A METHOD OF OBTAINING BLOOD FROM WHITE MICE

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White mice are widely used for biological research. The possibility of obtaining large numbers of animals, their cheapness, and the ease of rearing and care all go to make mice irreplaceable experimental animals. White mice are no less widely used in the manufacture of bacterial preparations as controls of nontoxicity of vaccines and sera, the antigenicity of vaccines, and so on. It has recently been shown that the testing of the antigenic properties of tetanus [3, 4] and gas gangrene [1] toxoids may be successfully carried out also in white mice.

One of the disadvantages of work with white mice is the impossibility of obtaining blood for determination of the antibody level. For this reason tests of the antigenicity of vaccines and toxoids are based either on computation of the LD_{50} [5] or the effective index [2], or on determination of the rate of survival after inoculation of the animals with definite doses of toxins [1, 3, 4]. By puncture of the heart, amputation of part of the tail, or puncture of the paws or ear it is possible to obtain 2-3 drops of blood, which is insufficient for titration, and only by decapitation of mice may 0.3-0.5 ml of blood be obtained. In cases in which it is necessary to observe the course of antibody production, all investigations must therefore be carried out on other animals – guinea pigs and rabbits – and not on mice.

We considered it to be of practical importance to develop a method, suitable for general use, of obtaining blood from white mice in a sufficient quantity for serological and immunological investigations. For this purpose we adopted the method of obtaining blood from rats described by Woodruff and coworkers [6], based on the use of a lowered pressure.

EXPERIMENTAL METHOD

The glass receiver which we used (Fig. 1 and 2) has three openings. Into opening A, firmly closed with a cork, is inserted the mouse's tail; the opening B is connected to a manometer and motor for aspiration of air, and to opening C is attached the tube for collecting the blood.

The technique of obtaining blood is simple: The mouse's tail is smeared with xylene in order to dilate the caudal veins, the tip of the tail is cut off with sharp

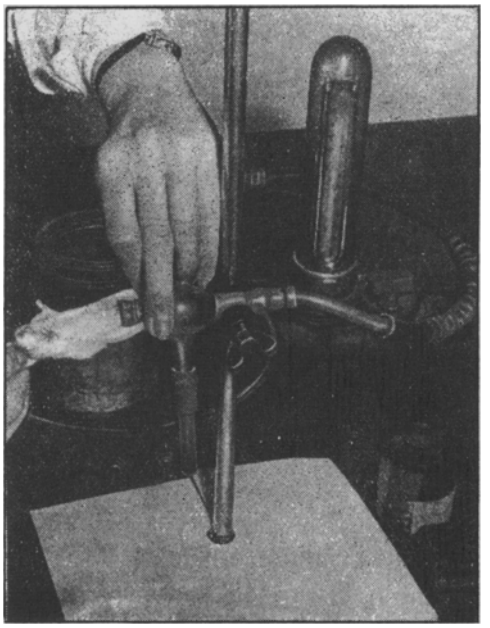

Fig. 1. Glass receiver for taking blood from white mice (general view).

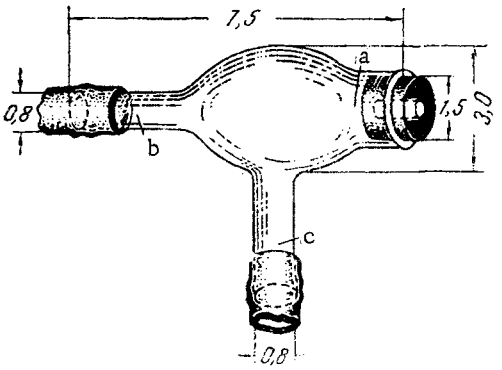

Fig. 2. Diagram of the glass receiver for taking blood from white mice. A) Opening into which the mouse's tail is placed; B) opening to the motor for aspiration of air; C) opening to the tube for collecting the blood (the internal dimensions are given in cm).

scissors, and to it is applied a drop of 5% sodium citrate solution. The mouse is then fixed firmly to the apparatus so that its tail falls into the opening C. The motor is set in motion and the collection of blood begins.

The first series of experiments was carried out on white mice in order to clarify the details necessary for the satisfactory obtaining of blood. It was discovered that the posterior part of the mouse's body must be firmly pressed against the opening A so that a pressure of 20-40 mm Hg was created in the receiver. At a higher (over 40 mm Hg) or lower (below 20 mm Hg) pressure, blood is obtained very slowly or not at all. If the opening A is closed with a rubber stopper or its size is reduced so that it fits closely to the posterior part of the mouse's body, a very low pressure (2-5 mm Hg) is created in the receiver, the vessels are compressed and blood does not flow. It was therefore found most suitable to use a cork stopper, which does not compress the vessels of the posterior part of the mouse's body and permits the entry of air into the receiver so that the pressure inside varies between 20 and 40 mm Hg. The mouse's tail must either be on the wall of the receiver or fall towards the opening C; should it enter the constricted part of the receiver, towards the opening B, blood will also cease to flow. In order to obtain a translucent, nonhemolyzed serum, it is essential that the walls of the receiver and tube be smooth; this is achieved by coating them with paraffin wax and by the use of short tubes (up to 5-9 cm). Under these conditions a perfectly translucent serum was obtained over a compact blood clot (Fig. 3).

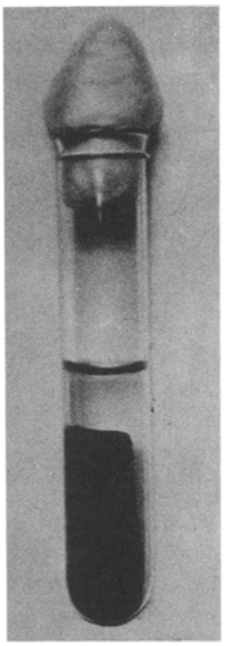

Fig. 3. Tube containing blood taken from white mice.

EXPERIMENTAL RESULTS

The results of taking blood from 100 mice by the method described are given in Table 1 and compared with those obtained by decapitation.

It may be seen from the data (Table 1) that, by the method described, it is possible to obtain from mice 0.2-0.5 ml of blood, with an average of 0.3 ml from each animal, or the same volume as by decapitation. The time required for taking blood is from 2 to 5 minutes per animal. It must be emphasized that the taking of blood by this method does not affect the condition of the mice—they remain active and healthy; not once have dyspnea, fits or death of the animals been observed. This is a considerable advantage of the method described, for the mice may be used for subsequent investigations.

In the next experiment, carried out on 10 mice which were immunized with 1 ml of tetanus toxoid, we investigated the possibility of taking blood repeatedly (every 5 days). As a check on the condition of the animals, they were systematically weighed and the blood examined. In order to compensate for the loss of blood taken, an injection of twice the volume of physiological saline was given to the animals soon after bleeding. The results obtained are given in Table 2.

The results given in Table 2 show that it is possible for blood to be taken repeatedly from white mice.

On each occasion from 3.1 to 3.8 ml of blood could be taken from 10 mice. In the course of five takings of blood from each mouse, 1.5-2.0 ml of blood was obtained; the animals' condition nevertheless remained satisfactory and they did not lose weight. It is true that these repeated

TABLE 1. Results of Taking Blood from Mice by Various Methods

No. of mice	Site of taking blood	Volume of blood from 1 mouse (in ml)	Time (in min)	Pressure (in mm Hg)
100	Caudal vessels	0.2–0.5 (average 0.3)	2–5 (average 2½)	20–40
10	Cervical vessels by decapitation	0.3–0.5 (average 0.4)	2–3 (average 2½)	–

TABLE 2. Results of the Investigation of Repeated Taking of Blood (ever 5 Days) from Mice

Index		Taking of blood every 5 days				
		1st	2nd	3rd	4th	5th
Volume of blood from 10 mice (in ml)		3.1	3.6	3.2	3.8	3.5
Time required for taking blood (in min)		35	30	28	23	22
Length of tail cut off (in mm)		8-12	3-4	3-4	3-4	3-4
Condition of mice		Satisfactory				
Average weight of each mouse (in g)		16.5	16.5	16.5	16.5	16.5
Mean Hemoglobin level (in %)		96	67	69	–	37
Mean red cell count (per mm^3)		6,520,000	6,420,000	5,960,000	–	3,920,000
Mean leukocyte count (per mm^3)		9,950	9,750	9,650	–	9,450
Leukocyte formula (mean)	Lymphocytes	64	66	65	–	59
	Neutrophils	27	27	26	–	35
	Monocytes	8	7	9	–	6
	Eosinophils	1	–	–	–	–
	Basophils	–	–	–	–	–

TABLE 3. Effect of Repeated (everv 48 Hours) Taking of Blood on the Condition of Mice

Index		Taking blood every 48 hr				
		1st	2nd	3rd	4th	5th
Volume of blood from 10 mice (in ml)		3,4	3,1	3,2	3,9	3,3
Time required for taking blood (in min)		30	33	41	27	21
Length of tail cut off (in mm)		8—12	3—4	3—4	3—4	3—4
Condition of mice		Satisfactory				Dyspnea, quickening of the heart rate; 2 mice died
Average weight of each mouse (in g)		17,6	16,9	16,2	14,6	13,5
Mean hemoglobin level (in %)		60	—	37	—	32
Mean red cell count (per mm^3)		5 245 000	—	3 580 000	—	3 740 000
Mean leukocyte count (per mm^3)		7 300	—	7 560	—	8 630
Leukocyte formula (mean)	Lymphocytes	64	—	65	—	64
	Neutrophils	33	—	29	—	32
	Monocytes	3	—	4	—	4
	Eosinophils	—	—	2	—	—
	Basophils	—	—	—	—	—

takings of blood led to the development of anemia: The hemoglobin fell to 37%, the red cell count to 3,000,000, and the leukocyte count and formula remained practically unchanged.

A similar experiment was carried out on ten mice, immunized with 0.5 ml of perfringens toxoid, in order to see if it was possible to take blood more frequently (every 48 hours). The results obtained are shown in Table 3.

As the results in Table 3 show, more frequent taking of blood is possible. In these circumstances a volume of blood sufficient for titration—from 3.1 to 3.9 ml—may be obtained each time from 10 mice. Despite such frequent taking of blood, the animals' condition was satisfactory; only after the fifth taking of blood did the mice show dyspnea and a quickening of the heart rate; 2 mice died. It must be pointed out that such frequent taking of blood leads to a considerable loss of weight and to the rapid development of a severe anemia.

SUMMARY

The authors developed a method of obtaining blood (nonhemolyzed serum) from the caudal blood vessels of white mice in amounts sufficient for serological and immunological examinations. A glass bulb was connected to a suction motor to reduce the pressure. This method permits systematic and repeated blood drawing (3.1—3.9 ml of blood from 10 mice)—5 times at intervals of 2-5 days. Blood drawing performed by method does not affect

the condition of the mice and permits them to be used for other investigations.

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