term crushing of soft tissues in rats. The high blood enzyme levels were due mainly to activity of acid nucleases and aryl sulfatases A and B starting from the 3rd hour of tissue crushing and until the end of the experiment.

LITERATURE CITED

1. A. I. Basaeva, I. N. Pidevich, and M. F. Vyalykh, Byull. Éksp. Biol. Med., No. 10, 35 (1970).
2. V. N. El'skii, Vopr. Med. Khim., 23, 365 (1977).
3. A. M. Zubovskii, Vopr. Med. Khim., 14, 152 (1978).
4. V. I. Kocherga, "Effect of serotonin on nucleic acid metabolism in the brain of animals," Candidate's Dissertation, Kiev (1963).
5. G. N. Kryzhanovskii and S. Ya. Ostryi, Patol. Fiziol., No. 5, 24 (1967).
6. V. K. Kulagin, The Pathological Physiology of Trauma and Shock [in Russian], Leningrad (1978).
7. A. A. Pokrovskii, in: The Chemical Basis of Physiological Processes [in Russian], Moscow (1962), p. 311.
8. A. A. Pokrovskii and V. A. Tutel'yan, Biokhimiya, 33, 809 (1968).
9. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
10. V. I. Ovsyannikov, Kardiologiya, No. 1, 53 (1970).
11. L. F. Semenov, Experimental Prevention of Acute Radiation Sickness [in Russian], Leningrad (1967).
12. V. Yu. Urbakh, Biometric Methods [in Russian], Moscow (1964).
13. Zh. A. Chotoev, "Effect of serotonin on oxidative and carbohydrate-phosphorus metabolism of heart muscle," Candidate's Dissertation, Moscow (1964).

EFFECT OF HETEROLOGOUS PROTEINS ON THE INITIAL STAGES OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

E. G. Alksnis

UDC 616.13-004.6-036.4-036.65-02:615.373.3

KEY WORDS: atherosclerosis; human γ-globulin.

Human γ-globulin is widely used in medical practice for preventitive and therapeutic practice, especially in children. Injection of large doses of heterologous proteins (250 mg/kg body weight or more) into animals has been shown to increase the permeability of the endothelium of arteries and to accelerate the development of atherosclerosis in animals fed with cholesterol [6, 12, 13, 15]. Bovine serum albumin or whole blood serum was used as antigens in these investigations, and human γ-globulin, which is used in medical practice in the form of parenteral injections, was never once used for immunization. Yet there are indications in the literature that experimental atherosclerosis is not accelerated if animals are given injections of smaller doses of heterologous proteins [9, 14].

It was therefore decided to study how immunization with various doses of human γ-globulin (HGG) and also of bovine serum albumin (BSA), most frequently used in investigations of this kind, affects the development of experimental atherosclerosis in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 57 male rabbits weighing 2.5 kg. Hypercholesteremia was induced by daily administration of 500 mg cholesterol, dissolved in sunflower oil, via gastric tubes [3].

Department of Biochemistry and Department of Microbiology and Immunology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 8, pp. 33-35, August, 1982. Original article submitted January 27, 1982.

0007-4888/82/9408-1048$07.50

TABLE 1. Blood Levels of β- and Pre-β-LP (I), Total CH (II), and TG (III) in Blood (in mg/dl) and Severity of Changes in Aortas of Rabbits Immunized with Two Large Doses of Antigens (M ± m)

Antigen	Number of animals in group	Parameter studied	Day after beginning of immunization (duration of cholesterol feeding in days shown in parentheses)				Percent of involvement of aorta
			1	14 (3)	28 (17)	74 (63)	
Control	9	I	65±10	350±35	825±160	3625±365	13,5±1,6
		II	38±6	173±29	342±60	1349±190	
		III	76±8	68±8	57±7	273±52	
BSA	8	I	70±5	315±30	830±80	4120±905	27,6±6,3*
		II	39±2	177±17	382±40	1429±263	
		III	64±4	104±5*	69±6	269±17	
HGG	8	I	70±5	208±30	750±90	3870±220	7,4±1,5*
		II	43±3	134±11	369±41	1404±138	
		III	62±4	60±7	51±8	197±16	

*P < 0.05 compared with control.

TABLE 2. Blood Levels of β- and Pre-β-LP (I), Total CH (II), and TG (III) and Severity of Changes in Aortas of Rabbits Immunized with Repeated Small Doses of Antigens (M ± m)

Antigen	Number of animals in group	Parameter studied	Day after beginning of immunization (duration of cholesterol feeding in days shown in parentheses)					Percent of involvement of aorta
			1	14	28	70 (42)	91 (63)	
Control	10	I	70±5	80±5	70±10	2625±420	3855±555	13,6±1,8
		II	34±4	41±3	35±4	1011±119	1412±151	
		III	73±4	72±6	64±8	173±21	269±26	
BSA	10	I	80±10	170±10**	80±5	4590±605*	4290±925	15,4±1,9
		II	32±3	62±3**	43±3	1833±225*	1665±308	
		III	79±8	111±11*	66±5	466±98*	394±25*	
HGG	12	I	65±10	100±15	80±15	2590±255	4160±475	5,2±0,6**
		II	29±4	38±4	37±5	1108±89	1601±194	
		III	84±8	76±8	74±11	209±22	233±30	

*P < 0.05 compared with control.
**P < 0.001 compared with control.

For immunization, medical standard HGG and a 10% solution of BSA (from Sigma, USA) in 0.9% NaCl were used. The level of sensitization was judged from the area of hyperemia 24 and 48 h after skin testing (2 mg protein in 0.02 ml), which was done before and 7, 14, 28, and 42 days after immunization. The presence of antibodies in the rabbits was determined by the complement fixation test [5] in samples of blood serum obtained 2, 7, 11, 14, 21, 28, and 42 days after the beginning of immunization.

Some animals received two intravenous injections of large doses of BSA or HGG (500 mg, followed by 250 mg 10 days later) and received the cholesterol diet for 9 weeks after the end of immunization. Other animals were given 20, 25, and 30 mg of BSA or HGG on three consecutive days. The course of injections was repeated after 7 days. The last dose (30 mg) was injected intravenously (total dose of antigen injected 150 mg). At the time of maximal development of the skin reactions, 28 days after the beginning of immunization, the rabbits were given a cholesterol diet for 9 weeks. Unimmunized animals, kept for 9 weeks on the atherogenic diet, served as the control.

The concentration of β- and pre-β-lipoproteins (LP) were determined by the method in [7] and total cholesterol (CH) and triglycerides (TG) were determined on an AA-II automatic analyzer (Technicon) in the blood serum taken from rabbits at intervals during development of the immune response and experimental atherosclerosis. At the end of the experiments, the aortas of the animals were stained with Sudan III in order to determine the area of the atherosclerotic lesion [1].

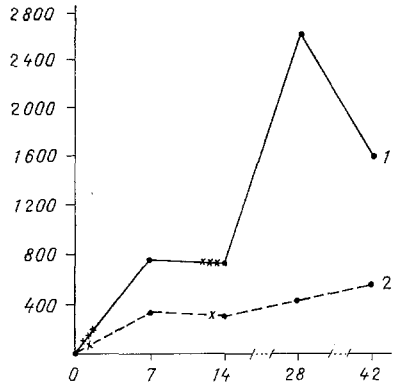

Fig. 1. Skin tests after injection of HGG into rabbits (area of hyperemia 24 h after skin testing). 1) Repeated subcutaneous injection of small doses of HGG (total dose 150 mg); 2) intravenous injection of two large doses of HGG (total dose 750 mg). Abscissa, time after beginning of immunization (in days); ordinate, area of hyperemia (in mm^2). Crosses indicate repeated injections of protein.

The results were subjected to statistical analysis [2, 10].

EXPERIMENTAL RESULTS

As Table 1 shows, cholesterol feeding was accompanied by a parallel increase in the concentration of LP and lipids in the blood of the unimmunized animals and of rabbits receiving large doses of antigens. However, these groups differed significantly in the area of atherosclerotic changes in the aorta at the end of the experiment. Administration of large doses of BSA led to the more rapid development of atherosclerosis, whereas immunization with HGG had no potentiating effect on the development of atherosclerosis when the lesions were assessed visually and planimetrically.

Subcutaneous immunization of rabbits with smaller doses of HGG was not followed by the more intensive development of hyperlipidemia compared with the control, whereas injection of BSA in the same doses led to an increase in the blood lipid concentration in animals receiving a high cholesterol diet (Table 2). Injection of small doses of HGG, incidentally, delayed the development of experimental atherosclerosis, whereas injection of small doses of BSA had no such action.

It is a noteworthy fact that when the animals were injected with smaller doses of antigen, the skin reaction was stronger than when large doses were used (Fig. 1). Meanwhile, the maximal blood titer of antibodies in rabbits receiving two injections of large doses of antigen (1:64-1:128) was higher than when small doses of protein were injected subcutaneously (1:16-1:32). This result is perhaps attributable to the mode of injection of the antigen, for subcutaneous immunization stimulates the mechanisms of cellular immunity by a greater degree than intravenous injection. It is particularly important to note that after repeated subcutaneous injection of small doses of HGG, the area of atherosclerotic changes in the aorta was significantly reduced ($P < 0.001$).

The results are evidence of the importance of the effect of immunization on the development of experimental atherosclerosis, depending on the character of the antigen and the dose and mode of its injection. It must be particularly emphasized that immunization with HGG, unlike with BSA under the same experimental conditions, not only did not accelerate but, in the case of subcutaneous injection of relatively small doses of HGG, it significantly retarded the development of atherosclerosis.

A comparison can be drawn between the effect of small doses of γ-globulins and that of Bogomolets' species-nonspecific serum on the development of atherosclerosis [4, 8], although this sheds no light on the mechanism of the protective action of the latter. It must also be pointed out that when equal doses of heterologous antigens are used, the immune response of

rabbits to γ-globulin, despite its greater molecular weight, was weaker than to serum albumin [11]. The possibility cannot be ruled out that γ-globulin, even of heterologous origin, normalizes the immunologic reactivity of an animal fed with cholesterol on account of the complement-fixing function of the Fc-fragment.

LITERATURE CITED

1. G. G. Avtandilov, Classification and Planimetric Assessment of Atherosclerotic Lesions in Blood Vessels [in Russian], Nal'chik (1960).
2. I. P. Ashmarin, N. N. Vasil'ev, and V. A. Ambrosov, Rapid Methods of Statistical Analysis and Experimental Planning [in Russian], Leningrad (1971).
3. K. G. Volkova, Arkh. Biol. Nauk, 30, 269 (1930).
4. I. M. Gandzha, I. P. Myagkaya, and N. K. Furkalo, Vrach. Delo, No. 3, 36 (1973).
5. M. Seifart, in: Immunologic Methods [Russian translation], ed. H. Friemel, Moscow (1979), p. 136.
6. Yu. A. Kashirin, V. I. Petrovskii, and V. A. Nagornev, Patol. Fiziol., No. 5, 30 (1976).
7. A. N. Klimov, T. N. Lovyagina, and É. B. Ban'kovskaya, Lab. Delo, No. 5, 276 (1966).
8. I. P. Myagkaya, Vrach. Delo, No. 5, 39 (1971).
9. I. P. Myagkaya and A. S. Stupina, in: Cytotoxins in Modern Medicine [in Russian], ed. O. A. Bogomolets, No. 6, Kiev (1972), p. 34.
10. I. A. Oivin, Patol. Fiziol., No. 4, 76 (1960).
11. F. G. Germuth, C. Flanagan, and M. R. Montenegro, Bull. Johns Hopkins Hosp., 101, 149 (1957).
12. A. N. Howard, J. Patelski, D. E. Bowyer, et al., Atherosclerosis, 14, 17 (1971).
13. L. Levy, Adv. Exp. Biol. Med., 1, 426 (1967).
14. C. R. Minick and G. E. Murphy, Am. J. Pathol., 73, 265 (1973).
15. C. R. Minick, G. E. Murphy, and W. G. Campbell, J. Exp. Med., 124, 635 (1966).

CHANGES IN PLASMA CORTICOSTERONE LEVELS OF INBRED MICE AFTER STRESS

S. B. Seredenin, B. A. Badyshtov, M. M. Nikitina, and V. B. Rozen

UDC 616.154.453:577.175.53]-06:613.863

KEY WORDS: corticosterone; blood plasma; stress.

Many experiments on inbred animals have shown that their behavior under stress is genetically controlled [3, 4]. Other investigations have shown that the hormonal response of the pituitary-adrenal system is genetically determined [7, 8].

The object of this investigation was to study differences in the plasma corticosterone level in mice of different strains reacting differently to an emotional-stress situation simulated in the "open field" (OF) test.

EXPERIMENTAL METHOD

C57BL/6 (6), CBA, and BALB/c (C) mice weighing 20-22 g were used. The conditions under which the animals were kept and the modification of the OF method used were described previously [3]. Corticosterone in blood plasma obtained after decapitation was determined by the method in [6] in the writers' own modification in two series of experiments 10 and 20 min after the end of the experiment in OF (+OF+10, +OF+20). In three control experiments, the hormone level was measured in intact mice (IM) and again after the same manipulations as with the experimental animals, except they were not placed in OF (—OF+10, —OF+20). The results were subjected to statistical analysis by Student's t test.

Laboratory of Pharmacologic Genetics, N. I. Pirogov Second Moscow Medical Institute. Laboratory of Endocrinology, Faculty of Biology, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 8, pp. 36-37, August, 1982. Original article submitted April 21, 1982.