mean Difference. Vol. RBC per 100 cc. Blood	E/H Ratio. Mean A-V Difference ±S.E. Mean Difference. Eosinophils per 10^{-5} cc. RBC
Operation + Epinephrine 0.2 mg./Kg. I.A.				
1	50.8	+3.3	−2.9 ± 2.4	—
2	—	—	—	—
3	—	—	—	—
4	40.5	0	+0.50 ± 0.60	−1.1 ± 1.5
5	35.5	+2.8	−7.9 ± 2.5	−2.0 ± 3.1
6	47.5	0	+9.0 ± 4.5	−2.6 ± 0.73 (PS)
7	49.0	+16	−4.1 ± 0.61 (S)	−1.8 ± 0.54 (S)
Operation + ACTH 24 mg. I.M.				
8	37.2	+4.8	−0.83 ± 0.67	−3.4 ± 1.1 (S)
9	46.0	+18	+0.07 ± 0.60	−0.08 ± 0.29
Operation Alone				
10	48.0	+12	+0.50 ± 1.34	—
Mean	44.3	+7.1	−0.71 ± 1.71	−1.82 ± 0.47 (PS)

S = $P < 0.01$ PS = $P < 0.02$

Under the conditions imposed in the present experiments, splenic vein eosinophil concentrations and their ratios to neutrophils and hematocrit averaged significantly higher than arterial values during the period when eosinopenia developed. These findings, in the presence of a spleen which was not increasing in size, can only mean that eosinophils were being added to the blood in its passage through the spleen. Since no measurements of splenic blood flow were made, the negative A-V differences cannot be directly converted into estimates of the actual rate of eosinophil output. Alterations in splenic blood flow would greatly affect the estimate of magnitude of splenic eosinophil output but would not alter the demonstration that some degree of output occurred.

There is no evidence in the present work which bears on the role of the adrenal cortex in the observed splenic output of eosinophils, since the effects of the operation itself could not be controlled. However, Recant et al.[4] have shown that the adrenalectomized rat develops eosinophilia after epinephrine, but not after

ACTH. Therefore, it seems likely that epinephrine or operative stress or both have a dual effect on eosinophils, a pituitary-adrenal-mediated stimulus for eosinopenia and a nonadrenal-mediated effect on the spleen leading to prolonged eosinophil output.

Significantly negative A-V differences in the ratio of eosinophils to neutrophils and to hematocrit were found in the present experiments. In the case of the neutrophils, the higher ratio of eosinophils to neutrophils in the splenic vein was usually dependent not merely on differences in proportion but on actual differences in direction of the A-V differences for the two cells. This suggests that the spleen is capable of storing one cell type while releasing another, but the presence of this mechanism cannot be definitely established from the present study since the overall trend toward neutrophil storage was not statistically significant. It is certain only that marked quantitative differences can exist between the splenic action upon eosinophils and its simultaneous effect on neutrophils and erythrocytes.

The mechanism of the eosinopenia induced by 11,17-oxysteroids remains unknown. Theoretically, it could be accomplished by (1) decreased rate of production or release of eosinophils from bone marrow, (2) increased rate of destruction of eosinophils in blood or tissues, (3) the entrapping of eosinophils in peripheral sites with subsequent liberation during the recovery stage or (4) a combination of certain of these effects. The first possibility seems unlikely in that it presupposes an extremely short life cycle for the eosininophil. The second has not been supported by in vitro studies with pure adrenal steroids.[11] The third proposal therefore seems a most probable one. It is consistent with the very rapid restoration of eosinophil counts to normal after maximum eosinopenia. It is further supported by the observation that the percentage of single-lobed, "young" eosinophils in 1000 to 3000 cell differential counts does not rise during the recovery period when eosinophils are returning to the peripheral blood.[12] The sites where eosinophils may be trapped in significant numbers have as yet escaped detection. It is to be hoped that further studies along these lines may answer the question posed here and that information relative to the function of the eosinophil cell may be a by-product of such studies.

Summary and Conclusions

1. Arteriovenous eosinophil differences across the spleen were measured in 10 dogs during the development of adrenal-mediated eosinopenia. In 7 instances the stimulus was epinephrine, in 2 ACTH, and in 1 operation alone.

2. In no case was significant net splenic uptake of eosinophils indicated. In 3 out of 7 epinephrine experiments and in 1 ACTH experiment, a significant net splenic output of eosinophils was found. For all 10 experiments averaged, the splenic output of eosinophils was significant.

3. Arteriovenous neutrophil and hematocrit differences were insignificant and were independent of simultaneous eosinophil differences.

4. It is concluded that the spleen is not a significant site of uptake or destruction of eosinophils during the development of adrenal-mediated eosinopenia. Under certain conditions, it actually extrudes eosinophils into the general circulation at a time when other factors are causing eosinophil disappearance.

5. The results suggest but do not establish the presence of a discriminatory splenic mechanism for the control of blood cells, capable of storing cells of one type while it releases others into the circulation.

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