THE EFFECT OF ACUTE BLEEDING ON CATECHOL AMINE SECRETION

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Saito, in 1928, studied the effect of bleeding on epinephrine secretion from the adrenal gland and found a close relation between the amount of withdrawn blood and the augmentation ratio of epinephrine in adrenal vein blood. [1] Using his own fluorometric method, Lund, in 1951, observed in two dogs an increase in release of epinephrine and norepinephrine from the adrenal gland following bleeding. Secretion rates returned to normal after retransfusion of the withdrawn blood. [2] He also noticed that epinephrine and norepinephrine maintain a constant ratio during the experimental period. Watts, using rat uterus as a test medium, found significant quantities of epinephrine in dog arterial blood after a hemorrhage amounting to 40 ml per kg of body weight. [3] Recently, Waud has reported that following hemorrhagic hypotension in dogs, blood pyruvate, lactate, and sugar values rose. When normal pressures were re-established by transfusion with dextran, these values fell to normal. [4] As far as the writer knows, no systematic studies have been made to determine simultaneously the E and NE contents of both adrenal vein blood and peripheral blood following withdrawal of varying amounts of blood. The output of catechol amines from the adrenal glands returns to normal after transfusion of withdrawn blood, as stated above, but we do not know what happens after saline or dextran infusion. The purpose of the present study is to measure E and NE contents in both adrenal vein blood and peripheral circulation, before and after removal of various quantities of blood, and to establish the effect of various therapeutic agents such as blood, saline and dextran infusions, on the secretion of catechol amines following bleeding. We believe this to be a more extensive study than hitherto undertaken, and improved techniques have enabled us to obtain more accurate information.

Materials and Method

Thirteen healthy dogs ranging in weight from 14 to 20 kilograms were used for this study. After anesthetization with pentobarbitol (Nembutal), a poly-

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ethylene catheter was placed in the right lumbo-adrenal vein, following the procedure described by Satake.[5] A polyethylene catheter was placed in the right lumbo-adrenal vein and a polyethylene choker was placed around the vein proximal to the gland in such a fashion as to make possible the intermittent collection of adrenal venous effluent.

Another catheter was passed into the left femoral artery and connected to a mercury manometer for blood pressure recordings. This same catheter was also used for removing blood. Blood from the right adrenal vein was collected in 15 ml graduated tubes containing one drop of heparin. Peripheral blood samples were collected from the left femoral artery and vein. The left adrenal glands of three dogs (numbers 11, 12, and 13) were removed, and right adrenal vein blood was withdrawn continuously so that no adrenal vein blood passed through the body. Blood samples were kept under refrigeration until centrifuged. The collected plasma was removed and stored at -20 C, and thawed out immediately before the catechol amine determination.

Two control samples, to check the resting secretion of catechol amines, were drawn from the adrenal vein and peripheral artery of each animal. The dogs were then bled in amounts varying from 200 ml to 600 ml, during a period of 15 to 65 minutes. The total blood volume of a dog is equal to 9.72 per cent of its total body weight, according to Meck and Gasser.[6] Blood pressures were recorded and samples of adrenal vein and peripheral artery blood were collected at regular intervals for catechol amine determination. When catechol amine output reached what was considered to be peak levels, isotonic saline was given to dogs 1 and 2; blood to dogs 6 and 8; and 6 per cent dextran solution to dogs 9 and 10, in amounts somewhat greater than the volume of withdrawn blood. These solutions were infused intravenously over a period of five to 15 minutes and blood samples were continuously collected.

Determinations of E and NE in blood plasma were made according to the method developed by Weil-Malherbe and Bone,[7,8] as modified by Aranow[9] and Goldfein et al.[10] The results are expressed in micrograms per minute of E and NE output for adrenal vein plasma, and micrograms per liter for peripheral plasma.

Results

In general, blood pressure promptly fell during bleeding, from normal values (between 110 and 145) to 25 to 55 mm Hg. The initial fall in blood pressure was, however, soon followed by some recovery in most cases, and decreased again for a period of several hours, until therapeutic agents were infused. A fall in blood pressure always resulted in an increase of catechol

amine secretion from the adrenal gland (Table 1). Dogs bled rapidly developed a peak level of catechol amine secretion more quickly than the dogs bled more slowly. In dogs 1, 2, 6, and 8, two or three peaks of epinephrine secretion were observed at approximately 30, 75, and 120 minutes. Dogs 6 and 8, bled again at the end of the experiment, after the originally withdrawn blood had been replaced, showed a similar rise in adrenal vein catechol amine output, but the elevation observed at this time was not as great as it had been initially (Figure 1).

There was a relatively close correlation between the magnitude of the bleeding and the augmentation ratio (A. R.)* of catechol amine output in adrenal vein blood. Bleeding of one-tenth of the total blood volume gave an A.R. for NE and E of 41.4 and 11.6, respectively; bleeding of one-fourth of total blood volume gave values of 15 and 54, respectively; and bleeding of one-third of total blood volume gave values of 6 and 93, respectively (Table 2, Figure 2). This means that extreme bleeding increases the A.R. of epinephrine more than norepinephrine.

The concentration of NE and E in the adrenal vein blood exhibited considerable variations during the experiment. In calculating the augmentation ratios it was found that epinephrine output exceeds norepinephrine output in large hemorrhages, whereas the reverse is true in small hemorrhages. The time factor must also be considered: following bleeding there occurs a prompt increase and then a relatively rapid decrease in epinephrine secretion, while norepinephrine during the same period, increased and decreased more slowly. Thus, E/NE ratio shows considerable variation during the experiment (Table 3).

There was a significant reduction in the adrenal vein blood flow following bleeding but the adrenal vein catechol amine output did not seem to be influenced to any great extent by the reduction in blood flow through the gland (Table 1).

In all cases peripheral NE and E levels increased, the former somewhat less than the latter, except in dogs 2 and 9, in arterial blood, when the output of catechol amines from the adrenal vein reached its peak. The arterial blood epinephrine level was much higher than that of venous blood, but the norepinephrine levels did not show a significant arterial-venous difference (Figure 3).

In three dogs with unilateral adrenalectomy, the adrenal glands released large amounts of catechol amines, but peripheral E and NE levels did not change.

* The augmentation ratio is the ratio of basic catechol amine level to highest level after bleeding. It is obtained by dividing the highest level by the initial basic level (highest level/basic level=A.R.).

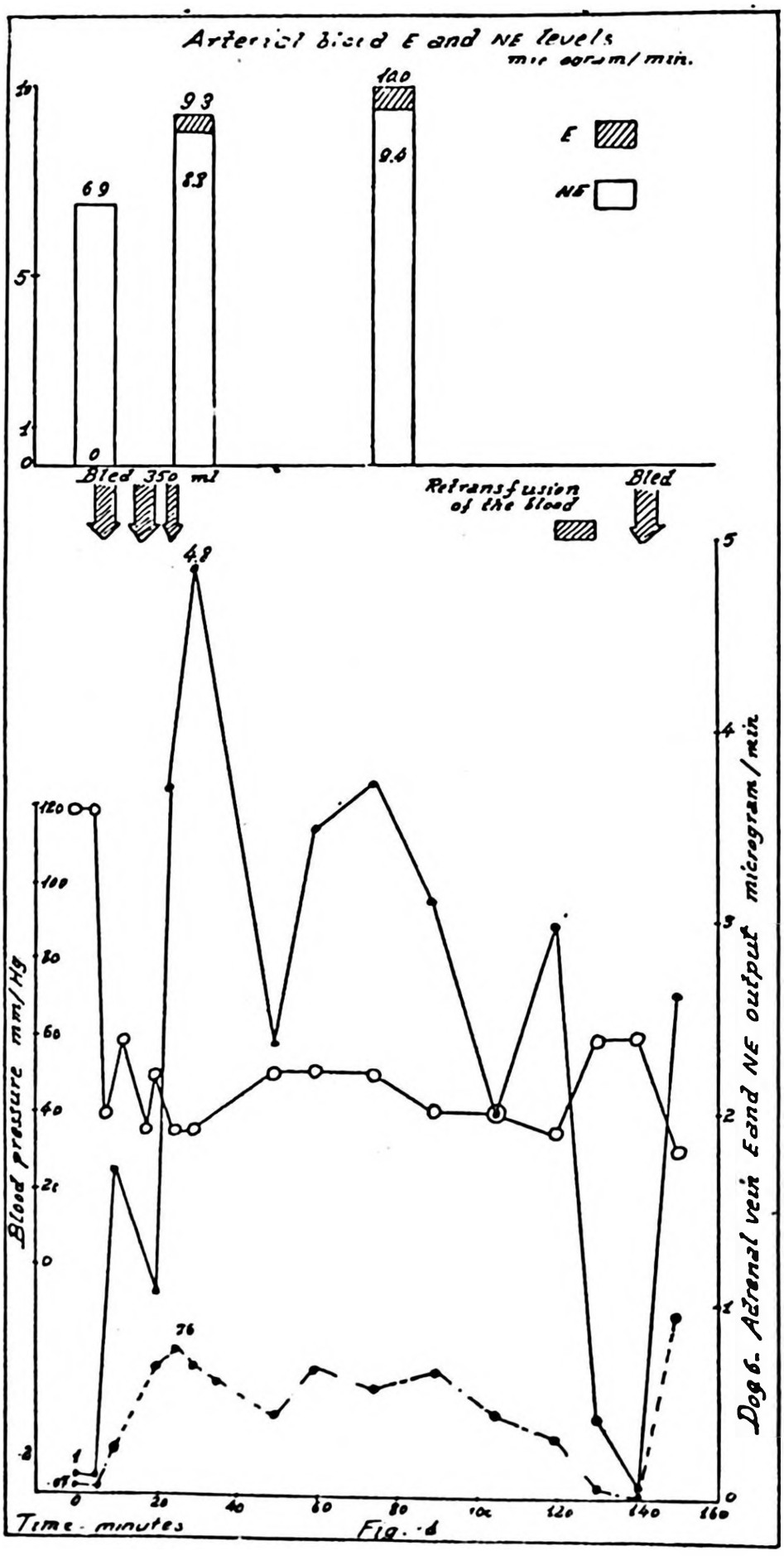
Arterial blood E and NE levels
mic ogram/min.
E
NE
6.9
9.3
8.3
12.0
9.6
Bled 350 ml
Retransfusion of the blood
Bled
4.9
7.6
Blood pressure mm/Hg
Dog 6. Adrenal vein E and NE output microgram/min
Time - minutes

Fig. 6

Table 1

No of dogs	Duration of the bleeding, minutes	Time from the bleeding to the peak of adrenal vein catecholamine output, min.	Withdrawn blood volume, ml	Adrenal vein catecholamine output ng/minute, kg BW		Adrenal vein blood flow, ml/min		Blood Pressure mm Hg		Arterial blood catecholamine level ng/l plasma			
				Control	Highest	Control	Flow at maximum catecholamine output	Control	lowest BP during experiment	Control		Highest level after bleeding	
										NE	E	NE	E
1	45	60	200	0.0139	0.1910	2.5	1.0	120	25	2.4	2.8	6.8	7.6
2	15	17	200	0.1210	0.1870	4.0	0.8	120	40	2.0	0.9	2.1	1.4
3	15	50	400	0.0049	0.0937	1.6	0.6	110	50	4.5	0.8	8.9	12.1
4	15	30	480	0.0103	0.2221	6.9	1.8	140	40	0.9	0.2	3.0	6.9
5	10	50	450	0.0120	0.2725	4.3	0.4	140	38	1.1*	0.8*	9.5*	9.5*
6	15	20	350	0.0108	0.4313	6.5	4.0	140	35	6.9	0.0	9.4	10.0
7	60	65	450	0.0028	0.1016	3.2	0.4	115	44	1.7	2.0	5.0	8.5
8	45	75	600	0.0009	0.0496	2.4	0.7	115	40	2.3	3.5	6.3	9.0
9	30	55	350	0.0012	0.0415	2.8	1.5	140	50	1.4	0.3	2.6	0.3
10	65	70	550	0.0026	0.2161	4.1	1.3	145	40	1.1	0.8	2.7	9.4

* These values represent femoral vein epinephrine and norepinephrine levels

Table. 2

No. of dogs	Weight of dogs, kg.	Volume of withdrawn blood, ml	Withdrawn blood volume / Total blood volume	Adrenal vein NE output ng/min plasma per kg of body weight			Adrenal vein E output ng/min plasma per kg of body weight		
				Control	Highest	Highest / Control	Control	Highest	Highest / Control
1	20.5	200	1/10	0.0012	0.0424	35.4	0.0127	0.1486	11.7
2	18.0	200	1/9	0.0013	0.0630	47.4	0.0108	0.1240	11.5
3	17.5	400	1/4	0.0009	0.0292	32.4	0.0040	0.0645	16.1
4	20.0	480	1/4	0.0011	0.0395	34.3	0.0094	0.1826	19.5
5	18.0	450	1/4	0.0021	0.0620	30.0	0.0099	0.2105	21.2
6	13.0	350	1/3.5	0.0040	0.0590	15.0	0.0068	0.3723	54.7
7	14.0	450	1/3	0.0008	0.0032	4.0	0.0020	0.0984	49.2
8	13.0	600	1/3	0.0006	0.0042	7.4	0.0003	0.0454	133.2
9	11.0	350	1/3	0.0007	0.0056	7.7	0.0005	0.0358	79.0
10	16.0	550	1/3	0.0007	0.0037	5.0	0.0019	0.2124	113.6

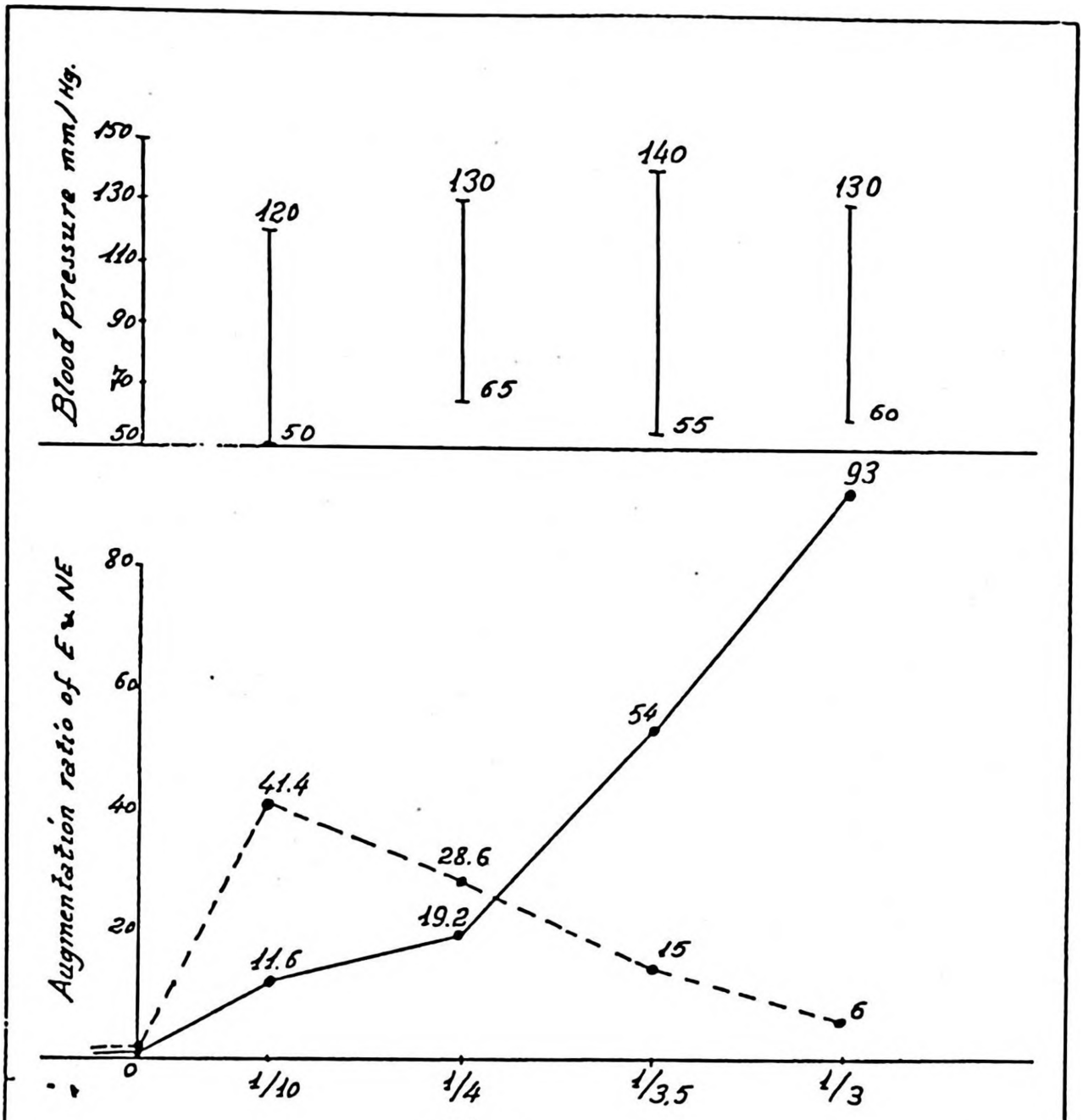

Fig. 2

Relationship between blood pressure and the augmentation ratio ($\frac{\text{highest output}}{\text{basic output}}$) of E and NE output following removal of 1/10, 1/4, 1/3,5 & 1/3 of the total blood volume.

I Blood pressure levels before bleeding and at height of catechol amine output.

Augmentation ratio of NE

" " " " E

Fig. 2

Table 3.

The ratio of withdrawn - blood to total blood volume	E/NE Control	E/NE after bleeding in minutes					
		10	20	40	70	110	150
1/10	8.0	4.0	2.7	1.0	2.7	3.2	–
1/4	4.9	7.6	5.8	20.6	2.2	3.0	1.5
1/3.5	1.7	6 3	7.0	5.7	6 3	5 0	0.8
1/3	2.1	7.3	26.5	65.0	71	70	55

The ratio of E to NE in adrenal vein blood before and after bleeding

TABLE 4

	Adrenal vein catechol amine output, micrograms/min. per animal				Arterial blood catechol amine levels, micrograms/L of plasma			
No. dogs	control NE	E	highest NE	E	control NE	E	highest NE	E
11	0.030	0.248	1.452	2.856	2.0	0.9	2.1	1.4
12	0.053	0.074	1.211	3.429	2.9	1.3	2.9	1.3
13	0.006	0.002	0.048	0.530	2.1	0.0	2.5	0.0

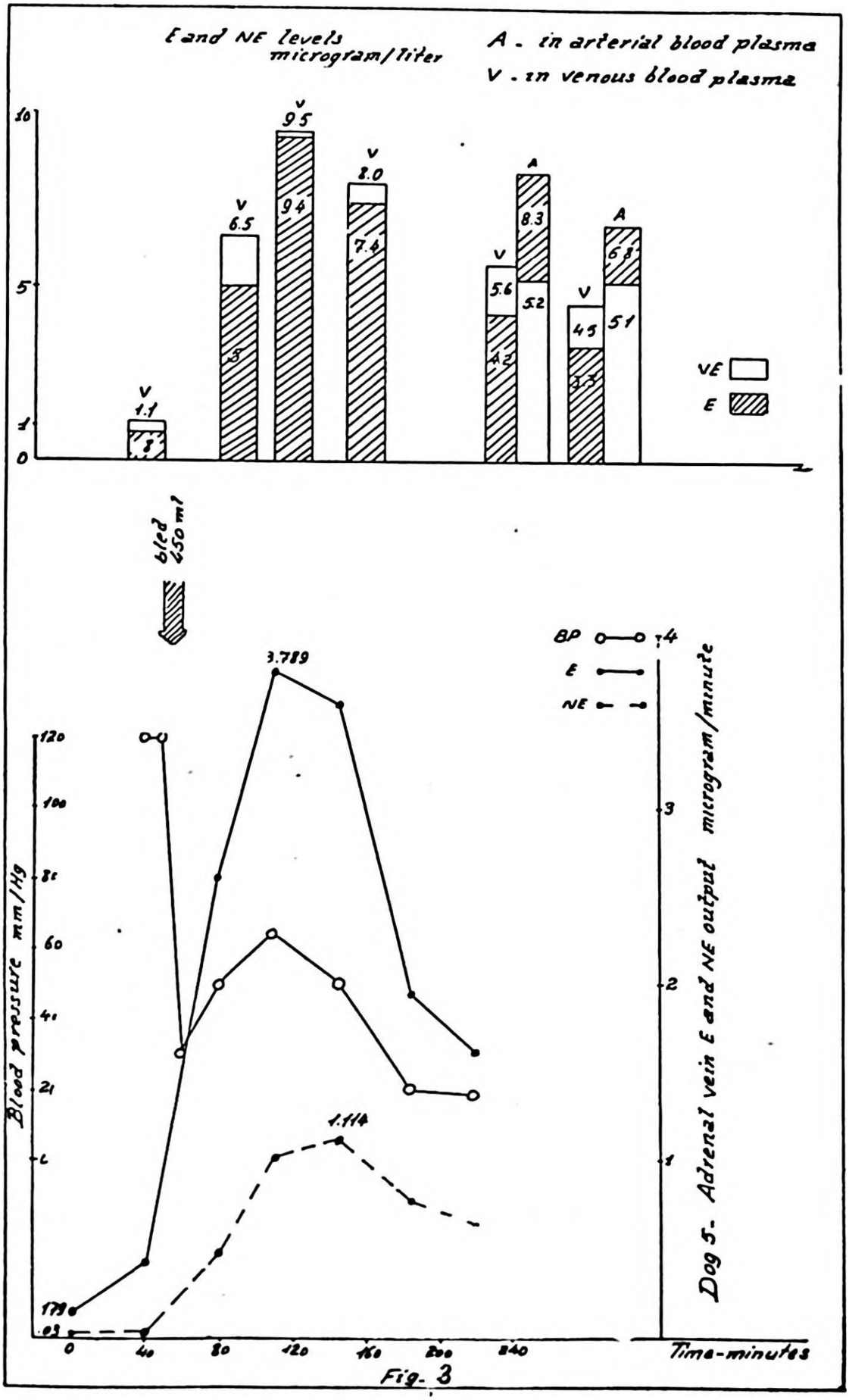
E and NE levels microgram/liter
A - in arterial blood plasma
V - in venous blood plasma
10
5
1
0
V 1.1
V 6.5
V 9.5
V 8.0
V 5.6
A 8.3
V 4.5
A 6.8
9.4
7.4
5.2
5.1
VE
E
bled 450 ml
BP
E
NE
3.789
1.114
120
100
80
60
40
20
0
.179
.03
4
3
2
1
Blood pressure mm/Hg
Dog 5- Adrenal vein E and NE output microgram/minute
0
40
80
120
160
200
240
Time-minutes

Fig. 3

After infusion of 500 ml isotonic saline (dogs 1 and 2) adrenal vein catechol amine output declined sharply, but remained higher than the resting, prehemorrhagic level (Figure 4). Infusion of withdrawn blood (dogs 6 and 8) or 6 per cent dextran solution (dogs 9 and 10) at the peak of catechol amine output, caused a sharp decline in catechol amine output to the basic level (Figure 5).

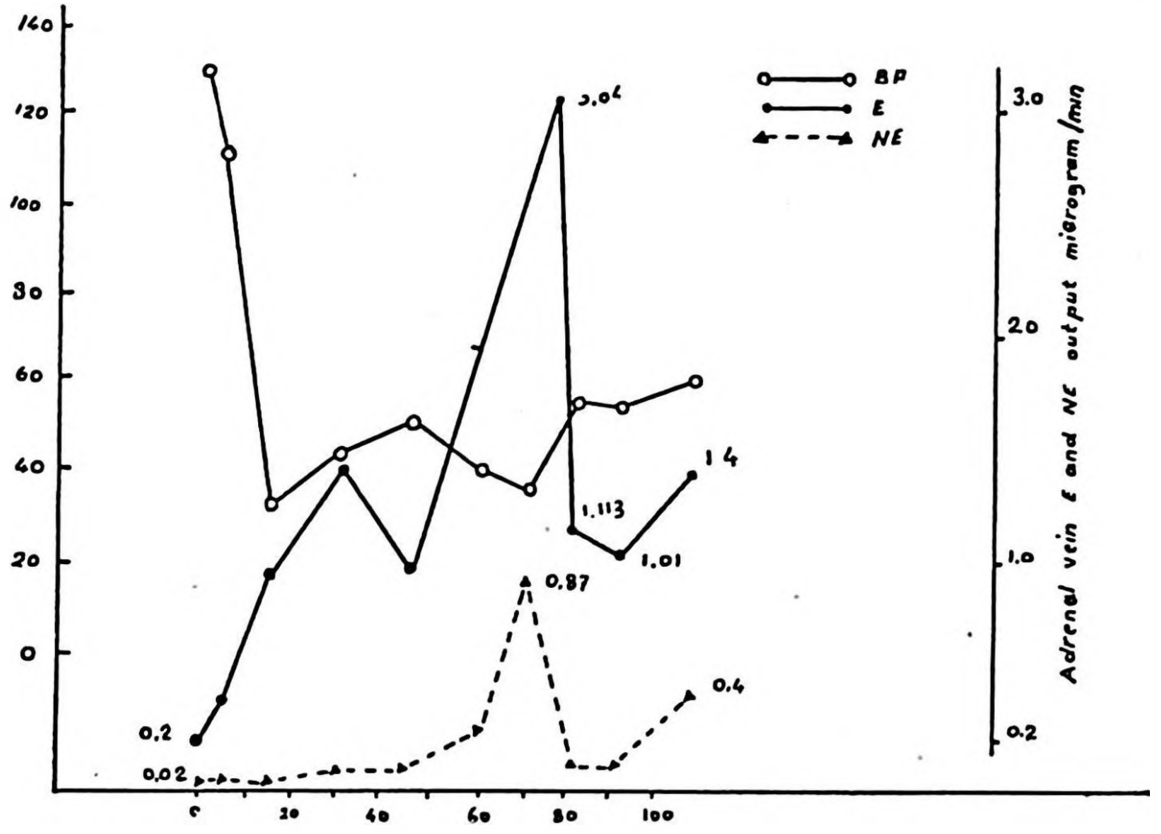

Fig. 4

Discussion

A decrease in blood pressure and blood volume, after hemorrhage, was always followed by an increase in NE and E secretion from the adrenal gland. A fall in blood pressure or decrease in blood volume or both might be the main factor in the adrenal gland response to the bleeding. As far as I know, no work has been done in this special field. In relatively small bleedings, a fall in blood pressure was the main factor, since blood volume did not change significantly, and it brought about an increased augmentation ratio of both NE and E, but greater in the former. On the other hand, in large bleedings, where there was a diminished blood volume as well as a fall in pressure, epinephrine secretion increased much more than norepinephrine secretion. Thus, blood volume may play the most important role in the augmentation of epinephrine secretion. On the other hand, blood pressure seems to be more important in determining the level of norepinephrine secretion.

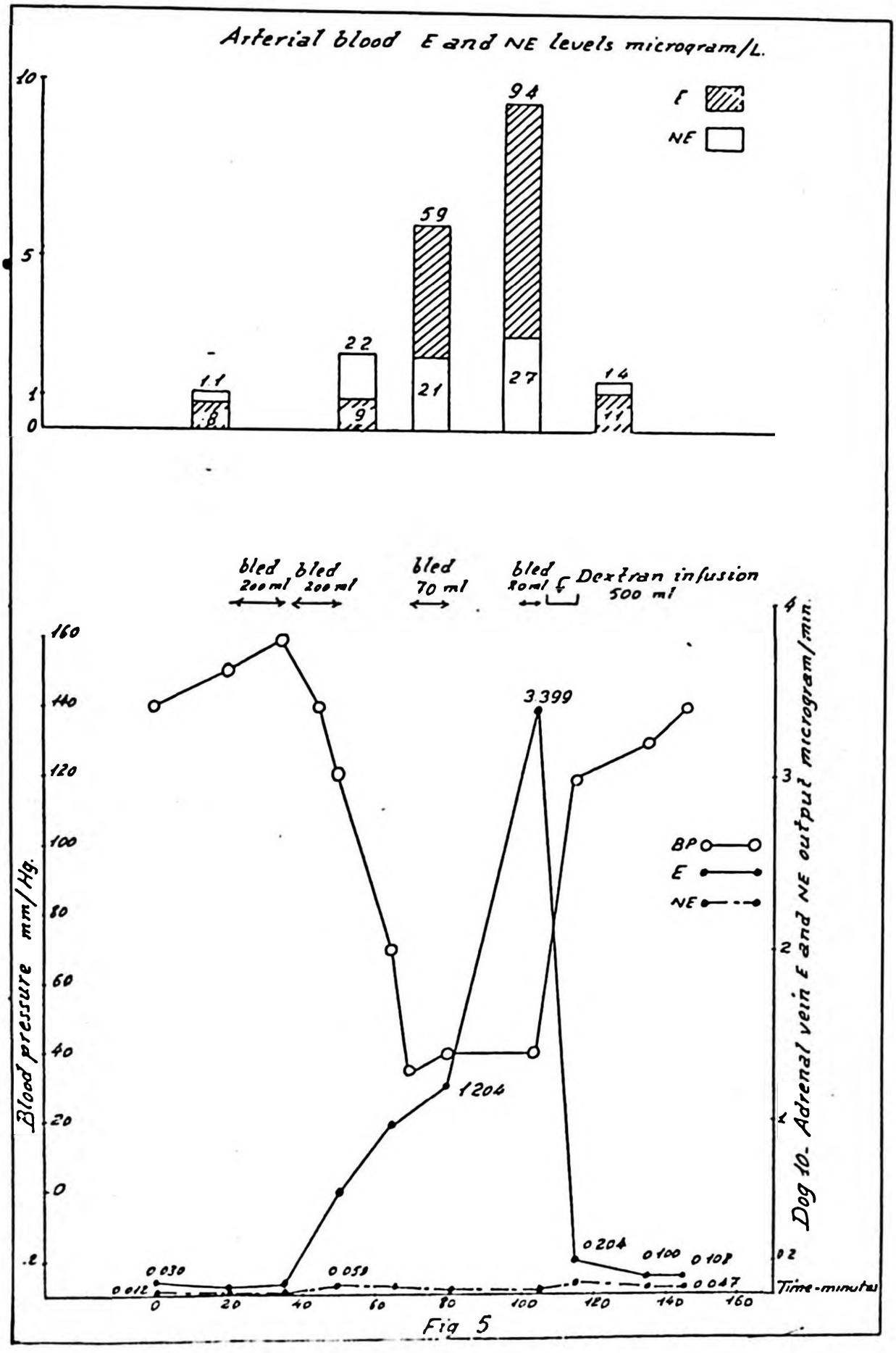
Arterial blood E and NE levels microgram/L.
E
NE
94
59
22
14
11
21
27
bled 200 ml
bled 200 ml
bled 70 ml
bled 10 ml
Dextran infusion 500 ml
BP
E
NE
3.399
1204
0.204
0.100
0.107
0.047
0.030
0.012
0.059
Blood pressure mm/Hg.
Dog 10- Adrenal vein E and NE output microgram/min.
Time-minutes

Fig 5

Undoubtedly animals need more epinephrine than norepinephrine when the blood volume is greatly decreased in order to increase heart rate and cardiac output. Following norepinephrine infusion, there is a decrease in heart rate and no prominent change in cardiac output has been observed by many investigators. [11–17] Therefore, in the presence of a massive hemorrhage, very high levels of norepinephrine might be harmful. [18–19] Indeed, hemorrhagic dogs treated with norepinephrine were found to have more anatomic lesions than those not receiving the drug. [20] It may be that the small increase in NE secretion following a large hemorrhage is caused by the conversion of NE to E in the adrenal gland. Bülbring and Burn [21] have shown that this can occur. It is also possible that, in hemorrhagic shock, infused NE may be converted to E very rapidly in the adrenal gland.

It is apparent that the source of bleedings in not important since in two dogs the same response in catechol amine secretion and blood pressure fall was obtained from arterial as from venous bleedings.

In a study of two hemorrhagic dogs, Lund observed that the concentration of NE in the adrenal vein maintained a constant ratio with E concentration, irrespective of the duration of the experiment. On the contrary, in our experiments, we found that the ratio of E to NE was not constant, but exhibited considerable variation. If the augmentation ratio is calculated in the case of large hemorrhages, it is found that the output of epinephrine exceeds that of norepinephrine. The reverse is true in the case of small bleedings. There is also an important time factor which affects catechol amine secretion rates. Following bleeding, a prompt increase and a relatively rapid decrease in epinephrine secretion occurs, whereas norepinephrine, during the same period shows a slow increase and decrease. As a result of this, the E/NE ratio increased at the beginning and decreased at the end of the experiment.

Satake noticed two peaks in adrenal vein catechol amine output after bleeding, the first occuring in half an hour, and the second after two or more hours. In most cases we observed a similar pattern. We believe, however, that Satake's observation may have been partially the result of repeated bleedings, since after each bleeding in our animals the catechol amine output reached a peak and then fell only to become elevated again following further bleeding.

In four dogs both blood pressure and increased catechol amine output in adrenal vein plasma reached the basic level immediately following infusion of blood or dextran. Lund has reported the same observation following blood transfusion. Infusion of saline solution, however, in amounts equal to or double the volume of withdrawn blood, neither corrected the blood pressure nor returned the catechol amine secretion to basic levels. After saline infusion there

was some improvement in blood pressure and a drop in catechol amine output, but it was not comparable to the results observed with blood or dextran infusion. The blood pressure again decreased and the catechol amine output became elevated once more.

Two dogs bled again at the end of the experiment showed a rise in adrenal vein catechol amine output but the elevation observed now was not nearly as much as that seen initially. The relatively poor response of the adrenal gland to another bleeding following so closely upon previous bleedings might be caused by the depletion of catechol amine stores.

In most dogs, arterial epinephrine and norepinephrine levels increased following bleeding. Generally, arterial plasma epinephrine levels exceed those of norepinephrine, whereas normally the reverse is true, when adrenal vein epinephrine output reaches its peak.

Arterial and venous blood specimens were obtained after bleeding. The arterial blood E level was much higher than that of venous blood, but the norepinephrine levels did not show a significant difference. This may be due to the fact that E is mainly produced by the adrenal medulla, whereas NE is produced by both the adrenal medulla and sympathetic nerve endings. Both E and NE are destroyed rapidly in the liver and other tissues. Therefore arterial blood might show higher epinephrine levels since there would be less chance for its destruction in the tissues.

In three dogs with unilateral adrenalectomy, the right adrenal vein blood was collected continuously, so that no adrenal vein blood passed through the body. In these circumstances, the adrenal glands released large amounts of catechol amines, but peripheral E and NE levels did not change. In other words, a good response to bleeding by the gland occurred, but there was a relatively poor response by the peripheral nerve ends. It appears that the existence of adrenal gland secretion itself is necessary to increase peripheral catechol amine secretion in these dogs.

The greatly increased catechol amine secretion by the adrenal glands following hemorrhage may possibly influence the blood levels of glucose, lactate, and pyruvate, all of which Waud found to be elevated.

REFERENCES

1. Saito, S.: Effect of haemorrhage upon rate of liberation of epinephrine form suprarenal gland of dogs, Tohoku J. Exper. Med. **11**: 79, 1928.
2. Lund, A.: Release of adrenaline and noradrenaline from suprarenal gland, Acta pharmacol. et toxicol. **7**: 309, 1951.

3. Watts, D. T.: Arterial blood epinephrine levels during hemorrhagic hypotension in dogs, Amer. J. Physiol. **184**: 271, 1957.
4. Waud, R. A.: Blood and hemodynamic changes in dogs following hemorrhagic hypotension and its treatment with dextran, J. Pharm. Exp. Ther. **119**: 85, 1957.
5. Satake, Y.: Secretion of adrenaline and sympathins, Tohoku J. Exper. Med. (supp.2) **60**: 1, 1954.
6. Meek and Gasser, quoted from Satake, Y.: Secretion of adrenaline and sympathins, Tohoku J. Exper. Med. (supp. 2) **60**: 1, 1954.
7. Weil-Malherbe, H., and Bone, A. D.: Chemical estimation of adrenaline-like substances in blood, Biochem. J. **51**: 311, 1952.
8. Weil-Malherbe, H., and Bone, A. D.: Adrenergic amines of human blood, Lancet **1**: 974, 1953.
9. Aronow, L., and Howard, F. A.: Improved fluorometric technique to measure changes in adrenal epinephrine-norepinephrine output caused by veratrum alkaloids, Fed. Proc. **14**: 315, 1955.
10. Goldfein, A., Zileli, Ş., Goodman, D., and Thorn, A. W.: The estimation of epinephrine and norepinephrine in human plasma, J. Clin. Endocr. **21**: 281, 1961.
11. Goldenberg, M., Pines, K. L., Baldwin, E. F., and Green, C. E.: Hemodynamic response of man to nor-epinephrine and epinephrine and its relation to problem of hypertension, Am. J. Med. **5**: 792, 1948.
12. Barcroft, H., and Konzett, H.: On actions of noradrenaline, adrenaline and isopropyl noradrenaline on arterial blood pressure, heart rate and muscle blood flow in man, J. Physiol. **110**: 194, 1949.
13. Swan, H. J. C.: Effect of noradrenaline on human circulation, Lancet **2**: 508, 1949.
14. Kappert, A., Sutton, G. C., Reale, A., Skoglund, C. H., and Nylin, G.: Clinical response of human beings to l-nor-adrenaline and its clinical applicability, Acta cardiol. **5**: 121, 1950.
15. Zileli, Ş., Hamlin, J. T., Reutter, F. W., and Friend, D. G.: Evaluation of catechol amine levels in renal insufficiency, J. Clin. Invest. **37**: 409, 1958.
16. Barcroft, H., and Starr, I.: Comparison of actions of adrenaline and noradrenaline on cardiac output in man, Clin. Sc. **10**: 295, 1951.
17. Duane, H., Meyers, F. H., and Schmitt, G. H.: Hemodynamic effects of infusions of epinephrine and arterenol in normal and shocked dogs, Amer. J. Physiol. **189**: 224, 1957.
18. Moyer, J. H., Handley, C. A., and Huggins, R. A.: Effect of adrenergic blockade and norepinephrine on renal and cardiovascular hemodynamics following hemorrhage, Circulation Res. **2**: 441, 1954.
19. Moyer, J. H., Morris, G. C. Jr., and Beazley, H. L.: Renal hemodynamic response to vasopressor agents in treatment of shock, Circulation **12**: 96, 1955.
20. Hackel, D., and Catchpole, B.: Effect of L-norepinephrine on the pathologic changes occurring in hemorrhagic shock, Amer. J. Path. **33**: 624, 1957.
21. Bülbring, E., and Burn, J. H.: Formation of adrenaline from noradrenaline in perfused suprarenal gland, Brit. J. Pharmacol. **4**: 245, 1949.