endotoxin is a nonhepatic factor mediating the tolerance of phagocytes to a phlogogenic stimulus upon reparative regeneration of the liver. The endotoxin stimulates macrophagal production of IL-1 and IL-6 [7] that enhance the synthesis of the acute phase proteins in hepatocytes. The endotoxin also elevates blood concentration of glucose [10] and stimulates macrophagal production of prostaglandin E_2 [8]. This may inhibit phlogogenic and hemopoiesis-stimulating activities of lung and liver macrophages and suppress granulomatous inflammation in regenerating liver.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, Yu. M. Zakharov, *et al.*, *Gematol. Transfuziol.*, No. 3, 20-23 (1990).
2. L. D. Liozner, *Regeneration and Development* [in Russian], Moscow (1982).
3. A. N. Mayanskii and D. N. Mayanskii, *Reviews on Neutrophil and Macrophage* [in Russian], Novosibirsk (1989).
4. D. N. Mayanskii, V. I. Shcherbakov, and T. G. Komlyagina, *Byull. Eksp. Biol. Med.*, **97**, No. 5, 620-624 (1984).
5. I. V. Plyushch, D. D. Tsyrendorzhiev, A. A. Zubakhin, and D. N. Mayanskii, *Ibid.*, **119**, No. 5, 477-479 (1995).
6. B. Benaceraff, G. Biozi, and B. Halpern, in: *Physiopathology of Research*, Springfield (1957), pp. 52-79.
7. M. Callery, T. Kamei, and M. Flye, *Circ. Shock*, **37**, 185-188 (1992).
8. M. Callery, T. Kamei, M. Mangino, and M. Flye, *Hepatology*, **14**, 368-372 (1991).
9. R. Cornell, *Ibid.*, **11**, 923-931 (1990).
10. M. Geisterfer, C. Richards, M. Baumann, *et al.*, *Cytokine*, **5**, No. 1, 1-7 (1993).
11. K. Hamazaki, S. Sato, M. Yunoki, *et al.*, *Res. Exp. Med. (Berl.)*, **194**, No. 4, 237-246 (1994).
12. G. Higgins and R. Anderson, *Arch. Pathol.*, **12**, 186-202 (1931).
13. D. Kazansky, N. Nastoyashchaya, M. Lomakin, and N. Artsimovich, *Immunol. Lett.*, **33**, No. 1, 93-98 (1992).
14. D. Mayanski, Y. Schwartz, S. Kutina, *et al.*, *Int. J. Exp. Pathol.*, **74**, 229-236 (1993).
15. S. Wahl, G. Costa, M. Corcoran, *et al.*, *J. Immunol.*, **150**, No. 8, 3553-3560 (1993).

p-Aminobenzoic Acid as a Stimulator of Angiogenesis

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p-Aminobenzoic acid (0.002 and 0.005% in a volume of 0.1 ml) stimulates angiogenesis in the yolk sac of chick embryos. It is more effective in a concentration of 0.005%, significantly increasing the length and branching of large vessels (by 27-33% and 35-43%, respectively) in comparison with the control (injection of 0.1 ml normal saline).

Key Words: *p-aminobenzoic acid; angiogenesis; yolk sac; chick embryos*

Angiogenesis is a reactive process occurring not only during embryogenesis but also in adult organism. It is triggered under various conditions by different pathophysiological factors. Angiogenesis has been regarded as a positive (for example, wound healing) or negative process (carcinogenesis, tissue ischemia, etc.). Modulation of angiogenesis with the use of stimulators and inhibitors may be helpful in the correction of various pathologies. In was reported that neovascularization is stimulated by heparin and copper-containing substances [8] and inhibited by protamine [10], cartilaginous extracts [9], emoxipine [4], α-interferon, etc.

We attempted to modulate angiogenesis with p-aminobenzoic acid (PABA). This substance stimulates phenotypic traits in animals [2] and plants [7]. Of special interest is the ability of PABA to induce selective modifications during ontogenesis; for example, it stimulates the development of the external segments of retinal photoreceptor cells [6] and promotes proliferation of stromal cells during healing of corneal wounds [5]. In the present

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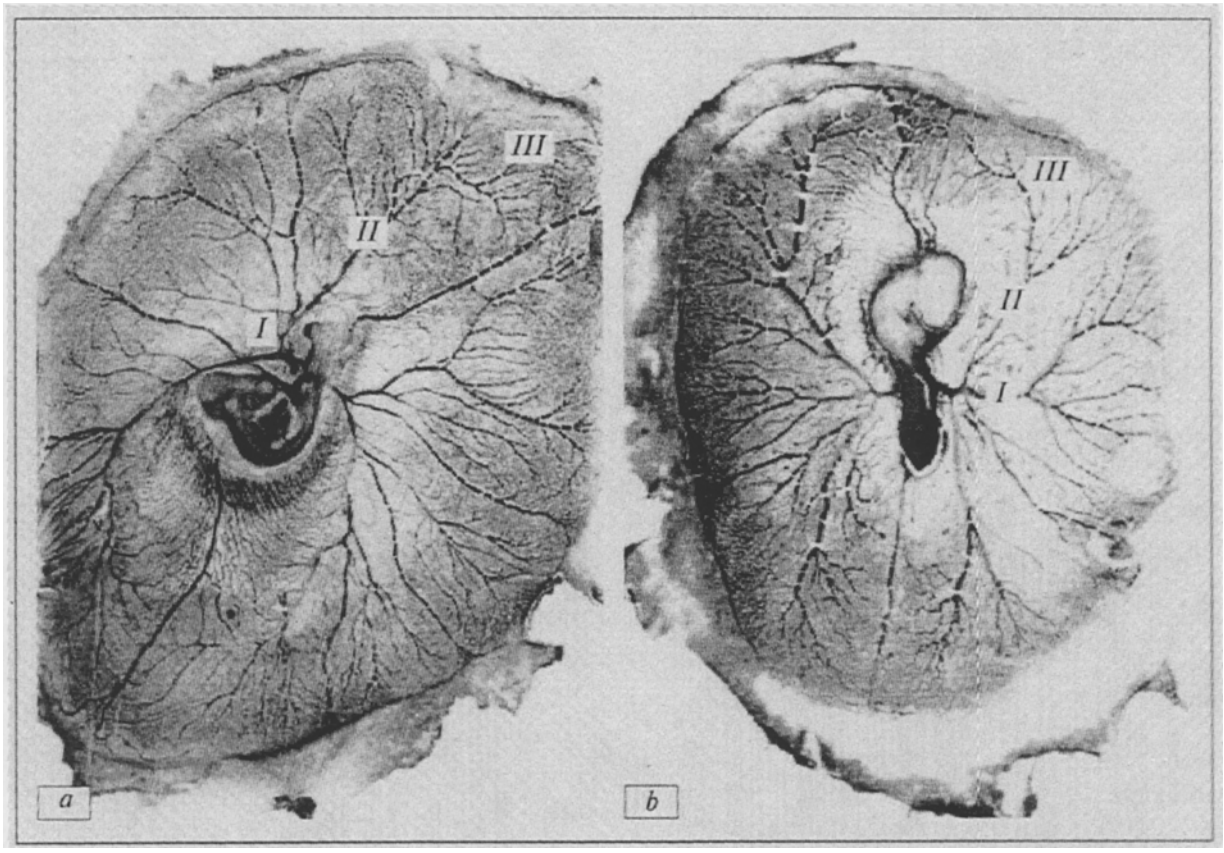

Fig. 1. Total preparation of chick embryo with the yolk sac after a 72-h incubation. *a*) experiment; *b*) control; *I*, *II*, and *III*) branching of the yolk sac blood vessels.

study we examined the effect of PABA on angiogenesis.

MATERIALS AND METHODS

The effect of PABA on the angiogenesis in the yolk sac of 48-h Leghorn chick embryos (embryonic stage 13 [1]) was studied. PABA was injected as normal saline solution (0.1 ml) in concentrations of 0.002% (14 embryos) or 0.005% (19 embryos) with a pipet through a hole in the eggshell; 15 embryos received an equal volume of normal saline (control). Biological activity of these concentrations was tested previously [5,6]. The results were evaluated 24 hours postinjection (stages 18-19 [1]) on fixed whole mount preparations. To this end each embryo with yolk sac was removed and a whole mount was prepared. The mounts were fixed for 20 min in 12% neutral formalin, 20 min in 96° ethanol, then passed through descending ethanol concentrations to pure water, and stained with hematoxylin by the method of Carazzi. The total length and the number of branches of the large yolk sac vessels (orders I, II, and III) and total vascularized and embryonic areas were evaluated on photographs of the total mounts using a Mini-Mop setup (Opton) (Fig. 1). Statistical data were obtained using a TgA10 Opton particle autoanalyzer. The significance of differences was evaluated using the Student test. The correlation between the vascularized area, on the one hand, and the length of vessels and the number of branches, on the other, was assessed using a correlation coefficient calculated from the conventional formula [3]. Experiments were repeated two times: at the beginning of October (Table 1) and at the end of November (Table 2) of the same year. Due to certain season peculiarities, the data of these series were not combined. In order to compare the results obtained in these experiments the index R was calculated from the following formula: $R=(E-C)/C$, where E and C are the means for each parameter in experimental and control group.

RESULTS

A single injection of PABA in both concentrations stimulated angiogenesis in the yolk sac of chick embryos. In both series, PABA proved to be more effective at 0.005% than at 0.002%, significantly increasing the total length of large vessels (by 27-33%) and the number of branches (by 35-43%) in

TABLE 1. Effect of PABA on Chick Embryos and Yolk Sac Vessels in October ($M \pm m$)

Object	Control (n=5)	PABA, 0.002% (n=6)	R, %	PABA, 0.005%(n=10)	R, %
Number of order I, II, and III branches	132.3±17.0	174.5±7.0**	31.9	178.0±15.0*	34.5
Total length of order I, II, and III vessels	2975.0±192.0	3794.0±320.0**	27.5	3873.0±149.0***	30.0
Vascularized area	1065.0±95.0	1630.0±100.0***	53.0	1756.0±96.0***	64.9
Embryonal area	92.8±9.5	115.5±14.0	24.8	133.5±9.8***	43.9

Note. Here and in Table 2: *p<0.02, **p<0.05, ***p<0.001 in comparison with the control.

TABLE 2. Effect of PABA on Chick Embryos and Yolk Sac Vessels in November ($M \pm m$)

Object	Control (n=10)	PABA, 0.002% (n=8)	R, %	PABA, 0.005% (n=9)	R, %
Number of order I, II, and III branches	79.3±9.5	97.5±5.8	22.8	114.1±10.2*	43.7
Total length of order I, II, and III vessels	1076.0±56.6	1250.0±81.0**	16.2	1432.0±91.4**	33.1
Vascularized area	4375.0±250.9	4583.0±243.3	4.7	4601.0±127.3	5.1
Embryonal area	159.4±9.0	172.5±9.9	8.2	193.4±18.3**	21.3

comparison with the control. The size of embryos (area) was also increased.

The vascularized area of the yolk sac varied to a greater extent. Both concentrations of PABA slightly increased this parameter; however, the differences between the control and experimental groups were not always statistically significant (Tables 1 and 2). There was no correlation between the vascularized area and the length of blood vessels (r=0) and the number of branches (r=0.494). This suggests that in contrast to the length of blood vessels, the vascularized area of the yolk sac is not a valid parameter for evaluating the effect of some preparations. In the present study we did not analyze the mechanism of the embryo enlargement that may result from better vascularization of the yolk sac and direct influence of PABA on the embryo.

Thus, our results show that PABA stimulates angiogenesis. This paves the way for further investigations of PABA as a modulator of angiogenesis.

This and our previous findings [4] indicate that a total preparation of chick embryos with the yolk sac is a suitable model for morphometric evaluation of various effects on blood vessels.

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REFERENCES

1. *Objects of Developmental Biology* [in Russian], Moscow (1975), pp. 470, 473.
2. I. A. Rapoport, in: *Phenogenetic Analysis of Independent and Dependent Differentiation*, Proceedings of the Institute of Cytology, Histology, and Embryology [in Russian], Vol. 2, Moscow (1948), Issue 1, pp. 51-52.
3. P. F. Rokitskii, in: *Fundamentals of Variational Statistics for Biologists* [in Russian], Minsk (1961), pp. 104-113.
4. A. A. Sologub, S. I. Akberova, and G. G. Ziangirova, *Byull. Eksp. Biol. Med.*, **114**, No. 12, 620-622 (1992).
5. A. A. Sologub, I. G. Panova, and O. G. Stroeva, *Ontogenesis*, **25**, No. 6, 54-59 (1994).
6. O. G. Stroeva, V. A. Poplinskaya, I. P. Khoroshilova-Maslova, and I. A. Rapoport, *Dokl. Akad. Nauk SSSR*, **31**, No. 2, 483-487 (1990).
7. I. A. Rapoport (Ed.), *Chemical Mutagens and Para-Aminobenzoic Acid in Raising the Crop Yield* [in Russian], Moscow (1989).
8. G. Alessandry, K. Raju, and P. M. Gullino, *Cancer Res.*, **43**, 1790-1797 (1983).
9. S. B. Goren, R. Eisenstein, and E. Choromokos, *Am. J. Ophthalmol.*, **84**, 305-309 (1977).
10. S. Taylor and I. Folkman, *Nature*, **197**, 307-312 (1982).