MECHANISM OF FORMATION OF SOME TRUE AND FALSE ALLERGIC REACTIONS

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In experiments on rabbits after unilateral coagulation of the posterior hypothalamic nucleus, reciprocal relationships were observed between the area of the local allergic lesion during reproduction of the Arthüs (weakening) and Schwartzmann (intensification) phenomena, while after bilateral coagulation this reaction was completely absent. After coagulation of the posterior hypothalamic nucleus the serum antibody level did not correlate with the intensity of the local allergic reaction (Arthüs phenomenon). Despite absence of the local reaction during reproduction of the Schwartzmann phenomenon, phasic changes were observed in the clotting system of the blood and in the blood level of adrenal hormones.

The attention of many investigators has recently been attracted to the mechanisms of regulation of immunogenesis, and particular interest has been shown in the hypothalamus. Antibody formation [3, 6, 12, 22], certain nonspecific factors of immunity [14, 16, etc.], anaphylactic shock [2, 7, 19], the phenomena of Arthüs and Schwartzmann [1, 4, 23], and reactions of transplantation immunity [8, 10, 13] have been studied from this standpoint. However, it must be emphasized that the results given by different workers are contradictory, making further systematic research essential.

The object of this investigation was to study the character of formation and the course of the Schwartzmann and Arthüs phenomena, diametrically opposite in their pathogenetic mechanisms, when reproduced after destruction of the posterior hypothalamic nuclei.

EXPERIMENTAL METHOD

In experiments on 60 rabbits of both sexes, weighing 2.5-3.5 kg, the posterior hypothalamic nucleus was destroyed unilaterally or bilaterally under pentobarbital (1 ml 2% pentobarbital/kg body weight) anesthesia by a direct electric current (1 mA for 30 sec) using a monopolar platinum electrode, insulated throughout its length except at the tip, which was exposed for a distance of 0.8-1 mm. The electrode was inserted under the control of a stereotaxic apparatus of the Horsley–Clark type.

The site of electrocoagulation was verified macroscopically and microscopically in sections identified in accordance with the atlas [18], with measurement of the zone of destruction by means of a type MOV-1-15 micrometer in paraffin sections stained by Nissl's method and with hematoxylin-eosin (Fig. 1).

The Arthüs phenomenon was reproduced in the classical manner by giving five subcutaneous injections of horse serum in a dose of 3 ml with intervals of 5 days between injections. The area of the allergic skin reaction was expressed in square millimeters. The precipitin titer was determined in parallel experiments by the ring precipitation test.

The Schwartzmann phenomenon was reproduced by intradermal injection of sensitizing (0.2 ml) and reacting doses (0.3 ml/kg body weight) of the filtrate of a 6-day culture of Escherichia coli at intervals of 24 h.

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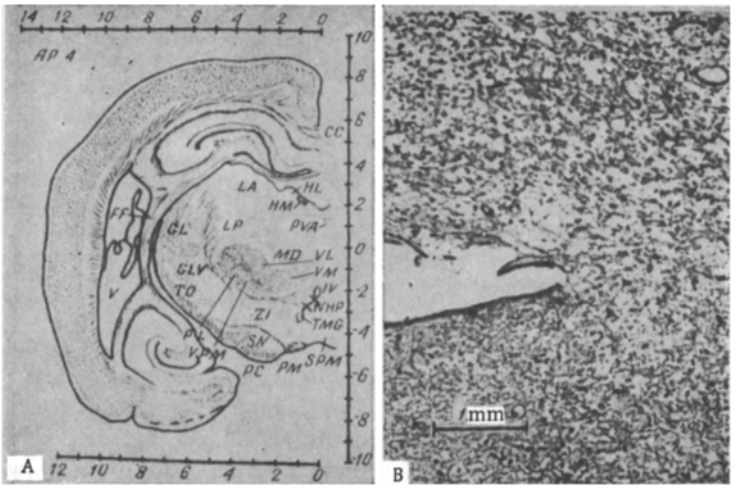

Fig. 1. Location of zone of damage to posterior hypothalamus: A) in schematic section of the atlas of Fifkova and Marsal [18]: crosses denote zone of destruction of posterior hypothalamic nucleus (NHP); B) in histological section: unilateral coagulation of NHP, including wall of third ventricle. Hematoxylin-eosin, 400 ×.

The Arthüs and Schwartzmann phenomena were reproduced 4-5 days or 3 months after coagulation of the posterior hypothalamic nucleus. The 11-hydroxycorticosteroids (11-HCS) in the blood were estimated at the same time by a fluorometric method [10] and the thromboelastogram was recorded on the Tromb-1 thromboelastograph in order to monitor changes in the clotting system of the blood. The thromboelastograms were read and interpreted as described by Pinkus [9].

A Minsk-12 computer was used for the statistical analysis of the results.

EXPERIMENTAL RESULTS

In the intact rabbits the 4th subcutaneous injection of 3 ml horse serum during reproduction of the Arthüs phenomenon induced the formation of an extensive zone of infiltration at the site of injection, with a larger area of central necrosis and considerable edema of the surrounding tissue. The area of infiltration when tested on the 10th day was 65.0 ± 10.8 mm^2, on the 15th day 265.8 ± 17.85 mm^2, on the 20th day 544.2 ± 23.33 mm^2, on the 25th day 760 ± 24.43 mm^2, and on the 30th day 944.2 ± 20.64 mm^2 (Fig. 2A, I). The increase in area of the zone of infiltration was statistically significant ($P < 0.001$).

During reproduction of the Arthüs phenomenon after unilateral destruction of the posterior hypothalamic nucleus, the area of infiltration which was formed was less than the control (Fig. 2A, II), and no signs of necrosis or edema could be found. The area of the zone of infiltration when tested on the 5th day was 7.6 ± 2.15 mm^2, on the 10th day 20.0 ± 1.04 mm^2, on the 15th day 69.2 ± 3.54 mm^2, on the 20th day 104.8 ± 4.72 mm^2, on the 25th day 187.8 ± 13.54 mm^2, and on the 30th day 284.09 ± 69 mm^2.

Latent manifestations of the Arthüs phenomenon also were observed in rabbits in which coagulation of the hypothalamic nucleus was preceded by the first injection of antigen (Fig. 2A, III). The area of the zone of infiltration when tested on the 10th day was 31.6 ± 2.33 mm^2, on the 15th day 95.8 ± 2.74 mm^2, on the 20th day 205.2 ± 6.52 mm^2, on the 25th day 312.6 ± 4.83 mm^2, and on the 30th day 413.2 ± 15.88 mm^2.

A weak manifestation of the Arthüs phenomenon was observed when it was reproduced 3 months after coagulation (Fig. 2A, IV). The area of the zone of infiltration in this series of experiments on the 10th day was 32.6 ± 4.79 mm^2, on the 15th day 124.2 ± 9.56 mm^2, on the 20th day 235.6 ± 21.58 mm^2, on the 25th day 365.8 ± 22.93 mm^2, and on the 30th day 658 ± 45.3 mm^2.

Attempts to reproduce the Arthüs phenomenon after bilateral destruction of the posterior hypothalamic nucleus were completely unsuccessful. Both the first and the subsequent injections of horse serum were absorbed without trace. No zones of infiltration at the site of injection could be palpated. On morphological examination of the skin in the area of injection of horse serum only an ill-defined lymphocytic infiltration of the deep layers of the dermis, localized in character, could be found.

TABLE 1. Dynamics of Serum Precipitins during Reproduction of the Arthüs Phenomenon

Group	Statistical index	Days of investigation								
		5-th	10-th	15-th	20-th	25-th	30-th	35-th	40-th	45-th
I	$n=5$									
	M	2,1	2,4	2,5	3,2	3,2	3,4	3,7	3,4	2,7
	$\pm m$	0,15	0,17	0,17	0,27	0,3	0,13	0,28	0,25	0,31
	P	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
II	$n=5$									
	M	0,52	1,0	2,5	2,5	3,3	4,0	3,5	2,8	2,5
	$\pm m$	0,031	0,044	0,07	0,29	0,13	0,25	0,22	0,18	0,22
	P	>0,1	>0,05	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
III	$n=5$									
	M	2,38	2,38	2,84	3,34	4,35	4,32	5,4	4,7	3,94
	$\pm m$	0,17	0,11	0,17	0,11	0,21	0,25	0,26	0,13	0,2
	P	<0,01	<0,001	<0,001	<0,001	<0,001	0,001	<0,001	<0,13	<0,001
IV	$n=5$									
	M	2,8	2,92	2,98	3,34	3,48	3,86	4,4	3,8	3,0
	$\pm m$	0,27	0,11	0,15	0,14	0,14	0,11	0,09	0,24	0,15
	P	<0,001	<0,001	<0,001	<0,001	<0,001	0,001	<0,001	<0,001	<0,001
V	$n=5$									
	M	0,6	1,88	2,3	2,9	2,75	2,5	2,6		
	$\pm m$	0,26	0,20	0,15	0,11	0,67	0,59	0,64		
	P	<0,05	<0,001	<0,001	<0,001	<0,001	0,001	<0,001		

Legend. I) Control group; rabbits after unilateral coagulation of posterior hypothalamic nucleus; II) reproduction of the phenomenon immediately after coagulation; III) the first injection of antigen precedes coagulation; IV) reproduction of the phenomenon 3 months after coagulation; V) rabbits after bilateral coagulation.

TABLE 2. Changes in Thromboelastographic Indices of Blood Clotting System during Reproduction of the Schwartzmann Phenomenon

Series of experiments		Statistical index	r	K	r+K	T	ma	Σ	Ci
Control (A)	Initially	*M*	31,1	20,2	51,5	311,0	57,7	147,7	2,0
		±*m*	5,25	2,18	5,36	25	3,6	16,41	0,32
		P							
	1 h	*M*	22,2	12,2	34,4	211,6	58	153,8	1,75
		±*m*	3,01	2,87	3,12	36,25	5,85	29,41	0,25
		P	⩾0,1	<0,02	<0,01	<0,01	>0,8	>0,6	>0,6
	5 h	*M*	20,2	11	31,2	174,6	55,6	128,6	1,86
		±*m*	2,12	1,23	3,39	66,9	2,78	12,28	0,18
		P	>0,05	<0,001	<0,01	>0,05	>0,6	⩾0,5	>0,7
	1 day	*M*	32,4	13,2	45,8	227,6	61,8	172	1,84
		±*m*	10,44	3,34	15,55	37,5	2,68	29,02	0,48
		P	>0,9	>0,1	>0,8	>0,05	<0,03	⩾0,1	>0,8
	3 days	*M*	19,6	7,2	26,8	210,6	67,4	211,4	2,92
		±*m*	2,12	1,23	2,68	66,9	3,34	37,5	0,45
		P	>0,05	<0,001	<0,001	>0,3	<0,001	>0,05	⩾0,1
	7 »	*M*	18,8	16,8	35,6	315,6	63,8	182,8	1,77
		±*m*	3,39	2,24	2,12	33,9	4,45	20,31	0,18
		P	>0,05	>0,6	<0,02	>0,9	>0,2	<0,05	⩾0,5
Unilateral coagulation of posterior hypothalamic nucleus (B)	Initally after coagulation	*M*	34	16	48	275	60	162	2,11
		±*m*	9,87	5,2	7,46	14,6	5,2	33,96	0,71
		P	⩾0,9	⩾0,5	>0,7	>0,1	>0,7	>0,8	>0,9
	1 h	*M*	25	11	36	232	73	286	2,04
		±*m*	4,34	1,44	3,32	45,37	3,9	40,80	0,14
		P	>0,5	>0,7	>0,9	⩾0,4	<0,05	<0,02	>0,9
	5 h	*M*	29	10	39	159	71	253	2,04
		±*m*	7,8	1,4	7,15	26,88	2,16	24,56	0,2
		P	>0,7	>0,3	>0,8	<0,001	<0,05	<0,05	>0,9
	1 day	*M*	81	18	99	245	71	255	0,89
		±*m*	27,08	5,2	34,68	60,85	2,3	26,45	0,4
		P	>0,5	>0,8	>0,1	>0,6	<0,05	<0,02	>0,05
	3 days	*M*	48	10	58	200	79	299	1,4
		±*m*	4,26	0,86	5,35	40	1,25	56,0	0,06
		P	>0,2	>0,1	>0,1	⩾0,05	<0,001	<0,05	>0,3
	7 »	*M*	30	9	40	157	70	250	1,9
		±*m*	6	0,93	7,25	39,5	4,37	33,75	0,5
		P	⩾0,8	>0,2	>0,4	<0,02	⩾0,2	<0,02	>0,7
Bilateral coagulation of posterior hypothalamic nucleus (C)	Initially after coagulation	*M*	31,36	39,64	72,82	192,27	40	75,55	0,89
		±*m*	3,52	7,71	10,72	16,06	3,99	11,89	0,24
		P	⩾0,7	<0,02	<0,05	<0,001	<0,001	<0,001	<0,05
	1 h	*M*	21,0	39,33	60,33	167,66	44,66	98	1,62
		±*m*	7,38	21,31	30,74	28,69	10,66	45,08	0,92
		P	>0,2	>0,9	<0,02	>0,5	>0,6	>0,5	⩾0,6
	5 h	*M*	21,48	42	119	248,66	51,66	113,33	0,56
		±*m*	7,7	17,4	41,7	27,05	6,15	26,59	0,18
		P	>0,05	>0,5	>0,2	⩾0,1	>0,1	⩾0,2	⩾0,1
	1 day	*M*	34,25	32,25	66,5	223,38	48,87	104	0,92
		±*m*	5,07	9,2	11,47	16,1	3,37	16,6	0,17
		P	⩾0,5	>0,5	⩾0,5	⩾0,1	>0,05	⩾0,5	⩾0,9
	3 days	*M*	25,75	33	58,75	202,25	42,62	78,62	0,89
		±*m*	5,6	8	12,13	14,84	3,33	10,8	0,13
		P	>0,5	⩾0,5	<0,05	>0,5	>0,5	>0,5	>0,9
	7 »	*M*	23,17	15,67	38,83	202,33	54,5	139,83	1,99
		±*m*	6,2	3,37	8,42	26,1	4,24	21,9	0,37
		P	>0,1	<0,01	<0,05	>0,5	>0,05	<0,05	<0,05

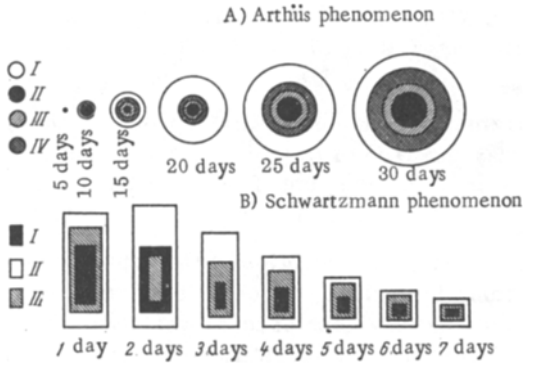

Fig. 2

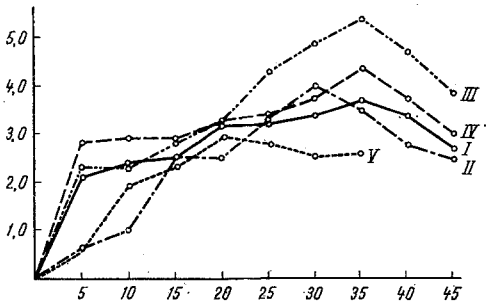

Fig. 3

Fig. 2. Area (in mm^2) of skin lesion during reproduction of allergic phenomena: A) Arthüs phenomenon: I) control; II) rabbits after preliminary coagulation of posterior hypothalamic nucleus; III) rabbits in which coagulation was preceded by first injection of horse serum; IV) rabbits in which phenomenon was reproduced 3 months after coagulation of posterior hypothalamic nucleus. B) Schwartzmann phenomenon: I) control rabbits; II) rabbits in which phenomenon was reproduced after unilateral coagulation of posterior hypothalamic nucleus; III) rabbits in which phenomenon was reproduced 3 months after coagulation of posterior hypothalamic nucleus.

Fig. 3. Dynamics of serum antibodies during reproduction of the Arthüs phenomenon. I) control animals; II) rabbits after unilateral destruction of the posterior hypothalamic nucleus; III) rabbits in which coagulation was preceded by the first injection of horse serum; IV) rabbits in which the phenomenon was reproduced 3 months after coagulation of the posterior hypothalamic nucleus; V) rabbits in which the phenomenon was reproduced after bilateral destruction of the posterior hypothalamic nucleus. Abscissa, days of testing; ordinate, mean titers (in log).

During reproduction of the Arthüs phenomenon the dynamics of the precipitins was investigated. The need for detecting precipitins was due to the fact that, according to the literature, the increase in tissue sensitivity to protein is directly proportional to the precipitin titer. Some workers [5, 15, 17] point out that the Arthüs phenomenon develops in rabbits in the presence of antibody titers of 1:200 or higher. This was confirmed during reproduction of the Arthüs phenomenon in intact animals. However, during sensitization after unilateral destruction of the posterior hypothalamic nucleus, even though high (1:6,000-1:8,000) titers of antibodies were found (Fig. 3, Table 1) no clearly defined local allergic reaction could be detected. Meanwhile, after bilateral coagulation the phenomenon could not be reproduced, not withstanding the presence of antibodies in a titer of 1:200-1:1600. This was evidently because for the Arthüs phenomenon to develop, besides antibodies in sufficient quantity, the participation of complement is essesntial [21, 24]. The writers showed in their previous experiments [11] that repeated immunization, especially after destruction of the posterior hypothalamic nucleus, leads to inhibition of the complementary activity of the blood serum. This could account for the abortive manifestations of the Arthüs phenomenon when it was reproduced after coagulation of the posterior hypothalamic nucleus. Another factor with an important role was the reactivity of the cells, which without doubt must have been altered by these varied procedures.

The next step in the investigation was to study a conventional allergic reaction of the type in whose formation serum antibodies play no part (the Schwartzmann phenomenon).

In the control group of animals (Fig. 2B, I), 1 day after injection of the reacting dose of toxin the area of the reaction was 461.4 ± 23.86 mm^2, and after 2 days it showed a statistically significant increase ($P < 0.001$) to 501.6 ± 30.23 mm^2, after which a decrease in the hyperergic reaction was observed. Starting from the 3rd day, there was a statistically significant decrease in the area of inflammation: on the 3rd day 359.2 ± 16.22 mm^2, the 4th day 207.8 ± 9.41 mm^2, 5th day 190.8 ± 10.25 mm^2, 6th day 130.6 ± 11.2 mm^2, and 7th day 74.4 ± 7.94 mm^2.

After unilateral destruction of the posterior hypothalamic nucleus the reacting dose of toxin evoked a more marked reaction than in the control (Fig. 2B, II). The area of inflammation 1 day after injection of

the reacting dose of toxin was 920.6 ± 26.46 mm^2, 2 days after it was 945.1 ± 23.25 mm^2, 3 days after 741.8 ± 39.4 mm^2, 4 days 542.4 ±45.12 mm^2, 5 days 368.8 ± 19.12 mm^2, 6 days 276.4 ± 16.55 mm^2, and 7 days after injection it was 213.8 ± 9.74 mm^2.

Even 3 months after coagulation, when the Schwartzmann phenomenon was reproduced the inflammatory reaction of the skin was stronger (Fig. 2B, III). After 1 day the area of inflammation was 669.4 ± 6.78 mm^2, after 2 days 491.4 ± 38.17 mm^2, after 5 days 368.8 ± 19.12 mm^2, after 6 days 276.4 ± 16.55 mm^2, and after 7 days 213.8 ± 9.74 mm^2.

In the writers' opinion the most striking fact is that during reproduction of the Schwartzmann phenomenon after bilateral destruction of the posterior hypothalamic nucleus the local Schwartzmann phenomenon did not develop at all. Not until 1 day after injection of the reacting dose of toxin was a very slight hyperemia observed, and this disappeared without trace 2 days later. During investigation of the blood clotting system, one of the leading pathogenetic mechanisms of the Schwartzmann phenomenon, signs of hypercoagulation were found in the animals subjected to unilateral destruction of the posterior hypothalamic nucleus 1 and 5 h and 1 week after injection of the reacting dose of toxin (Table 2B) and in animals subjected to bilateral destruction of the nucleus 1 h and 1, 3, and 7 days after injection of the toxin (Table 2C): shortening of the time of clot formation (K) the reaction time + time of clot formation (r + K), the total blood clotting time (T) (only 1 h after injection in the case of bilateral destruction), an increase in the maximal amplitude (ma) and elasticity of the clot (Σ). Signs of hypocoagulation also were observed: an increase in the reaction time + time of clot formation (r + K) and a decrease in the thromboelastographic coagulation index (Ci), which were recorded on the 1st and 3rd day after injection in the case of unilateral destruction and on the 5th day after injection in the case of bilateral destruction.

During reproduction of the Schwartzmann phenomenon in control rabbits the changes in the clotting system showed evidence of hypercoagulation at all times of the investigation (Table 2A).

In the study of the blood clotting indices it will be noted that not only were significant differences found during comparison of the control results with those of both experimental series, but the differences were also significant when results obtained during reproduction of the Schwartzmann phenomenon in animals subjected to unilateral destruction of the posterior hypthalamic nucleus were compared with the corresponding results obtained after bilateral coagulation.

It is important to emphasize that the changes in the blood clotting system on the 5th day after unilateral and bilateral coagulation, i.e., before reproduction of the Schwartzmann phenomenon, were inadequate to the situation and significant in magnitude. For example, after unilateral destruction the phases I and II of blood clotting were shortened (moderate hypercoagulation, see Table 2C), while after bilateral destruction all three phases of blood clotting were delayed (marked hypocoagulation, see Table 2C).

Another interesting result was that during reproduction of the local Schwartzmann phenomenon after bilateral destruction of the posterior hypthalamic nucleus injection of the sensitizing dose of toxin induced a sharper rise in the 11-HCS concentration (28.5 μg%) in the blood of the experimental animals compared with the control group (21.3 μg%). After injection of the reacting dose the decrease in 11-HCS level in the experimental animals took place earlier than in the controls, so that the initial values were reached after 24 h (7.4 μg%), whereas in the control animals a second and considerable increase in the 11-HCS concentration was observed (18.7 μg%). Later in the investigation the change in the 11-HCS concentration was no longer significant.

It can be concluded from these facts that a complex correlation exists between the state of certain hypothalamic nuclei and the formation and intensity of allergic phenomena which differ in their pathogenetic mechanisms.

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