PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CONCERNING THE REFLEX MECHANISM OF ANTIBODY FORMATION

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The question concerning reflex formation of antibodies without circulating antigen, first posed by R. Pfeiffer [14] and then by E. Friedberger [9-12] is at present being elaborated by A. N. Gordienko [2].

The present work introduces experimental material obtained during the study of the processes of antibody formation under conditions of antigen action on chemoreceptors of the isolated carotid sinus in rabbits (75 experiments). The data presented below constitute the results of verification of A. N. Gordienko's experiments but, unlike Gordienko who ligated all the vessels and tissues of the carotid sinus (excluding the sinocarotid nerve) en masse with one common ligature, we carried out very careful, conservative preparation of the blood vessels in the area of the bifurcation of the common carotid artery, aiming not to impair circulation in the carotid glomus and nerve. It is known that thrombosis of glomus vessels leads to loss of excitability of its chemoreceptors [1, 13, etc.). Our method of isolation allows the chemoreceptors to preserve their functional properties, i.e., excitability in response to physiologically adequate stimuli, and therefore for the sake of brevity this method will henceforth be referred to as "physiologic."

EXPERIMENTAL METHODS

Under light ether anesthesia a single thick ligature is passed under the common carotid cephalad to the thyroid artery; another such ligature is passed under the external carotid artery at the site of branching of the lingual artery. By means of these 2 ligatures an assistant manipulates the area of the bifurcation upwards and medially. The internal carotid, occipital, superior pharyngeal, lingual and other small (2-3) branches are exposed in this position. All the vessels, including the common carotid and the external carotid arteries are tied with 2 ligatures. To prevent accidental access of antigen to the surface of the wound the latter is packed with sterile tampons. Antigen (dysentery vaccine, complete typhoid or dysentery antigen) is introduced into the sinus through a puncture in the common carotid artery; the amount used is from 0.06 to 0.1 ml. At the moment of withdrawal of the syringe a third ligature is tightened around the common carotid artery, this ligature being the most cephalad. After the injection the blind sac formed by the ligature of the vessels in the sinus area should show noticeable distension. The tampons are carefully replaced by fresh ones. The wound is covered with a dry tampon and left for 5 minutes after which all the vessels are divided between the ligatures, the sinocarotid nerve being the last to be sectioned, as far from the sinus as possible, and the carotid sinus is then excised. The wound is closed in two layers. Blood for antigen titer is withdrawn from the pinna vein or from the heart 15 minutes, 1 hour, 2 hours and 24 hours after the injection of antigen into the sinus. The presence of antibodies in the blood is determined on the 4th, 7th, 10th and 14th day following antigen introduction.

The sinocarotid nerve is dissected away from the carotid sinus removed from the body. The sinus is cut up with scissors into 1 ml sterile physiologic solution, "extracted" in the refrigerator for 18-20 hours and the amount of antigen in the "extract" determined. The sinocarotid nerve, separated from the sinus, is treated similarly. Antigen is determined by means of complement fixation reaction in the cold.

Reaction ingredients: antigen (being sought) – blood serum, "extracts"; antibody – precipitating typhoid or dysentery serum (precipitin titer –1:800,000, complement fixation (cold) titer –1:1,000,000) prepared at the I. I. Mechnikov Institute of Vaccines and Sera (typhoid serum series 85, dysentery series 147); complement-desiccated complement prepared at the same Institute (series 98-105), hemolytic serum prepared at the same

TABLE 1

Control Experiments

No. of rabbit	Experimental conditions	Antigen in the blood		Agglutinins in the blood	
		Initial titer	Maximal titer	Initial titer	Maximal titer
7	A. Saline introduced into sinus and sinus removed 5 min later	0	0	1/10	1/10
8	The same	0	0	1/10	1/20
53	" "	0	0	1/20	1/20
54	" "	0	0	1/5	1/10
5	B. Saline introduced into sinus and sinus removed; heated dysentery vaccine (2,000,000 bodies) given intravenously	0	1/2	1/20	1/640
6	The same	0	Trace	1/10	1/960
9	" "	0	»	1/10	1/800
10	" "	0	»	1/30	1/500
11	" "	0	1/2	1/10	1/300
12	" "	0	1/2	1/20	1/600
1	C. Dysentery vaccine (2,000,000 bodies) given intravenously	0	Trace	1/5	1/320
2	The same	0	»	1/10	1/960
3	" "	0	1/2	1/10	1/480
4	" "	0	1/2	1/5	1/640
39	" "	0	Trace	1/20	1/1000
40	" "	0	1/2	1/10	1/400
25	D. Complete dysentery antigen (0.01 mg) given intravenously	0	1/4	1/20	1/1000
26	The same	0	1/4	1/10	1/4000
27	" "	0	1/2	1/20	1/2000
28	" "	0	1/2	1/10	1/1200

Institute (series 14, titer 1:1600), sheep erythrocytes 3% suspension prepared ex tempore by the usual method.

The reaction was carried out in the refrigerator at 2° over a period of 18-20 hours, the results being read after allowing the material to stand in a thermostat at 37-38° for 25-30 minutes.

Blood antibodies were determined by agglutination reaction.

EXPERIMENTAL RESULTS

First series – control experiments. Preliminary control experiments were carried out in order to discover the following: the effect of operative removal of the carotid sinus on the initial antibody titer (4 experiments); effect of operative removal of the carotid sinus on the process of antibody formation on intravenous injection of antigen (6 experiments); dynamics of antibody formation on intravenous injection of small amounts of dysentery antigens (10 experiments). The amount of antigen for intravenous injection was chosen in accordance with our supposition as to the quantity that could pass from the isolated sinus into the blood if such a possibility existed.

The results of the experiments are presented in Table 1.

Second Series – investigation of antibody formation under conditions of "physiologic" isolation of the carotid sinus. "Physiologic" isolation of the carotid sinus differs favorably from the method proposed by A. N. Gordienko in that it preserves the excitability of the carotid glomus receptors. Absolute isolation, according to A. N. Gordienko, can only be achieved with loss of sensitivity of chemoreceptors as the result either of thrombosis of vessels supplying the glomus or by ligation of these vessels. Since it is absolutely essential to preserve receptor

TABLE 2

Introduction of Antigens into the Isolated Carotid Sinus

No. of rabbit	Experimental conditions	Antigen in the blood		Agglutinins in the blood	
		Initial titer	Maximal titer	Initial titer	Maximal titer
13	A. Heated dysentery vaccine ($2 \cdot 10^8$ bodies)	0	Trace	1/20	1/320
14	The same	0	»	1/10	1/640
15	" "	0	»	1/10	1/480
16	" "	0	0	1/10	1/20
19	" "	0	Trace	1/20	1/300
20	" "	0	»	1/5	1/800
21	" "	0	0	1/20	1/10
22	" "	0	Trace	1/10	1/400
23	" "	0	Not clear	1/10	1/800
24	" "	0	» »	1/20	1/300
29	" "	0	Trace	1/30	1/500
30	" "	0	»	1/20	1/600
31	" "	0	»	1/10	1/800
35	" "	0	0	1/10	1/10
36	" "	0	Trace	1/10	1/600
37	B. Complete dysentery antigen (0.06-0.1 mg)	0	»	1/10	1/800
38	The same	0	1/2	1/20	1/500
41	" "	0	Trace	1/10	1/1200
42	" "	0	»	1/10	1/600
43	" "	0	»	1/10	1/1800
48	C. Complete typhoid antigen (0.06-0.1 mg)	0	»	0	1/320
49	The same	0	0	1/5	1/20
50	" "	0	1/2	0	1/5000
51	" "	0	Trace	0	1/1000
52	" "	0	»	1/5	1/5000
57	" "	0	1/2	0	1/1200
58	" "	0	Trace	0	1/600
59	" "	0	»	1/5	1/1000
60	" "	0	0	1/10	1/20
61	" "	0	Trace	0	1/400
17	D. Dysentery vaccine introduced after ligation of sinocarotid nerve	0	0	1/30	1/30
18	The same	0	0	1/10	1/20
32	" "	0	0	1/10	1/10
33	" "	0	0	1/20	1/10
34	" "	0	0	1/5	1/10
62	E. Complete typhoid antigen (0.06-0.1 mg) administered after ligation of sinocarotid nerve	0	0	0	0
63	The same	0	0	0	0
64	" "	0	0	1/5	1/10
65	" "	0	0	0	0
66	" "	0	0	1/10	0
67	" "	0	0	1/5	1/20
68	" "	0	0	0	0

excitability to antigen in order to prove the reflex nature of antibody formation, we made use of "physiologic" isolation.

The following experiments were undertaken in this series:

1) introduction of 0.06-0.1 ml heated dysentery vaccine into the isolated carotid sinus (15 experiments);

2) introduction of 0.06-0.1 mg complete dysentery (5 experiments) or typhoid (10 experiments) antigen;

3) introduction, for control purposes, of the corresponding antigens into the isolated carotid sinus after its denervation; denervation was effected by tying the sinocarotid nerve with two ligatures and subsequent division (12 experiments).

The experimental results are presented in Table 2.

As can be seen from Table 2, 25 out of the 30 rabbits taken for the main experiments showed minimal amounts of antigen in the blood following introduction of the antigen into the physiologically isolated carotid sinus. The appearance of antigen in the blood was accompanied by subsequent rise in antibody titer.

We wish to draw particular attention to the results of 5 experiments (rabbits No. 16, 21, 35, 49 and 60); complete isolation of the carotid sinus occurred in the course of these experiments (as the result of thrombosis of glomus capillaries, most probably) as proved by absence of antibody from the blood, shown by repeated search for it by the cold complement fixation method. Absolute isolation of the carotid sinus area from blood and lymph circulation of the body as a whole thus completely excludes the possibility of reflex antibody formation under the influence of antigen action on glomus chemoreceptors. Antibodies are only formed when the antigen passes from the sinus into the blood; in those cases in which no antigen is found in the blood there is also no antibody formation.

What is the manner in which antigen passes from "isolated" sinus into the blood? A 3rd series of experiments was undertaken in order to answer this question.

Third series – resorption of antigen from the isolated carotid sinus. 0.06-0.1 mg complete typhoid antigen in 1:1000 dilution was introduced into the isolated carotid sinus. Contact with the antigen lasted for 5 minutes

TABLE 3

Antigen Resorption from the Isolated Carotid Sinus

No. of rabbit	Time of sinus–carotid contact, min.	Amt of antigen introduced into sinus, mg	Amt. of antigen detected			
			In the sinus		In nerve γ	In blood γ
			mg	% of introduced		
50	5	0,1	0,09	90	4	6
51	5	0,1	0,08	80	2	18
52	5	0,08	0,06	75	2	18
57	5	0,09	0,06	67	4	26
58	5	0,06	0,05	84	4	6
59	5	0,08	0,06	75	2	18
60	5	0,06	0,05	84	0	10
61	5	0,10	0,06	60	Trace	40
69	15	0,06	0.03	50	2	28
70	15	0,1	0,06	60	Trace	40
71	15	0,1	0,07	70	1,5	28,5
74	15	0,08	0,05	63	4	26
75	15	0,1	0,06	60	2	38
72	30	0,1	0,04	40	3	57
73	30	0,1	0,05	50	2	48
76	30	0,08	0,025	32	2	73
77	30	0,1	0,05	50	1,5	48,5
78	30	0,08	0,03	38	Trace	50

(8 experiments), 15 minutes (5 experiments) and 30 minutes (5 experiments). The excised sinus and sinocarotid nerve were then extracted and their antigen content determined.

The results of these experiments are given in Table 3.

It follows from Table 3 that after 5 minutes' contact 60-90% of the administered amount of antigen remains in the isolated sinus (10-40% antigen is resorbed); after 15 minutes' contact 50-70% antigen remains (30-50% is resorbed) and after 30 minutes' contact only 32-50% of the introduced antigen remains in the isolated sinus. Therefore as early as the first 5 minutes a considerable amount of the introduced substance is resorbed from the isolated carotid sinus. The speed of resorption during the first 5 minutes is enhanced by increased pressure within the sinus; during the subsequent 15-30 minutes resorption continues, but less intensely, owing to lowering of pressure within the sinus.

Similar results were obtained by Iu. V. Sergeev in I. A. Oivin's laboratory; he showed that half the radioactive isotope (NaI^{131}) introduced into the isolated carotid sinus (rabbit) was resorbed in 29 minutes on average.

Parallel determination of antigen content in the sinocarotid nerve and the cellular tissue around it showed that it contained small amounts of antigen which could nevertheless be determined by the cold complement fixation method; these amounts varied from traces to 4 γ. The amount of antigen in the nerve does not depend substantially on the duration of resorption; consequently in this case the perineural lymphatic and hematogenic pathways of the nerve serve as a peculiar "cable" along which the antigen leaks from the isolated sinus into the blood.

The difference (leakage of antigen from the sinus – antigen in the sinocarotid nerve) could furnish data for determination of the amount of antigen resorbed into the blood. It amounted to 6-40 γ after 5 minutes.

The number of antigen molecules contained in 6 γ can be determined on the basis of Avogadro's number. The number of molecules in 1 g/mol (Avogadro's number) is equal to $6.023 \cdot 10^{23}$; 1 g/mol antigen equals 1,000,000 g, therefore 1,000,000 g antigen contains $6.023 \cdot 10^{23}$ molecules, 1 g antigen contains $6.023 \cdot 10^{17}$ molecules, 1 γ antigen contains $6.023 \cdot 10^{11}$ molecules, and 6 γ antigen contains $36.138 \cdot 10^{11}$ molecules.

It is known that each molecule of antigen (diphtheria toxoid) introduced into the body is associated with the formation of a minimum of 1,000,000 molecules of antibody within 3 weeks [7]. Clearly then the passage of such a seemingly small amount of antigen as 6 γ from the sinus into the blood is more than sufficient for successful sensitization of the animal organism.

We conclude on the basis of our experiments and in agreement with the conclusions of P. F. Zdrodovskii, I. A. Oivin, D. F. Pletsityi and many others that experiments on the isolated carotid sinus do not give convincing proof of the reflex mechanism of antibody production. A. N. Gordienko's assertion that the carotid sinus receptors participate in the mechanism of antibody production results from the erroneous methods used by him and his collaborators in their work on the study of the reflex mechanism of antibody production.

SUMMARY

It was demonstrated in this work that the complete isolation of the carotid sinus from the blood and the lymph circulation excludes the possibility of the reflex production of antibodies when the antigen acts on the glomus chemoreceptors alone. It was established by investigation of antigen resorption from the isolated carotid sinus by the leakage determination method that on the average about 24% of the antigen injected into the sinus is resorbed within the first 5 minutes. The determination of the antigen content in the sinocarotid nerve under these conditions demonstrated that one of the important paths of resorption was the perineural lymphatic spaces, and possibly the blood capillaries.

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