A STUDY OF THE CATECHOL AMINE CONTENT OF RED BLOOD CELLS AND PLASMA IN HEMORRHAGIC AND HYPOGLYCEMIC SHOCK

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The catechol amine output of adrenal vein plasma is greatly increased in response to conditions of stress. [1,2,3,4,5,6] These elevations of epinephrine (E) and norepinephrine (NE) quickly return to normal values when stress is removed. [5,7,8] Infusion studies also have shown that the elevation of circulating E and NE produced by intravenous infusion of these substances rapidly returns to preinfusion values when the infusion is stopped. [9,10] The rapid disappearance of circulating catechol amines, in both conditions, may be due either to storage or to destruction in the tissues. Von Euler [11] observed no increase in the catechol amine contents of tissues such as liver, spleen, muscle, heart and kidney following intravenous infusion. On the other hand Raab [12] found an increase in tissue catechol amine content following the injection of extremely high doses, 10 mg/kg of body weight, in dogs. The writer knows of no study performed to date on the catechol amine content of red blood cells in conditions of stress.

The purpose of the present study is to investigate the E and NE contents of red blood cells and of plasma in the adrenal vein blood of dogs subjected to hypoglycemic and hemorrhagic shocks. If the storage of catechol amines in the tissues plays an important role in the disappearance of plasma sympathomimetic amines, then an elevation of these amines in the red blood cells would be expected, since they form part of the tissue component.

Method

Five dogs weighing between 20 and 30 kg were anesthetized with 30 mg of pentobarbital per kilo of body weight. A polyethylene catheter was placed in the right lumbo-adrenal vein, using the procedure described by Hume and Nelson. [13] A second catheter was inserted in the right femoral artery and connected to a mercury manometer for blood pressure recording in phlebotomized dogs. Samples of blood were collected into heparin, chilled in ice and the plasma separated by centrifugation, frozen and stored at -40° C. The separated red blood cells were washed twice with cold isotonic salt solution to remove plasma residue and then were frozen and stored in the same manner as the plasma.

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Hypoglycemia was produced by the intravenous administration of crystalline insulin, one unit per kilo of body weight. Hemorrhagic shock was produced by removing 300 to 500 cc of blood from each dog.

The method of Weil-Malherbe and Bone [14] as modified by Aranow [15] was used for the measurement of catechol amines in the blood plasma and red cells. The findings are expressed as micrograms per minute in adrenal vein blood. Blood glucose was determined by the Nelson modification of the Somogyi [16] procedure.

Results

Studies were performed on five dogs, of which two received insulin and three were bled to shock. One unit of crystalline insulin per kilo of body weight was administered intravenously to two dogs following the collection of control blood specimens. The results of E and NE and blood glucose determinations on samples of adrenal venous blood collected at 20 minute intervals are shown in Table 1 and Figure 1. A marked increase in plasma E output over control values occurred in each animal following insulin hypoglycemia. The peak outputs occurred at about 80 minutes after the injection of insulin and were 30 to 48 times greater than the control values. Epinephrine values appeared to correspond in an inverse manner to the changes observed in blood glucose concentrations. The peak of NE output was 6 to 16 times greater than the control figures.

The red blood cells, in contrast to the plasma, showed no increase in E and NE contents during insulin-induced hypoglycemia. In dogs 1 and 2, the epinephrine output in the adrenal vein via the red blood cells was 0.104 and 0.049 micrograms/min before insulin injection, and became 0.054 and 0.020 micrograms/min respectively during hypoglycemia. In the same dogs NE control values were 0.006 and 0.021 micrograms/min but dropped to 0.003 and 0.007 micrograms/min respectively during hypoglycemia.

Dogs 3, 4, and 5 were bled following the collection of control blood specimens. Table 2 and Figure 2 show the relationship between arterial blood pressure and the catechol amine output of adrenal vein blood. There is definite indication that the catechol amine output reached a maximum soon after the blood pressure dropped to shock levels. In these dogs the rate of augmentation of E was respectively 17, 54, and 46 times that of control values. On the other hand NE output showed increases respectively of 32, 15, and 23 times the control figures.

The red blood cells of adrenal vein blood, in contrast to the plasma, showed no increase in their E and NE values during hemorrhagic shock. In dogs 3, 4 and 5 the E output in the adrenal vein via the red blood cells was 0.020, 0.067, and 0.042 micrograms/min before bleeding and became respectively 0.008, 0.039, and 0.034 micrograms/min after bleeding. Norepinephrine output in these animals was respectively 0.005, 0.019, and 0.010 micrograms/min before hemorrhage but dropped to 0.002, 0.017, and 0.004 micrograms/min after hemorrhage.

Discussion

These results show that insulin-induced hypoglycemia and hemorrhagic hypotension in anesthetized dogs are accompanied by the appearance of large quantities of catechol amines in the adrenal vein blood plasma. The marked and relatively rapid increase in the adrenal medullary secretion occurring in response to hypoglycemia and hemorrhage was similar in degree and time relationship to that found by Satake[17] and Lund[5] and Goldfien et al,[7] in the dog and by Duner[1] in the cat. It was also observed by Duner[1] and Goldfien et al[7]. that the increased catechol amine output of the adrenal medulla returned toward control values following the administration of glucose in hypoglycemic dogs. Lund,[5] and Zileli et al.,[6] by reinfusing drawn blood into phlebotomized dogs, noticed an immediate drop in the output of catechol amines of the adrenal blood plasma. Satake[17] measured only total catechol amines, whereas Duner,[1] Goldfien et al.,[7] Lund,[5] and Zileli et al.,[6] estimated both E and NE. They found that the increased output was mainly due to E but NE also increased moderately. This last finding is in agreement with our results.

Our study, in addition, showed that the increased adrenal medullary secretion is carried mainly by the adrenal vein plasma. On the other hand the catechol amine content of the red blood cell dropped significantly at the time of maximal output of the adrenal medulla. It is difficult to state at the present time which factor might have contributed to the failure of the catechol amine content of the red blood cells to rise. Low concentrations of catechol amine in the red blood cells are not related to the washing procedure, since unwashed red cells showed only a little more amine, which came from the contaminating plasma. Two possibilities might be offered in explanation. First, it is possible that the cell membrane is not freely permeable to catechol amine. This hypothesis can be ruled out by the observation of Weil-Malherbe and Bone[18] who found that normal human red blood cells contain a little more E and a little less NE than plasma.

TABLE 1.
Dog No: 1

Minutes after insulin	Epinephrine output			Norepinephrine output		Blood sugar
	Plasma	Red cell		Plasma	Red cell	
	0.080	0.104	Control	0.021	0.006	75
20	0.315	0.104		0.075	0.006	61
40	1.637	0.099		0.243	0.005	48
60	1.587	0.099		0.257	0.004	30
80	2.212	0.066		0.289	0.003	32
100	2.046	0.054		0.332	0.003	48
120	1.820	0.070		0.243	0.003	52
140	1.335	0.080		0.258	0.003	52
160	0.648	0.100		0.180	0.004	55
180	0.378	0.107		0.106	0.005	57
Dog No. 2						
	0.047	0.049	Control	0.033	0.021	95
20	0.124	0.046		0.034	0.018	—
40	0.320	0.037		0.066	0.010	70
60	1.433	0.024		0.143	0.012	—
80	2.245	0.021		0.210	0.007	45
100	1.435	0.026		0.167	0.008	38
120	1.090	0.020		0.180	0.007	—
140	0.909	0.029		0.180	0.007	36
160	0.875	0.027		0.191	0.009	—
180	0.757	0.023		0.132	0.021	36
200	0.668	0.038		0.176	0.015	36
220	0.527	0.046		0.150	0.017	—
240	0.519	0.045		0.135	0.020	50

Adrenal vein catechol amine output in micrograms per minute, and blood sugar levels before and after intravenous insulin injection, one unit per kilo body weight.

We also observed the same finding in two dogs before insulin injection, but in other dogs both the E and NE contents of the red cell were lower than those of plasma. Second, it is possible that catechol amine may be metabolized rapidly by the red blood cell or may be changed into other inactive metabolites. The second hypothesis seems to be more logical. Thus the prompt disappearance of plasma catechol amines may be due to destruction partly in the red cell and mainly in other tissue cells. The dissappearance of these amines is not due to destruction in the plasma, since the blood contains such substances as amino acids, glutathione and ascorbic acid which all protect catechol amines against oxidation.[19]

TABLE II

Minutes after Bleeding	Epinephrine output		Dog No. 3	Norepinephrine output		Blood Pressure mm. Hg.
	Plasma	Red cell		Plasma	Red cell	
	0.070	0.020	Control	0.016	0.005	110
20	0.265	0.024		0.046	0.006	90
40	0.720	0.015		0.262	0.004	60
60	1.195	0.011		0.382	0.004	50
80	1.128	0.008		0.512	0.002	55
100	0.658	0.014		0.315	0.006	60
120	0.699	0.017		0.238	0.006	60
			Dog No. 4			
	0.089	0.067	Control	0.051	0.019	140
20	1.707	0.056		0.269	0.027	60
40	1.054	0.062		0.679	0.018	70
60	3.662	0.061		0.764	0.017	55
80	4.840	0.057		0.670	0.025	55
100	2.358	0.071		0.412	0.024	70
120	3.742	0.051		0.586	0.021	70
140	3.138	0.039		0.664	0.016	60
160	2.001	0.044		0.428	0.017	60
180	2.991	0.062		0.279	0.019	55
			Dog No. 5			
	0.074	0.042	Control	0.053	0.010	110
4	0.675	0.066		0.112	0.014	80
8	0.899	0.058		0.123	0.012	80
12	0.870	0.063		0.164	0.014	—
16	1.293	0.038		0.193	0.011	60
20	1.452	0.034		0.172	0.005	40
24	1.524	0.053		0.236	0.005	38
28	2.844	0.061		0.758	0.006	38
32	3.429	0.042		1.211	0.004	38

Adrenal vein catechol amine output in micrograms per minute, and blood pressure before and after bleeding.

Thus our finding in adrenal vein red blood cells is in agreement with that of Euler,[11] who observed no increase in the tissue E and NE contents following their infusion intravenously.

Summary

1. This study was performed on 5 dogs. Two animals received one unit of crystalline insulin per kilo of body weight. The other three dogs were bled to shock. The total catechol amine output of the

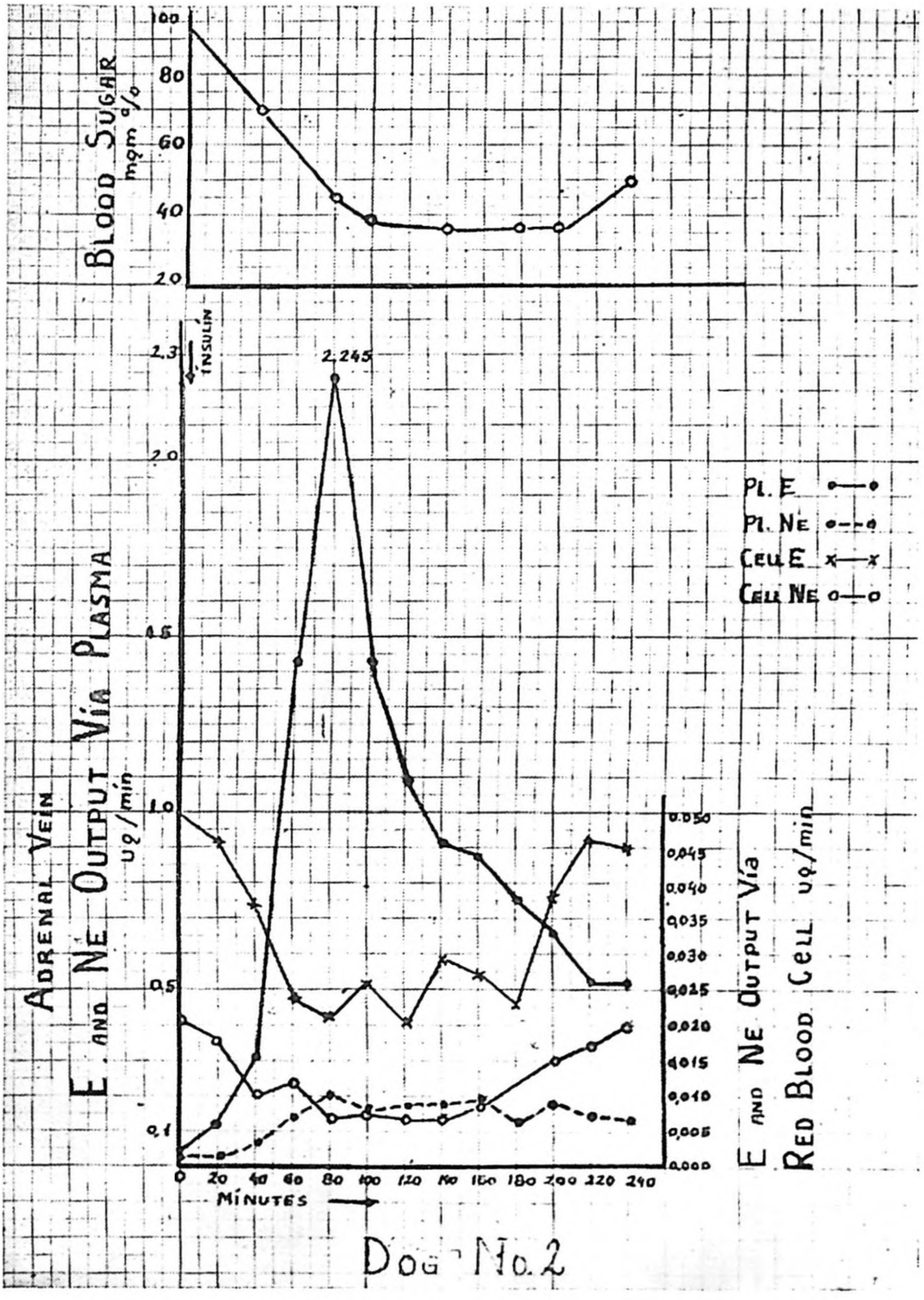
BLOOD SUGAR
mgm %
100
80
60
40
20
INSULIN
2.3
2.0
1.5
1.0
0.5
0.1
2.245
ADRENAL VEIN
E AND NE OUTPUT VIA PLASMA
uq/min
Pl. E
Pl. NE
Cell E
Cell NE
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
E AND NE OUTPUT VIA
RED BLOOD CELL uq/min
0 20 40 60 80 100 120 140 160 180 200 220 240
MINUTES
DOG No. 2

Fig. 1.

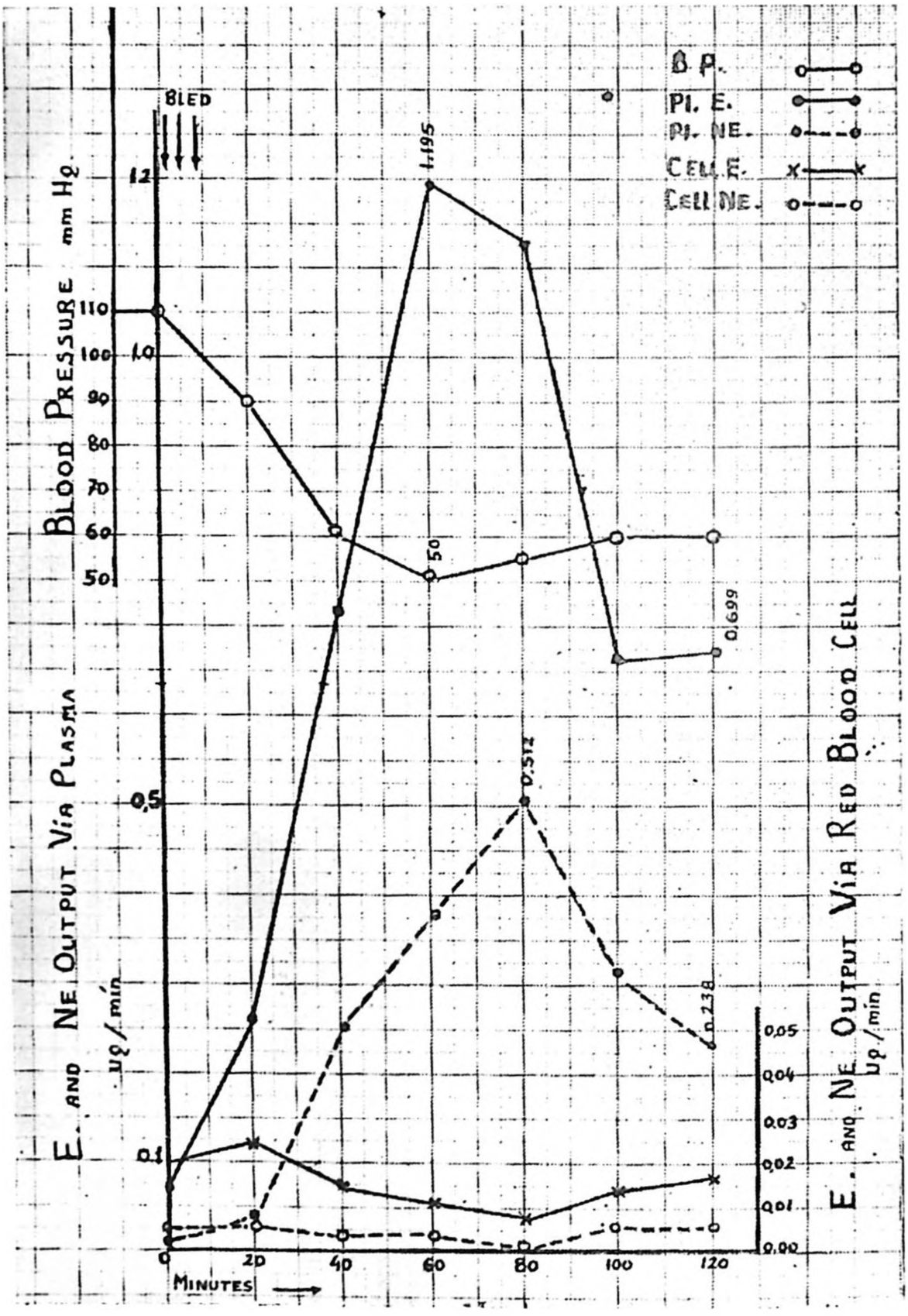
B.P.
Pl. E.
Pl. NE.
Cell E.
Cell NE.
BLED
BLOOD PRESSURE mm Hg.
E. AND NE. OUTPUT VIA PLASMA γ/min
E. AND NE. OUTPUT VIA RED BLOOD CELL γ/min
1.195
0.699
0.512
0.238
MINUTES
0
20
40
60
80
100
120
12
110
100
90
80
70
60
50
1.0
0.5
0.1
0.05
0.04
0.03
0.02
0.01
0.00

Fig. 2 : Dog No. 3.

adrenal gland was determined by analyzing the adrenal vein blood plasma and red blood cells separately.

2. The findings showed that hypoglycemia or hemorrhagic shock in dogs is accompanied by the appearance of large quantities of catechol amines in the adrenal vein blood plasma. On the other hand, the catechol amine content of the red blood cells dropped significantly at the time of stress.

3. It is suggested that the low catechol amine content of the red blood cells under experimental conditions is due to rapid destruction by the cell.

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