STIMULATION OF THE FORMATION OF NORMAL ANTIBODIES BY BLOOD LETTING

(UDC 615.811.3-092:612.017.1)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 11, pp. 82-86, November, 1964
Original article submitted February 15, 1963

Blood letting was used with success in medicine in antiquity, and in the Middle Ages it was regarded as a panacea for almost every disease; it is still used even today, especially with the use of bleeding cups. However, with the advent of the sulfonamides, antibiotics, and hormones, the importance of all the various types of therapeutic blood letting diminished, although their therapeutic value in certain diseases is without question. In the present study the effect of blood letting on the titer of normal dysentery agglutinins in the blood was investigated.

METHOD

After determination of the titer of normal dysentery agglutinins in 11 mongrel rabbits of different litters, weighing from 2.0 to 2.5 kg, each animal received a subcutaneous injection of 50 units of tetanus toxoid in the limb. Blood was taken from the auricular vein of 7 experimental animals in volumes of 15, 17, and 20 ml on 3 successive days, and this procedure was repeated after various intervals (5 or 10 days). The agglutinin titer of the experimental animals and of 4 control animals, from which blood was not taken, was investigated at these times. In the subsequent period blood letting was carried out only if the antibody titer fell. Control rabbit No. 27 and experimental rabbit No. 28, which received no tetanus toxoid, were sacrificed after two courses of blood letting at an interval of 5 days. Their serum was tested for its preventive properties; the spleen and the inguinal and axillary glands were weighed, and impressions were then taken from these organs, in which the plasmoblasts and plasma cells were counted separately by the method of Shumakova and Gurvich [1, 2].

TABLE 1. Reaction of Spleen and Lymph Glands to Blood Letting

Rabbit No.	Weight (in kg)	Weight of spleen (in mg)	Weight of lymph glands (in mg)				Total weight of lymph glands (in mg)	Number of plasmoblasts (numerator) and plasma cells (denominator)					
			RI	LI	RA	LA		Spleen	RI	LI	RA	LA	Lymph glands (total)
27 (control)	2.2	900	40	40	40	40	160	4/3	18/40	9/8	7/7	14/13	48/68
28 (experimental)	2.4	4600	150	120	220	210	700	69/9	97/10	133/34	71/31	90/23	391/98

Legend: RI) right inguinal lymph gland; LI) left inguinal lymph gland; RA) right axillary lymph gland; LA) left axillary lymph gland.

TABLE 2. Characteristics of Agglutinating Properties of Donors' Blood Sera

Titer	Antigens			
	Sh. dysent. Flxn.	Sh. dys. Sonne.	Salm. typhi.	Esch. coli
1:50	1	91	4	14
1:100	33	None	18	10
1:200	85	"	14	11
1:400	170	"	14	8
1:800	67	"	2	6
Negative	None	265	None	3
No. of persons examined	356	356	52	52
Confidence intervals	8.8(7.84-9.76)		7.5(7.3-7.7)	7.16(6.75-7.67)

TABLE 3. Characteristics of Agglutinating Properties of Blood Sera of Nondonors

Titer	Antigens		
	Sh. dysent. Flexn.	Salm. typhi.	Esch. coli
1:50	24	23	31
1:100	16	5	4
1:200	None	None	None
Negative	10	22	15
No. of persons examined	50	50	50
Confidence intervals	4.82(4.18-5.46)	4.45(3.75-5.15)	4.02(3.29-4.75)

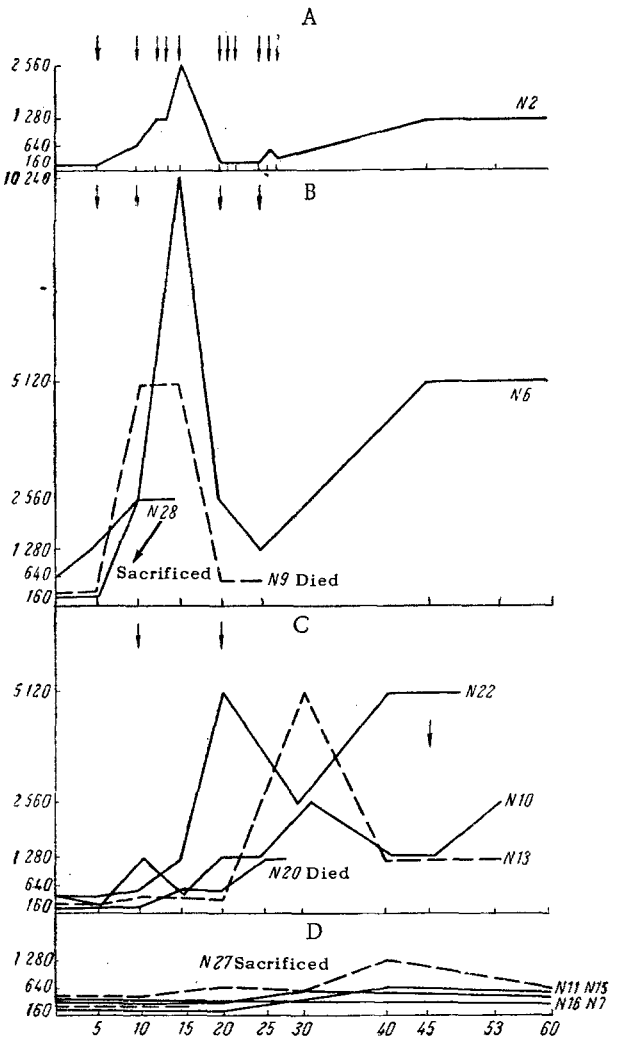
The blood sera of 356 donors were also tested for the presence of normal dysentery agglutinins with three different diagnostic cultures and with a living culture of Shigella flexneri. Of this number, 52 sera were also examined in the agglutination reaction with Escherichia coli and Salmonella typhi. Blood was taken only from persons who had not suffered from dysentery or other enteric diseases for the last 2 years and who had not been immunized against enteric infections during this period. Similar investigations were conducted with the serum of 50 healthy persons who were not donors.

RESULTS

Throughout the period of observation the titers of normal agglutinins in the control animals (without blood letting) were within the limits of the original values, with small variations (see figure, D).

The agglutinin titer of rabbit No. 2 (see figure, A) rose to four times the original level after two bleedings at an interval of 5 days. Three further blood lettings were carried out on 3 successive days, and this procedure was repeated 3 times in all at intervals of 3 days: the antibody titer was increased 16 times. Blood was again taken on 3 successive days; this cycle was performed twice after intervals of 2 and 4 days, and it was followed by a fall in the antibody titer of the blood. A further rest period of 17 days led to an increase (by 4 times the original level) in the agglutinin titer, although on this occasion no bleeding was performed.

In the rabbits subjected to blood letting once every 5 days (see figure, B), the agglutinin titer rose 2, 32, and 64 times over its initial level after the first two bleedings. Rabbit No. 28 was then sacrificed so that the plasma-cell reaction of the lymph glands could be studied. The next three bleedings led to a fall in the antibody titer of the serum. At this stage rabbit No. 9 died. After a rest of 20 days, during which no blood was withdrawn, the antibodies in rabbit No. 6 again reached a high level, at which they remained for 15 days (period of observation).



Changes in titer of normal antibodies in rabbits following blood letting. Numbers along vertical axis—dilutions of serum; along horizontal axis—days of observation; numbers on curves—Nos. of rabbits. The arrows denote blood lettings.

The stimulating role of blood letting was also clearly seen when blood was withdrawn every 10 days (see figure, C). After two bleedings the antibody titer of rabbit No. 10 rose 8 times, that of rabbit No. 20—16 times (the animal died), that of rabbit No. 13—32 times, and that of rabbit No. 22 after the first bleeding—16 times. During the next 5 days the antibody titer in rabbits Nos. 10 and 13 fell, but after the next blood letting the titer of rabbit No. 10 again rose (although not so intensively as at first), while that of rabbit No. 13 remained at its previous level. The agglutinin titer of rabbit No. 22 fell 10 days after the second bleeding, but during the next 10 days it again became maximal without further bleeding.

Mice treated with the serum of rabbit No. 28, sacrificed after two bleedings (titer 1:2560), survived after injection of 2, 3, and 4 LD_{50} of a culture of Shig. flexneri, while of the 8 control mice receiving the serum of control rabbit No. 27 (titer 1:320), 5 died.

The results given in Table 1 in turn demonstrate the very obvious stimulating effect of blood letting on the system of organs producing antibodies, as expressed by an increase in their weight and also in the numbers of plasmoblasts and plasma cells.

During the investigation of the donors' serum, normal dysentery agglutinins were found in the serum from all 356 persons investigated (Table 2). In all cases the agglutination was fairly strong. There were 442 reactions evaluated as ++++ with a diagnostic culture of Shig. flexneri, 323 were evaluated as +++, 276 as ++, 254 as +, and 33 as ±. Repeated experiments with exhaustion of the donors' sera with dysentery antigen demonstrated the specificity of the antibodies.

Different results were obtained from the study of the sera of healthy persons who were not donors (Table 3). In none of these were agglutinins to the above-mentioned antigens found in a dilution of 1:200.

We are inclined to attribute the increase in the titers of normal antibodies in the donors and experimental animals to the stimulating action of the blood letting on antigen formation. The increase in the antibody titer to a high level in almost all the rabbits after a rest, following a phase of reduction as a result of frequent bleedings, and the marked reaction of the spleen and lymph glands under the influence of the bleedings, indicate that in this case we are dealing with a process of stimulation of antibody production and not with their mechanical expulsion from the various body depots. When the titer of normal antibodies has been raised to a certain level by means of preliminary blood lettings, it may be maintained there for a fairly long period by occasional blood lettings, performed after a fall. This is the way in which we imagine the process of maintenance of the high concentration of antibodies in donors to take place.

Hence, in human donors and experimental animals, blood letting causes marked stimulation of the production of normal antibodies. In regard to donors this is confirmed by the high titers of dysentery antibodies, and in regard to rabbits, in addition, by the preventive properties of their serum, the change in the weight of the spleen and the subcutaneous lymph glands, and their plasmoblast and plasma-cell reactions. Two cycles of blood letting in rabbits in a dose of 15, 17, and 20 ml (0.6-0.8% of their body weight) at intervals of 5 or 10 days increases the titer of normal dysentery antibodies 4, 16, 32, and 64 times; the subsequent performance of similar or more frequent blood

lettings exhausts antibody formation, and is therefore undesirable. To maintain the titer of normal antibodies at a high level, it is advisable to perform successive blood lettings after the antibody concentration in the serum has started to fall, as demonstrated by the appropriate reaction. At the same time the schemes and doses of blood letting which were studied are possibly not the best for stimulating normal antibodies. However, we observed that the taking of only 2 ml of blood for the agglutination reaction invariably led to an increase in the agglutinin titer, although this increase was only slight.

LITERATURE CITED

1. G. A. Gurvich and G. V. Shumakova, Byull. éksper. biol., 10 (1957), p. 95.
2. G. V. Shumakova and G. A. Gurvich, Byull. éksper. biol., 11 (1958), p. 66.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
