PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE QUESTION OF THE ROLE OF THE CEREBRAL CORTEX IN THE PATHOGENESIS OF HEMOLYTIC ANEMIA

COMMUNICATION I. ATTEMPT TO REPRODUCE THE SYNDROME OF EXPERIMENTAL PHENYLHYDRAZINE ANEMIA

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Soviet Scientists, basing their views on a very broad experimental and clinical experience, have demonstrated the role of the central nervous system and, in particular, the cortex of the brain in the regulation of numerous functions of the organism in the normal as well as in the pathological state. In spite of this, and in spite of the appearance in the last few years of a considerable amount of experimental data testifying to the role of the nervous mechanisms in the regulation of "blood systems," this question cannot be said to have been completely resolved.

Along with this, a clear understanding of the role played by reflex mechanisms in the regulation of the blood system in the normal as well as in the diseased state has a very great significance both from the theoretical and practical standpoints.

In the laboratory of Professor D. I. Goldberg, there was studied the role played by the nervous system in the pathogenesis of experimental hemolytic anemias. In the works of K. I. Polkovnikova, V. S. Lavrov, N. I. Rosengurt, E. D. Goldberg, and their co-workers in this laboratory, there is a wealth of material testifying to the role of the receptor apparatus, spinal cord, and brain in the pathogenesis of phenylhydrazine anemia [4-5].

However, this question can be finally determined by the classic method of conditioned reflexes producing the syndrome of experimental phenylhydrazine anemia. The solution of this problem is of definite interest, as phenylhydrazine anemia is a classic experimental model of Addison-Biermer anemia. The study of the role played by the central nervous system in the pathogenesis of phenylhydrazine anemia would to a certain extent help clarify the role played by the central mechanisms in the pathogenesis of pernicious anemias.

At the present time there has been proven beyond argument the possibility of producing by way of conditioned reflexes various pathologic syndromes [6]. In the literature available to us we find no descriptions of the production of an anemic syndrome by way of the establishment of conditioned reflexes. Therefore we embarked on the experiment of causing phenylhydrazine anemia by the establishment of conditioned reflexes, using the classical methods.

EXPERIMENTAL

The experiments were performed upon rabbits, dogs and cats (altogether 25 animals). These experiments were broken into 3 series. Before creating the conditioned reflex in the animals it was necessary to eliminate the oriented reflexes of the experimental environment.

In the first series of experiments (animals Nos. 1-9, see table), the conditioning stimulus – metronome beat of a frequency of 60 beats to the minute – after having acted by itself for a definate period of time was

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reinforced by the subcutaneous introduction of a 1% solution of acidic phenylhydrazine in the quantity 1 cc per 1 kg weight of animal. After 23 such combinations of the specific irritant with a nonspecific stimulus the conditioning process was halted. After a 10 day intervel, during which blood factors returned to base levels, the experiment was repeated as before for 9 days, except that the animal received physiological saline instead of phenylhydrazine.

The control for Series I consisted of 2 rabbits (Nos. 1 and 5) who received during the entire series no phenylhydrazine but only physiological saline. In animal No. 9 (dog Gilda) the interval between the 2 experimental periods lasted 6 days, during which there was no attempt at producing the conditioned reflex. At the end of the 6 days the experiment was resumed under previous conditions but instead of phenylhydrazine, physiological saline was introduced.

In Series II experiments, (animal No. 10 - dog Sultan) the conditioner, a bell, was applied for a definite time (5 minutes), the ringing starting five minutes after the subcutaneous injection of phenylhydrazine.

This experimental procedure was based on the indications in the literature [4] that the anemia appears 3-7 minutes after the beginning of the hemolytic action upon the receptors. It might be thought that the conditioned reflex in this case falls closer in this series than in the experiments of the first series. It is known that simultaneous stimulations establish reflexes more readily than those that are separated. On the 6th day after the cessation of the introduction of phenylhydrazine in the previous conditions of the experiments we introduced the specific stimulus, the reinforcing introduction of physiological saline. We continued studying the blood picture.

In Series III experiments (rabbits Nos. 1-14) after introducing a needle into the ear vein of the animal the conditioning signal - a ring, lasting for 5 minutes - was given, and immediately thereafter there was introduced phenylhydrazine (0.1 cc 5% solution per 1 kg weight). After this the conditioning signal was continued for 5 more minutes.

With this procedure we attained complete uniformity during the time of action of both the specific and nonspecific stimuli.

After the 21st experiment, work on the conditioned reflex ceased. After 6 days, under previous experimental conditions, the rabbits received physiological saline instead of phenylhydrazine.

In the control animal (No. 14) the introduction of phenylhydrazine (and the substitution for it of physiological saline) was done at the same time intervals as in the experimental animals but without the prior use of the conditioning stimulus.

During the entire experimental period the blood pictures of experimental and control animals were under constant observation.

When the degree of anemia became so severe as to threaten life, work on establishing the conditioned reflexes was stopped, and then resumed after 3-4 days.

In order to observe the mode of action of phenylhydrazine, 11 acute experiments were performed on cats, into whom the hemolytic substance was introduced into a rear extremity vascularly isolated from the rest of the body, perfused with warm Ringer Solution, and connected with the animal by its femoral and sciatic nerves [9].

The controls had similar setups except that the blood vessels of the extremity remained connected, phenylhydrazine being introduced as before.

In individual experiments, the influence of narcosis and operational trauma upon the blood picture was studied.

In all animals we noted the r. b. c., hemoglobin content of blood, color index, leucocyte count, coagulation time, red blood smear, and the erythrometric curve.

In the acute experiment, on suitably stained slides, the number of erythrocytes with pathological structures was counted.

Blood Changes in the Experimental Animals During All Stages of The Investigation

Exp. Series	Number of animals	Number of erythrocytes (in millions)				Hemoglobin % (Sahli)				Color index				Number of leukocytes (in thousands)				Mean diameter of erythrocytes (in microns)			
		Before beginning exp.	At height of anemia	Before using conditioning signal	At end of exp.	Before beginning exp.	At height of anemia	Before using conditioning signal reinforced with physiologic saline	At end of exp.	Before beginning exp.	At height of anemia	Before using conditioning signal	At end of exp.	Before beginning exp.	At height of anemia	Before using conditioning signal	At end of exp.	Before beginning exp.	At height of anemia	Before using conditioning signal	At end of exp.
		5.2	—	5.3	5.2	68	—	73	71	0.9	—	0,7	0.9	10,1	—	10,1	11.5	6	—	6	6
	2	5.2	1.6	4.86	5.3	70	33	52	59	0.9	1,48	0,75	0,9	12,3	22,7	14,1	10.6	6	—	—	6
	3	4,7	0,99	4,6	5,18	58	21	57	58	1	1,6	0,9	0,8	10,7	24,3	12,1	12,6	6	—	—	6
I	4	5,2	1,6	4,8	5,1	65	40	52	64	0,87	1,7	0,8	0,8	8,3	17	11,5	9,6	6	—	—	6
	5	5,7	—	5,9	5,68	60	—	66	64	0,74	—	0,7	0,8	10,25	—	10,7	10,0	6	—	—	6
	6	5,4	1,53	5,6	5,1	58	30	66	60	0,7	1,4	0,8	0,8	8,75	23,0	12,3	8,9	6	8	—	6
	7	5,3	1,6	4,8	5,4	56	39	52	57	0,7	1,7	0,7	0,7	10,3	20,1	11,3	8,8	6	8	—	6
	8	5,1	1,2	4,6	5,5	54	27	48	56	0,8	1,5	0,7	0,7	9,6	18,6	11,7	8,3	6	8	—	6
	9	6,4	2,1	3,9	6,3	63	39	50	61	0,7	1,5	0,9	0,7	9,3	16,9	12,8	9,7	6	7	—	6
II	10	6,4	2,3	3,0	5,7	70	41	42	64	1	1,4	1,1	0,9	11,4	18,9	15,8	8,7	6	6*	—	6
	11	5,3	1,2	3,8	5,4	75	30	50	69	1	1,7	1,1	0,9	12,6	17,5	5,2	8,5	6	6*	6	6
	12	4,6	1,8	4,4	5,4	69	45	63	70	1	1,7	1	0,9	12,5	17,3	10	8,0	6	6*	6	6
III	13	4,8	1,6	4,3	5,2	60	33	60	66	0,89	1,4	1	0,9	9,9	18,3	11,2	10,3	6	6*	6	6
	14	5,4	1,45	4,0	5,8	65	31	58	66	0,8	1,5	1	0,8	9,3	19,3	10,3	9,3	6	7	6	6

* Large % of Macrocytes.

EXPERIMENTAL RESULTS

After only 2-3 injections of the hemolytic agent all animals developed the typical picture of phenylhydrazine anemia, detailed by many authors[2, 3, 4].

As can be seen from the table, before beginning the conditioning stimulus, reinforced by introduction of physiological saline, the number of erythrocytes and the percent of Hb in the animals of Series I reached almost base levels. In dog No. 9 and in animals of Series II and III the r. b. c. and percent of Hb did not reach base level. The color index, which at the height of the anemia was hyperchromic in most animals, at the beginning of this period of observation became hypochromic. Examination of blood smears revealed evidence of intensified regenerative activity in the bone marrow: numerous polychromatic erythrocytes being seen, Jolly bodies, and basophilically stippled erythrocytes. The mean diameter of the erythrocytes in all animals returned to normal and equaled 6 μ. In the majority of the animals a leucocytosis was observed; in the color index were the usual quantitative correlations.

Upon using the conditioning stimulus on all experimental animals for 9 days, reinforced by addition of physiological saline, we failed to note the appearance of any signs of a phenylhydrazine anemia (Series I), not observing even a delay in the intensity of the regenerative process (Series II and III) which ended in the experimental and control animals at the same time.

In animals Nos. 1-8 (Series I) the changes in the number of erythrocytes and percent of hemoglobin were small and well within the range of normal deviation. In animals Nos. 9-13 during the time of the entire experimental use of the conditioning stimulus, the r. b. c. count and percent of Hb continued to rise and attained base levels at the same time as the control animals, showing no tendency to diminution.

These same animals, along with normalization of the r. b. c. count and Hb% developed a definitely hypochromic index, this remaining so during the entire period of observation. The blood picture during the 9 days of the use of the conditioning signal, reinforced with physiological saline, became more and more normal and attained the levels usual for this breed of animal at the end of this period of time. Also, for this time interval there was no tendency for the mean diameter of the erythrocytes to become larger. The leucocyte count by the 2-3rd day after beginning use of the signal reached base levels and stayed there The leucocytic formula did not alter under the influence of the conditioning signal in the direction of findings seen in phenylhydrazine anemia.

As the literature proves that interoceptive conditioned reflexes are extinguished with great difficulty, it is not permissible to attribute this to a weakening of the reflex during the period of ceasing its reinforcement and the beginning again of the conditioning signal reinforced with physiological saline introduction [1, 7].

Having failed to obtain a phenylhydrazine type anemia by means of conditioned reflexes, we set ourselves a series of experiments using the above-described procedures, with the aim of verifying the statements in the literature that phenylhydrazine has mechanisms establishing reflexes. Within 5-15 minutes after the introduction of the hemolytic agent into a vascularly isolated extremity which still maintained its nervous connections, we were unable to identify any characteristics of phenylhydrazine type of anemia, and at the same time observed no evidence for reflex building activity on the part of the phenylhydrazine. Our results coincide fully with the results obtained by Sokolovskaya [8], who came to the conclusion that the first signs of a developing anemia appear not less than 3 hours after the introduction of the hemolytic agent.

LITERATURE CITED

[1] Bykov, K. M. Selected Articles*(Moscow, 1954), Vol. 2.

[2] Vysotsky, A. A. Med. Obozrenie Vol. 63 No. 4, pp. 247-256 (1905).

[3] Ginzburg, R. M. Works of the Central Institute of Hematology and Transfusion*(Moscow-Leningrad 1934)Nos. 7-8, pp. 136-145.

[4] Goldberg, D. I. Hematological Essays*(Tomsk, 1952).

[5] Goldberg, E. D. and Vedernikov, M. T. Reports of the 4th Pavlov Conference of the Tomsk Med. Institute* (Tomsk, 1954) pp. 59-66.

* In Russian.

[6] Dolin, A. O. Reports of the Joint Session Devoted to the 10th Anniversary of the Death of I. P. Pavlov* (Moscow 1948) pp. 207-217.

[7] Pavlov, I. P., in the work Sechenov, I. M., Pavlov, I. P. and Vvedensky, N.E. Physiology of the Nervous System, Selected Reports*(Moscow 1952) Vol. 4.

[8] Sokolovskaya, Ya. Ya., Arkh. Patol. No. 4 pp. 9-14.

[9] Chernigovsky, V. N., Yaroshevsky, A. Ya. Questions on the Nervous Regulation of the Vascular System*(Moscow 1953).

* In Russian.