FACTORS CONCERNED IN THE POLYCYTHEMIA PRODUCED BY THE SUBCUTANEOUS INJECTION OF EPINEPHRINE.

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INTRODUCTION.

Epinephrine polycythemia has recently been studied by Scott (1), Erlanger and Gasser (2), Lamson (3), and Edmunds and Stone (4). These authors are in agreement in a broad way in that they all view the phenomenon as, in part at least, due to a loss of plasma from the blood, resulting in a concentration of the formed elements. Sanguinetti (5) and Bostrom (6), on the other hand, believe that the concentration is an apparent one, occurring in the capillary blood only.

Scott followed the changes appearing in from 3 to 6 minutes after intravenous injection. According to him the increase in hemoglobin is entirely due to loss of fluid by filtration. The experiments of Erlanger and Gasser were related to to the production of shock and for that reason have little bearing on the subject at hand. Lamson gave large doses of epinephrine intravenously (0.9 mg. per kilo). He interprets the polycythemia as entirely one of concentration, occurring in the liver. His views have been criticized in the recent paper by Edmunds and Stone (4). Recently Chanutin, Smith, and Mendel (8) have shown that the water content of the liver does not bear out the idea that this organ serves as reservoir for excess fluid under such experimental conditions. Lamson at one time considered the possibility of addition of red cells to the circulation but apparently does not now hold to this view.

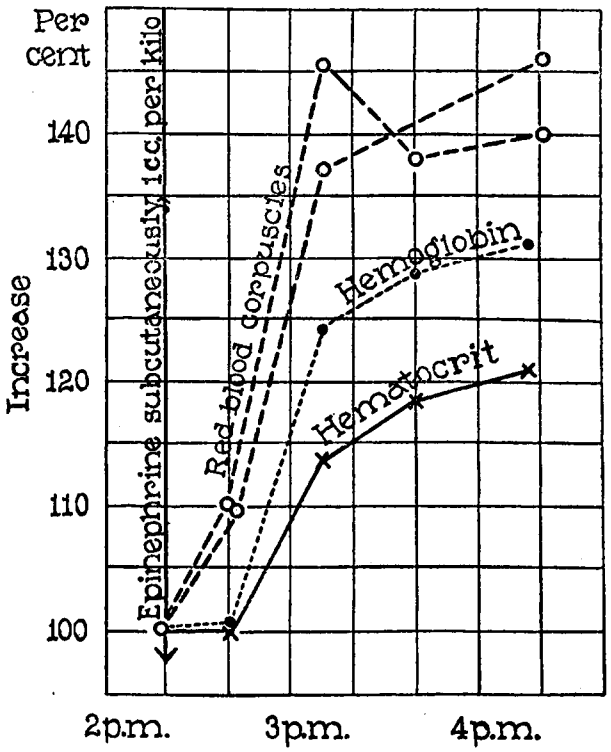
Edmunds and Stone expressed the belief that the effect of epinephrine was exerted *in part* upon the bone marrow. The present study represents an attempt at evaluation of the part that concentration by loss of plasma plays in the polycythemia. A preliminary report has been given recently (9). The views there expressed are somewhat modified in this paper.

Plasma volumes under the influence of epinephrine were first followed by Lamson and Keith (10), who reported that it was possible to obtain decreases in plasma volume and increase in hematocrit reading without change in cell count.

I.

The Plasma Volume Changes.

Experimental.—Plasma volume and red cell counts were made on anesthetized dogs using chloretone in warm cottonseed oil, injected intraperitoneally. We are aware that it has been demonstrated



TEXT-FIG. 1. Dog. Morphine and chloretone anesthesia. All blood drawn from the jugular veins. The two curves for the red cells are made from counts made in duplicate by two independent observers. The experiment shows the general agreement between the increase in volume, increase in hemoglobin, and increase in number of red cells produced by epinephrine. It also shows that this change occurs in the venous blood as well as in the capillaries.

that the induction of anesthesia of itself brings about an increase in the red cells, but the blood soon comes to have a constant concentration except as disturbed by other experimental procedures, so that we have not felt that the change of the first few minutes has disturbed

the later course of the experiments. Observations on the blood count were made at intervals of from 15 to 25 minutes. Previous experience had shown that the count reached its maximum at about 90 minutes after injection of the epinephrine, so that about this time interval was allowed between the first and second plasma volume determinations.

The blood for the red cell count was drawn from the capillaries of the nose. Earlier experiments by Edmunds and Stone had indicated that the changes in splenic vein blood and in blood drawn from the capillaries of the nose were parallel, so that it was felt that the capillary blood gave a fair index of the changes occurring throughout the circulation. In view of the results of Bostrom (6) who stated that the changes occurred in the capillaries, some experiments were carried out in which the counts were made on jugular blood instead of blood from the capillaries. The results of such an experiment are shown in Fig. 1.

The red cell counts in this experiment were made by two independent observers, each making two counts on each dilution. The counts, hemoglobin, and hematocrit determinations were all made on the same sample of blood, drawn from the jugular vein into a well oiled glass syringe. The first blood drawn before epinephrine was injected was used as a standard for the hemoglobin determinations, calling it 100 per cent. 1 cc. of this blood was diluted to 250 cc. in a volumetric flask, with 0.25 per cent HCl. After standing for 30 minutes it was read in a Duboscq colorimeter against a similar dilution of the successive samples drawn during the experiment.

This experiment clearly shows that there is a very definite change in the concentration of jugular blood. The parallelism of the curves is quite striking. The contention of Bostrom that the changes are chiefly capillary, is not upheld by this experiment. Unfortunately capillary blood counts were not made in this case, but since we have never failed to observe a polycythemia in the capillary blood from this dose in over fifteen experiments, we feel certain that one existed here.

Plasma volumes were determined by the dye method of Whipple as modified for successive determinations by Smith (12). The inadequacy of such methods has recently been emphasized by Lamson and Rosenthal (13). In spite of the justice of their criticisms it

was felt that the method might throw some light on the circulatory changes occurring under the conditions of experiment, even though not giving an actual measure of the volume of the plasma. The data to be given add point to Lamson's criticisms of the method.

Epinephrine solution (both Armour's suprarenalin and Parke, Davis and Company's adrenalin) was injected subcutaneously in doses of 1 cc. per kilo.

An abbreviated protocol of one experiment is given to indicate the details of the method.

Jan. 24, 1924. Dog, weight 47 pounds.

11.45 a.m. Morphine sulfate 75 mg.

1.20 p.m. Chloretone 4.5 gm., in oil, intraperitoneally; both jugular and both femoral veins exposed.

1.45 p.m. Red blood cells, nose capillaries, 6,110,000.

1.52 p.m. Drew 20 cc. blood, right jugular, red cells 6,670,000. 10 cc. of blood centrifuged (Tube 1); injected 4.26 cc. brilliant vital red 1 per cent solution into right femoral.

1.56 p.m. Drew 20 cc. blood, left jugular; centrifuged 10 cc. (Tube 2).

2.05 p.m. Injected 22 cc. epinephrine solution subcutaneously; Tubes 1 and 2 centrifuged for 20 minutes at 3,000 R.P.M.

2.30 p.m. Capillary blood, nose, red blood cells 6,780,000.

3.20 p.m. Drew 20 cc., right jugular, red blood cells 7,040,000; put 10 cc. in centrifuge Tube 3; injected 4.26 cc. dye as above.

3.24 p.m. Drew 20 cc. blood, right jugular; 10 cc. in Tube 4.

3.27 p.m. Red blood cells, nose capillaries, 8,000,000; Tubes 3 and 4 centrifuged for 20 minutes at 3,000 R.P.M.

The results of centrifugation follow:

Tube.	Red blood cells.	Plasma.	Red blood corpuscles.
	cc.	*cc.*	*per cent*
1	5.7	6.8	45.6
2	5.3	6.4	45.3
3	6.5	6.7	49.2
4	6.3	6.0	51.2

The amount of dye in the supernatant plasma was estimated, following in detail the method outlined by Smith. From these determinations it was estimated that the plasma at 1.56 p.m. was 1,133 cc., and at 3.24 p.m. it was 1,039 cc.

The results of nine such experiments are given in Table I. They may be summarized as follows: When plasma volume, hematocrit,

and red cell determinations are carried out simultaneously it has been found that the percentage decrease of plasma volume by determination is generally less than that calculated from the increase

TABLE I.

No.	Capillary blood increase.		Jugular blood increase.		Hematocrit.		Plasma volume.	Loss.	Required by red blood corpuscles increase.	Required by hematocrit.
		per cent		*per cent*		*per cent*	*cc.*	*per cent*	*per cent*	*per cent*
1	6,430,000–7,850,000	22	—	—	50–54	7.6	985–874	11	18	6.6
2	6,400,000–7,780,000	22	—	—	51.8–61.3	18	1,084–709	35	18	15
3	5,845,000–8,780,000	50	—	—	45–56	23	997–792	20	33	18
4	5,072,000–6,675,000	31	—	—	38–42	10	228–189	17	24	9
6	7,340,000–8,340,000	14	7,350,000 7,730,000	5	49.3–54.7	11	—	—	—	—
7	5,590,000–6,130,000	9	4,550,000 5,550,000	22	—	—	—	—	—	—
8	6,110,000–8,000,000	30	6,670,000 7,040,000	7	45.4–50.2	10	1,133–1,039	8	25	9
9	7,050,000–7,770,000	10	7,200,000 7,100,000	0	48.3–48.4	0	729–647	11	15	0
10	5,000,000–7,340,000	46	4,860,000* 6,840,000	40	34.8–47.7	38	—	—	—	—

*The red counts in No. 10 were all made on jugular blood, by two independent observers. The hemoglobin in this experiment also increased 32 per cent.

in number of cells in the capillary blood (Nos. 1, 2, 3, 4, 8, 9). In other words, taking the capillary blood as an index, there must be some other process occurring besides that of concentration by loss

of plasma. When, however, the percentage loss of plasma is compared with that calculated from the hematocrit readings from jugular blood, it is seen that the loss of plasma is quite adequate to account for the increase in number of red cells purely on the basis of concentration. Since there is no way, so far as we know, of evaluating the proportion of blood in the capillaries as compared with that in the larger vessels, it is not possible to say what part of the phenomenon of epinephrine polycythemia is the result of concentration, and what part is due to the action of other factors such as stimulation of the bone marrow. It becomes clear, therefore, that this method of attack cannot solve our problem.

There are certain other points in the results of these experiments to which we wish to call attention. The experiments above in part bear out the view of Bostrom that the polycythemia is more apparent than real. In Experiments 6, 8, and 9 there is a greater increase in capillary than in jugular blood. But there is at the same time a real increase in the jugular count, which is checked by the hematocrit readings (all except No. 9). Only in one experiment did there fail to appear an increase in the hematocrit reading, and in this experiment (No. 9) there was no increase in the jugular count.

Summary.—The subcutaneous injection of epinephrine in doses of 1 mg. per kilo in dogs is followed by an increase in number of red cells in both jugular and capillary blood. The increase is therefore real and not merely one of redistribution. The increase in hemoglobin and volume of red cells in the jugular blood runs parallel to the increase in the number of cells. There is associated with the red cell increase a loss of plasma from the vessels as indicated by the dye method. It is not possible to say what part of the red cell increase is due to actual loss of plasma, what is due to increased accumulation of red cells in the peripheral capillaries, and what due to other factors. This method of experimentation cannot, therefore, be used to determine whether or not there are red cells added to the circulation under the influence of epinephrine. It cannot be said that the data here reported offer any evidence either for or against the belief that at least part of the epinephrine polycythemia is due to stimulation of the bone marrow.

II.

Histological Changes Produced by Epinephrine.

The possible stimulation of the blood-forming organs suggested above has been thought of not as similar to that of pilocarpine on the salivary glands, in which there is an increased formation of secretion, but rather as analogous to that of pituitary extract on the mammary gland, which forces out secretion already present. In other words under the influence of epinephrine there is a discharge of those cells which are ready for entry into the general circulation.

The mode of response of the bone marrow in such an action is not altogether clear.

The recent work of Drinker, Drinker, and Lund (14) indicates that normally the delivery of red cells to the general circulation occurs because of growth pressure of groups of blood cells. This is pictured as a constant process, being stimulated only by those conditions which also stimulate growth of the red blood cell-forming tissue. The capillary walls are held to be intact, a closed system, though in animals whose bone marrow had been rendered hyperplastic by repeated hemorrhage, it was not always possible to detect endothelial walls between the growing red cell-forming tissue and the capillary spaces. These authors have also shown that there is a very efficient vasomotor mechanism to the marrow vessels, which responds to both nerve stimulation and epinephrine. Whether the capillaries played a part in the mechanism was not determined. It has been shown by a number of workers that contractile elements are present in the smallest vessels in other parts of the body, and that these elements respond to drugs (15). It is altogether conceivable that a similar apparatus is present in the bone marrow capillaries, though the careful studies of Drinker and his coworkers mentioned above did not reveal them.

Whatever the mechanism by which a sudden discharge of red cells from the marrow could be brought about, it is clear that the actual discharge should be brought into evidence by an increase in the number of red cells in the effluent blood, and in the general circulation as well, if the stimulation were sufficiently widespread, and also an increase in young forms in the peripheral blood. Evidence is here presented that there is actually an increase in young forms as the result of the injection of epinephrine.

Lamson (16) studying the polycythemia produced by epinephrine, advanced the view that new cells had been added as shown by the decrease in size and hemoglobin content, but he did not find any increase in nucleated red cells or

other direct evidence of the presence of young cells.[1] Drinker and Drinker found that when the marrow of the tibia was perfused with oxygenated physiological salt solution, the addition of epinephrine to the perfusion fluid resulted in a decrease in the number of nucleated red corpuscles in the effluent.

Experimental.—

In these experiments the following determinations were made on blood drawn from the capillaries of the nose: red cell count, reticulated cells, white cells, and nucleated red cells. After the normals were taken, the animals were given epinephrine in doses of 1 mg. per kilo, injected subcutaneously. Successive counts were then made at 15 to 20 minute intervals. Smears were made at the same time, stained with Wright's stain, and used for the differentials. The reticulocytes were stained with brilliant cresyl blue after the method recently described by Krumbhaar (17). A number of condensed protocols are given. No increase in nucleated red cells has been seen, so that figures for these cells are omitted. All counts have been converted to number of cells per c.mm.

May 23, 1923. Dog A4, weight 20 pounds. Chloretone 1.6 gm. intraperitoneally.

1.50 p.m. Red blood cells 7,420,000; reticulocytes 10,380.
2.00 " Adrenalin 8 cc. subcutaneously.
2.35 " Red blood cells 8,250,000; reticulocytes 24,750.
3.15 " " " " 8,520,000; " 23,000.
3.50 " " " " 9,060,000; " 20,876.

Apr. 30, 1923. Dog A1, weight 17 pounds. Chloretone 1.25 gm.

2.25 p.m. Red blood cells 6,180,000; reticulocytes 32,000.
2.30 " Adrenalin 7 cc. subcutaneously.
3.10 " Red blood cells 7,340,000; reticulocytes 73,000.
3.50 " " " " 7,650,000; " 150,000.

May 1, 1923. Dog A2, weight 30 pounds. Chloretone 3 gm.

2.30 p.m. Red blood cells 6,430,000; reticulocytes 19,200.
2.40 " Adrenalin 14 cc. subcutaneously.
3.10 " Red blood cells 8,010,000; reticulocytes 42,450.
4.00 " " " " 8,520,000; " 70,400.

It is obvious from these experiments that the number of reticulocytes in the capillary blood is very definitely increased under the influence of epinephrine. If one accepts the usual point of view that the reticulated red cells are young forms of red cells, then this is, we believe, clear evidence that there is some demand placed upon the bone marrow by the injection of this drug.[2]

[1] We are of the impression that Lamson no longer holds to the view that cells are added to the circulating blood under these experimental conditions.

[2] It is of interest to note in passing that the subcutaneous injection of physostigmine, which brings about a polycythemia, also increases the number of reticulocytes.

A second interesting line of evidence has been afforded by the study of smears made for differential counts in the course of some experiments similar to these. The details of the effect of epinephrine upon the white cells were reported in the paper by Edmunds and Stone and need not be considered in this connection. The present interest in these experiments lies in the tabulation of the polymorphonuclear leucocytes according to the number of lobes of the nuclei. A number of smears were submitted to Dr. Carl V. Weller of the Pathology Laboratory, without comment. The condensed protocol of one such experiment follows, with Dr. Weller's report appended.

Oct. 24, 1922. Dog, weight 7 kilos. Morphine 75 mg. at 11.00 a.m.
1.30 p.m Paraldehyde 15 cc. by stomach tube.
2.00 " Stomach, intestine, spleen removed; hepatic artery tied.
2.25 " Red blood cells 7,400,000; white corpuscles 19,650 (Smear 1).
2.30 " Epinephrine 6.5 mg. subcutaneously.
3.00 " Red blood cells 8,450,000;white corpuscles 25,000 (Smear 2).
3.15 " " " " 8,610,000; " " not counted.
3.45 " " " " 8,710,000; " " 26,650 (Smear 3).
4.15 " " " " 9,340,000; " " 20,050 (" 4).
4.45 " " " " 8,500,000; " " 17,000.

Report from Pathology Laboratory.

Cells.	Smear 1.	Smear 4.
	per cent	*per cent*
Lymphocytes, small	0.5 } 2.5	2.5 } 8.0
" large	2.0	5.5
Myelocytes		2.5
Large mononuclears		9.0
Transitionals	5.0	
Neutrophil polynuclears		
Three lobed	12.5 } 87	15.0 } 75
Four "	27.0	37.5
Five "	40.0	20.5
Six "	7.5	2.0
Eosinophils	2.0	4.5
Basophils		1.0
Indeterminates	3.5	
Letting transitionals and polynuclears = 100 per cent		
Transitionals	5+	11−
Polynuclears. Three lobed	14−	18−
Four "	29+	44+
Five "	43+	24+
Six "	8 +	2+

The view generally held is that the cells with the larger number of lobes are the older cells, since in infections or other conditions calling forth more polymorphonuclears, the percentage of the cells with two and three lobes increases. If this view be correct, then in the present experiment we have additional evidence that the proportion of young white cells in the peripheral circulation has been increased by the subcutaneous injection of epinephrine. It might be noted in passing that this is a case of polycythemia produced while the arterial circulation to the liver was occluded, a phenomenon which other observers have denied.

If as we believe the epinephrine is acting through the nervous mechanism to bring about the discharge of red cells from the bone marrow, then other methods of stimulation might conceivably bring about similar results. It has been shown by Scott, Herrmann, and Snell (18) that continued stimulation of the sciatics in a dog gave an increase in the red cell count. They attributed this, however, to the increased activity of the muscle. In order to eliminate this factor of exercise, we have carried out a similar experiment, with the dog completely under the influence of curare, so that no muscular contractions were evident. The condensed protocol follows.

Oct. 30, 1923. Dog, weight 7 kilos.
1.00 p.m. Morphine 50 mg.
2.30 " Chloretone in oil, 1.5 gm. intraperitoneally; right sciatic cut and peripheral end put on electrode; curare 75 mg. intravenously; artificial respiration.
3.00 p.m. Red blood cells 6,860,000.
3.02 " Started stimulation of sciatic.
3.15 " Red blood cells 6,980,000.
3.30 " " " " 7,770,000.
3.50 " " " " 7,250,000.
4.10 " " " " 7,860,000.

Both right and left tibias were then removed and sent to the Pathology Laboratory. They were examined by Dr. A. S. Warthin, who reported as follows: "..... If there is any difference between these, it is that the unstimulated one shows more cells of the myelocyte and lymphocyte type and also more megalokaryocytes than the stimulated one. There is no qualitative difference, only quantitative."

The polycythemia in this case can scarcely be looked upon as a concentration phenomenon, since the pressure was low from the

curare. It was not due to the curare, for curare has been shown not to produce a polycythemia. It could not be due to exercise, since the muscles were not contracting. The conclusion seems inevitable that it was due to stimulation of the bone marrow. The pathological findings, while not conclusive, seem to bear out this view. One would expect not a qualitative but rather a quantitative difference, as was reported by Professor Warthin.

Summary.—The subcutaneous injection of epinephrine in doses of 1 mg. per kilo brings about a polycythemia, an increase in reticulocytes, and an increase in young forms of polymorphonuclear leucocytes. The polycythemia is no doubt in part a concentration phenomenon, but the presence of these young cells in increasing numbers is believed to be evidence that there is at the same time a discharge of cells from the blood-forming organs, due to the effect of the drug upon them. A similar effect is obtained by continued stimulation of the peripheral ends of the cut sciatic nerves in the curarized animal.

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