ACCUMULATION OF ANTIBODIES IN THE CENTRAL NERVOUS SYSTEM

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The purpose of the present paper is to describe the accumulation of antibodies in the central nervous system of rabbits. Information on this subject may throw light upon several important problems: (1) the penetration of protein (globulin) from the blood into the spinal fluid, brain and cord, for antibodies cannot be separated from globulins experimentally, (2) the relationship between the cerebrospinal fluid and the tissue fluid of the brain and spinal cord and (3) the prevention and treatment of certain diseases of the central nervous system by the injection of immune serums.

The present work is a continuation of a series of studies (1) on the antibody content of the serum and organs of rabbits, which have yielded information as to the distribution of agglutinins in the serum, organs and lymph. Agglutinins against typhoid bacilli were selected as antibodies because they can be obtained in high concentration and can be measured easily and with relative accuracy. The rabbit was employed because the titers of the immune serums of rabbits are very much higher than in other small laboratory animals.* The experiments were carried out on actively and passively immunized animals. The active immunization consisted of a series of injections of killed typhoid bacilli into an ear vein. The passively immunized rabbits received one injection of immune serum into the blood stream. The antibody content of the organs was measured by extracting the organs (after grinding with sand) with salt solution and titrating the extracts. For the titration of agglutinins a method was used that is more sensitive than the usual routine method of agglutination. This method consists in centrifugalizing the tubes containing the serum or extract-dilutions and bacteria and reading the results while the tubes are being gently shaken and the sedimented bacteria resus-

* The dog—an animal extensively used in investigations on the central nervous system—does not furnish potent serum. Dogs could have been injected in the passive immunization experiments but by injecting immune serum obtained from rabbits into other rabbits the introduction of foreign protein is avoided.

pended. This technic was first used by Gathgens (2) in 1906 and has been employed by a number of investigators (3) since that time. For further technical details see previous publications of the present series.

The main results of the work, as already reported, can be summarized as follows: When serum containing antibodies is injected into an ear vein of the normal rabbit, antibodies accumulate in all the organs studied: the liver, spleen, kidney, lung, skin, muscle and uterus (smooth muscle). The rate of accumulation varies in the different organs. The final concentration is reached in the liver, spleen, lung and kidney in less than 10 minutes after the injection of immune serum, but in the uterus and skin only after several hours have elapsed. The antibodies penetrate most slowly into the skin. When the final amount of antibodies is accumulated there is a constant numerical relationship between the antibody content of the blood and organs. On the average, the highest dilution of extract prepared from 1 gm. of liver, spleen, kidney, lung or skin that agglutinated typhoid bacilli was one-tenth of the highest dilution of 1 cc. of serum that agglutinated typhoid bacilli.

Since there are less antibodies in the organs than in similar amounts of blood the question naturally arises whether the antibodies recovered from the organs are due to the blood present in them. That they are not derived mainly from the blood of the organs but from the extravascular part of the tissue is evidenced by the following observations. (1) Lymph obtained by cannulating the lymph ducts of the liver, leg, neck and thoracic duct contains antibodies in higher concentration than the organ extracts. (2) Perfusion does not reduce the antibody content of the skin and uterus. (3) More antibodies can be recovered in the perfusate from the living animal than were present in the blood before perfusion, showing that during perfusion antibodies penetrate from the organs into the blood vessels, an observation recently confirmed by Schwarzmann (4).

The equilibrium between the antibody content of the blood and of the organs can be reached from either the blood or the organs, for an identical relationship will establish itself between the antibody content of the serum and organs when the immune serum is injected either into the blood stream or into the skin.

EXPERIMENTAL

The experiments to be reported here were performed with both actively and passively immunized rabbits for these reasons. Passive immunization offers an opportunity to establish the rate of accumulation of antibodies in the organs and cerebrospinal fluid by examination of the rabbits at different intervals of time after the injection of immune serum. In actively immunized rabbits the titers of the blood and organ extracts are higher and therefore the observations are more striking. The technic of immunization was the same as in the previous work. The immune serum used was fresh, and was obtained and kept under sterile conditions.

Before describing the experiments, it must be emphasized again that the agglutination tests were made with the aid of centrifugalization, a method that is more sensitive than the routine method of agglutination. Without this technic, the results described in the present study cannot be duplicated.

I. Antibody-Content of the Brain, Spinal Cord and Cerebrospinal Fluid (from Cisterna Magna) of Actively Immunized Rabbits

The technic of these experiments differed from that of the earlier experiments in that urethane was not used for anesthesia because it is said that it promotes the

TABLE I

Agglutinin Titers of the Serum, Spinal Fluid, Brain and Spinal Cord of Actively Immunized Rabbits

Number of rabbit	Serum	Spinal fluid	Brain	Cord
1	1:150,000	1:640	1:2,500	1:1,300
2	1:150,000	1:420	1:600	1:384
3	1:128,000	1:160	—	—
4	1:102,000	1:512	1:576	1:1,200
5	1:102,000	1:256	1:1,500	1:362
6	1:102,000	1:512	—	—
7	1:102,000	1:512	—	—
8	1:102,000	1:512	1:583	1:290
9	1:50,000	1:128	—	—
10	1:32,000	1:80	1:200	1:104
11	1:32,000	—	1:192	1:60
12	1:32,000	1:40	—	—
13	1:26,000	1:48	—	—
14	1:20,000	1:52	—	—
15	1:13,000	1:26	—	—
16	1:3,200	1:5	—	—

passage of substances into the spinal fluid. The animals were narcotized with the minimum amount of ether necessary. Some of the rabbits were bled to death from the left carotid artery; some from the femoral arteries and the descending aorta; the site of bleeding did not influence the results. After the rabbits were bled to death cerebrospinal fluid was removed from the cisterna magna by means of a tuberculin syringe and skin-test needle (gauge 22). After some practice there was no difficulty in obtaining fluid free of blood. The samples of cerebrospinal fluid—in most cases 0.4 cc.—were centrifugalized and the sediment examined under a

microscope. Samples containing more than one red blood cell in 10 microscopic fields (seen with high dry lens) were rejected. From the brain and spinal cord the meninges were removed very carefully and the ventricles of the brain and central canal of the cord were then opened and carefully rinsed with salt solution. To ascertain whether any cerebrospinal or other fluid containing agglutinins was left on the surface of the organs, the last washing fluid was examined for agglutinins. Some of the washing fluid caused a trace of agglutination, but dilutions 1 in 2, 1

TABLE II

Agglutinin Titers of the Spinal Fluid and of Extracts of the Brain and Spinal Cord of Actively Immunized Rabbits*

Number of rabbit	Spinal fluid	Brain extract	Spinal cord extract
	per cent	*per cent*	*per cent*
1	0.43	1.70	0.87
2	0.28	0.40	0.26
3	0.12	—	—
4	0.50	0.56	1.17
5	0.25	1.47	0.35
6	0.50	—	—
7	0.50	—	—
8	0.50	0.57	0.28
9	0.26	—	—
10	0.25	0.62	0.32
11	—	0.60	0.18
12	0.12	—	—
13	0.18	—	—
14	0.26	—	—
15	0.20	—	—
16	0.20	—	—
Average.....	0.33	0.82	0.49

* The titers are expressed as percentages of the titers of the serum.

in 4 did not agglutinate typhoid bacilli. The washed organs were dried by pressing them lightly between filter papers, weighed, and ground with sea sand alone and with saline. The extracts were centrifugalized at high speed, the sediment discarded, and the supernatant fluid centrifugalized again until it became clear. In recording the results of titration, titer 1:100 means that when 1 gm. of brain was extracted with 9 cc. saline, the extract, diluted ten times, clumped typhoid bacilli and a dilution twice higher—1:200—did not agglutinate typhoid bacilli.

Tables I and II show the following.

1. The cerebrospinal fluid of all the rabbits immunized with killed

typhoid bacteria contained antibodies. The titer of the cerebrospinal fluid varied with the titer of the serum, the ratio of the titer of the serum to the titer of the cerebrospinal fluid being, on an average, 300:1, or 0.33 per cent. The variation in this numerical relationship exceeded in one case only the limit of accuracy of the method. One should bear in mind, in this connection, that in the agglutination test the serum, cerebrospinal fluid and organ extract are diluted by halves (1 in 100, 1:200).

2. The brain and the spinal cord of all the actively immunized animals contained agglutinins. The titer of the extracts of the brain and cord varied with the titer of the serums, but the numerical relationship between the titers of the serums and organ extracts was not so consistent as the numerical relationship between the titers of the serums and the cerebrospinal fluid. The ratio of the titers of the serums to the titers of the extracts of the brain and of the spinal cord ranged from 100:0.4 to 100:1.7 and from 100:0.18 to 100:1.17 respectively. The average agglutinin titer of brain extract was 0.82 per cent (of the titer of the serum) and that of the spinal-cord extract 0.49 per cent (of the titer of the serum). Therefore extracts prepared from 1 gm. of brain or spinal cord were more potent than the dilution of a similar amount of cerebrospinal fluid.

Summarizing the results of the observations on actively immunized rabbits, the following average numerical relationship was found between the agglutinin titers of the serum, spinal fluid, brain and spinal-cord extracts:

Blood serum	100. per cent
Cerebrospinal fluid	0.33 per cent
Brain extract	0.82 per cent
Spinal-cord extract	0.49 per cent

II. Antibody-Content of the Central Nervous System of Passively Immunized Rabbits

In studies of the distribution of antibodies in the blood and organs of passively immunized animals it is of great advantage to employ immune serums of high titers; therefore only very potent serums were used for passive immunization. The majority of the animals were injected with immune serums of which the titer was 1:64,000. The titer of one of the immune serums was as high as 1:200,000.

The titer of the serums and the amount injected are given in Table III. The serum was introduced slowly into an ear vein through a skin-test needle (gauge 22), the injection lasting from 3 to 4 minutes. Since it is generally believed that substances in solution injected into a peripheral vein are evenly distributed in the blood stream within 3 minutes, six rabbits were bled to death as early as 15 minutes after the immune serum had been injected. The results of the titrations of the serums, cerebrospinal fluids and organ extracts are tabulated in Tables III and IV.

TABLE III

Agglutinin Titers of the Serum, Spinal Fluid, Extracts of Brain and Spinal Cord of Passively Immunized Rabbits

Number of rabbit	Serum injected		Time between injection of serum and examination	Serum	Spinal fluid	Extract of brain	Extract of spina cord
	Amount	Titer					
1	10 cc.	1:128,000	15 minutes	1:25,000	0	1:80	1:80
2	10 cc.	1:128,000	15 minutes	1:25,000	0	1:50	1:50
3	25 cc.	1:64,000	15 minutes	1:16,000	1:5	1:96	1:29
4	25 cc.	1:64,000	15 minutes	1:24,000	less than 1:2.5	1:240	1:84
5	15 cc.	1:64,000	15 minutes	1:12,000	1:6	1:264	1:180
6	15 cc.	1:64,000	15 minute	1:12,000	less than 1:12	—	—
7	10 cc.	1:128,000	2 hours	1:18,000	1:1.8	1:68	1:18
8	10 cc.	1:128,000	2 hours	1:25,000	—	1:120	1:50
9	10 cc.	1:80,000	2 hours	1:10,000	1:6	1:120	1:80
10	25 cc.	1:64,000	3 hours	1:12,000	1:3.6	—	—
11	20 cc.	1:64,000	3 hours	1:12,000	1:32	—	—
12	15 cc.	1:200,000	4 hours	1:32,000	1:19	1:294	1:126
13	20 cc.	1:64,000	18 hours	1:6,400	1:11	1:29	1:10
14	25 cc.	1:64,000	18 hours	1:3,200	1:8	1:13	1:5
15	20 cc.	1:32,000	20 hours	1:3,200	1:3.2	—	—
16	20 cc.	1:64,000	20 hours	1:4,800	1:18	—	—
17	20 cc.	1:64,000	20 hours	1:3,200	1:10	—	—
18	20 cc.	1:64,000	24 hours	1:3,200	1:11.5	—	—
19	20 cc.	1:200,000	24 hours	1:40,000	1:160	—	—

The tables show that in four rabbits, which were bled beginning 10 to 15 minutes after the injection of immune serum and ending 10 minutes later, the undiluted cerebrospinal fluid failed to agglutinate typhoid bacilli. However, in two other rabbits a very small amount of agglutinins penetrated into the cerebrospinal fluid within that time. The penetration of the antibodies into the cerebrospinal fluid (obtained from the cisterna magna) proceeded at a slow rate, for the high-

est titers in the cerebrospinal fluid were found only after 15 hours had elapsed following the injection of immune serum. In these animals the titer of the cerebrospinal fluid ranged between 0.10 to 0.40 per cent of the titer of the serum; that is, it was about as high as in actively immunized rabbits.

TABLE IV

Agglutinin Titers of the Cerebrospinal Fluid and of Extracts of the Brain and Spinal Cord of Rabbits Following Intravenous Injection of Immune Serum*

Number of rabbit	Time between injection of serum and examination	Titer of cerebrospinal fluid	Titer of brain extract	Titer of extracts of spinal cord
		per cent	*per cent*	*per cent*
1	15 minutes	0	0.32	0.32
2	15 minutes	0	0.20	0.20
3	15 minutes	0.03	0.60	0.18
4	15 minutes	less than 0.01	1.00	0.35
5	15 minutes	0.05	2.2	1.5
6	15 minutes	less than 0.1	—	—
7	2 hours	0.01	0.38	0.10
8	2 hours	—	0.48	0.20
9	2 hours	0.06	1.20	0.80
10	3 hours	0.03	—	—
11	3 hours	0.27	—	—
12	4 hours	0.06	0.92	0.43
13	18 hours	0.17	0.45	0.15
14	18 hours	0.25	0.40	0.14
15	20 hours	0.10	—	—
16	20 hours	0.37	—	—
17	20 hours	0.31	—	—
18	24 hours	0.36	—	—
19	24 hours	0.40	—	—

* The titers are expressed as percentages of the titers of the serums.

Tables III and IV show that just as in actively immunized rabbits, the extracts of the brain and cord of passively immunized rabbits contained agglutinins. The numerical relationship of the titers of the serums and extracts of brain and cord are very similar to those found in the actively immunized animals, the titer of brain extract being on an average 0.70 per cent and that of the cord 0.71 per cent of the titers of the serum. The variation from the average was more marked than in the actively immunized rabbits.

It was expected that the accumulation of antibodies in the central nervous system would proceed at a slow rate. This was found true of the penetration of agglutinins into the cerebrospinal fluid. However it had not been expected that the titers of the organ extracts of rabbits examined 15 minutes after the injection of immune serum would be as high as those from rabbits that had been in contact with immune serum for 24 hours. This unexpected observation suggests either that the penetration of antibodies into the brain and spinal cord (tissue fluid of these organs) is as fast as into some organs, such as the spleen, liver and lung and faster than into the uterus and skin, or that the antibodies recovered were due mainly to the blood present

TABLE V

Antibody Titers of Serums and of Extracts of the Perfused Brain of Actively Immunized Rabbits

Number of rabbit	Titer of serum	Titer of brain extract	Titer* of brain
			per cent
1	25,000	270	1.0
2	25,000	180	0.7
3	12,800	770	0.6
4	250,000	1280	0.5
5	128,000	640	0.5

* Expressed as percentages of the titers of the serums.

in the brain and spinal cord. To throw light on this question, I perfused the brain of rabbits of which the blood serum had a high titer in the agglutinin test.

III. Antibody-Content of the Cerebrospinal Fluid, Brain and Spinal Cord after Perfusion

The perfusion experiments were performed as follows. An actively immunized rabbit was lightly narcotized with ether, and about 30 cc. of blood was obtained from the left femoral artery. Then 0.15 gm. of heparin was injected into an ear vein; 3 minutes later the rabbit was bled to death from the femoral arteries and the abdominal part of the descending aorta. Immediately after the bleeding was completed, the brachial arteries and descending part of the aorta were ligated, and Locke solution at 42°C. was introduced into the arch of the aorta with the purpose of perfusing the brain through the vertebral and internal carotid arteries. The

perfusion was usually continued for about 1 hour; about 500 cc. of perfusion fluid was used. The examination of cerebrospinal fluid, and of organ extract was carried out as described above.

Table V shows that perfusion did not diminish the antibody titers of the brain extract or the cerebrospinal fluid. However it was felt that before drawing a conclusion from this observation as to the presence of the antibodies in the extra-vascular brain tissue, it was desirable to obtain evidence of the adequacy of the perfusion experiments. To this end I compared histological sections of the brains of the exsanguinated rabbits with those prepared from rabbits whose brain was perfused. The comparison showed that in the unperfused brains the majority of the blood vessels contained red blood cells whereas in the perfused brains the blood vessels were distended and red blood cells were absent from the majority of them.

Final evidence for the view that the antibodies recovered from the brain by extraction are derived from the extra-vascular part of the tissue would be the demonstration of antibodies in the lymph flowing from the brain. However, no lymph duct draining the brain is known; therefore no direct evidence can be furnished at the present time for this view.

DISCUSSION

The antibody content of the cerebrospinal fluid of normal and diseased human beings and lower animals has been the subject of extensive clinical and experimental investigations. It has been very generally accepted that antibodies do not penetrate from the blood into the cerebrospinal fluid unless the meninges are inflamed. A survey of the literature, however, shows that several authors have reported the presence of antibodies in the cerebrospinal fluid without inflammation.

Hektoen and Carlson (5) found opsonins but no haemagglutinins in the cerebrospinal fluid of actively or passively immunized dogs. Becht and Greer (6) could not demonstrate agglutinins in the cerebrospinal fluid of rabbits immunized with typhoid vaccine. Kafka (7) found traces of hemolysins and bacterial agglutinins in the cerebrospinal fluid of immunized dogs. Starkenstein and Zitterbart (8) reported that only undiluted cerebrospinal fluid of dogs agglutinated typhoid bacilli, although the titer of their serum was as high as 1:10,000.

There are two reports in the literature on the relative titers of tetanus antitoxin in the serum and cerebrospinal fluid. Ransom (9), working in von Behring's laboratory, compared the antitoxin titers of the serum and of the cerebrospinal fluid of one very highly immunized horse. He found that the ratio of the antitoxin titer of the serum of this animal to that of the cerebrospinal fluid was 100:0.4. Lemaire and Debre (10), who studied the effect of morphine upon the permeability of the meninges, reported that in dogs injected with tetanus antitoxin the ratio of the titer of the serum to that of the cerebrospinal fluid was 100:0.2.

The experiments performed in the present study show clearly that antibodies are present in the cerebrospinal fluid of actively or passively immunized animals without inflammation of the meninges. The possible objection that the antibodies demonstrated were due to inflammation or contamination of the cerebrospinal fluid with blood as a result of faulty technic can be met as follows. (1) The specimens of cerebrospinal fluid did not contain red blood cells at all or only in a negligible number. (2) There was a constant numerical relationship of the titers of serums to the titers of cerebrospinal fluids. (3) In passively immunized rabbits the antibody titer of the cerebrospinal fluid increased during the first 15 hours following the injection of immune serum, although the titer of the blood decreased during this time.

It is pertinent to inquire whether the central nervous system of the rabbits used in these experiments was free of pathological changes. McCartney (11) at the suggestion of Flexner, examined the brains of a large number of apparently healthy rabbits and found histological evidence of meningo-encephalitic lesions in more than 50 per cent. His observation is in conformity with those made in other laboratories (Bull (12), Oliver (13)).

The brains of rabbits employed in the present work were not examined histologically, but it is reasonable to assume that the lesions found by McCartney occur in the brains of our stock rabbits.

McCartney, Bull and Oliver did not study the spinal fluid of their rabbits and therefore it is not known whether the lesions found by them in the brain are associated with a large number of leucocytes or with other signs of inflammation in the cerebrospinal fluid. Although the spinal fluid of the rabbits employed in the present work was free from an abnormal number of leucocytes, the possibility cannot be excluded that meningo-encephalitic lesions so prevalent in apparently normal

rabbits did not influence the accumulation of antibodies in the central nervous system in some of them. However antibodies were found in the spinal fluid, brain and spinal cord in all of the actively and all of the passively immunized rabbits 2 hours after passive immunization. It hardly seems possible that the accumulation of antibodies in the central nervous system of all the rabbits could have been due solely to the presence of meningo-encephalitis.

The reasons for the general belief that antibodies do not penetrate from the blood into the cerebrospinal fluid without the inflammation of the meninges are probably the following. (1) The antibody titer of the cerebrospinal fluid is relatively low, as would naturally follow from the circumstance that the antibody titers of the serums of animals examined in the course of the reported studies were not high. (2) A large number of studies dealt with normal hemolysins, whose titer in the serum is very low.

Amoss and Eberson (14) reported that "agglutinins were not found in the spinal fluid of normal monkeys which had received antimeningococcic serum intravenously." This observation can be readily explained by the experiments on rabbits reported here, which show a ratio of 300:1 between the titers of the serum and of the spinal fluid; whereas the serum of the monkey in the experiment of Amoss and Eberson contained only 100 units of agglutinins per cubic centimeter, and the amount of agglutinins present in the spinal fluid was, therefore, too small to be detected. Flexner, Clark and Amoss (15) found that "it is unusual for the neutralizing principles to be contained in the cerebrospinal fluid during convalescence from epidemic poliomyelitis," although neutralizing principles are present in the blood. It is, however, not probable that the serums of the convalescents contained the neutralizing principles or antibodies in quantities that could be demonstrated in three-hundred-fold dilution.

Although, as my experiments show, antibodies penetrate from the blood into the spinal fluid of rabbits even without inflammation, the fact remains that the antibody content of the spinal fluid is very small. The results of numerous clinical and experimental observations have shown the effectiveness of sterile inflammation and of injecting immune serum into the spinal fluid in raising the antibody content of the spinal fluid (Flexner). In experiments to be reported later this finding has been reobtained in rabbits.

Since antibodies are found in the globulin fraction of the serum, behave in many respects like globulins and may be expected to follow the distribution of globulins, it is interesting to compare the ratio of the antibody titers of the serum and the cerebrospinal fluid on

the one hand with the ratio of the globulin of the serum and that of the cerebrospinal fluid on the other hand. Mestezrat (16) stated that the globulin content of the cerebrospinal fluid is about 0.019 gm. per 100 cc., and it is said that 100 cc. of serum contains on an average 2.5 gm. of globulin. These data were obtained from the serum and cerebrospinal fluid of man, but it is possible that the globulin content of the serum and cerebrospinal fluid in the rabbit is at least of similar magnitude if not almost equal to that found in human beings. The ratio of globulin content of the serum to that of the cerebrospinal fluid,

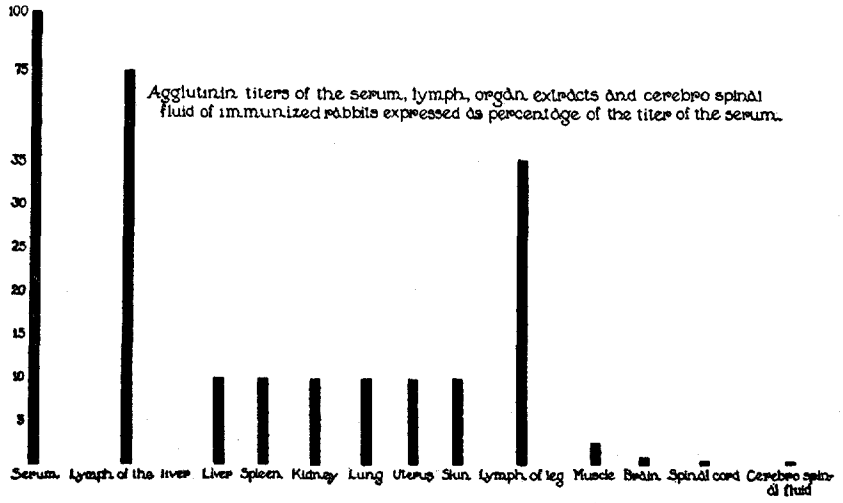

CHART 1

100:0.76, nearly equals that of the respective antibody titers, 100: 0.30. Considering the accuracy of the technic of agglutination tests a closer agreement can hardly be expected. Therefore it can be said there is some parallelism between the antibody titers of the serum and cerebrospinal fluid on the one hand and the globulin content of these fluids on the other hand.

These observations have an obvious bearing on the serum therapy of the central nervous system. They show that antibodies do accumulate in the tissue of the brain, spinal cord and cerebrospinal fluid even if the immune serum is not injected into the central nervous sys-

tem; furthermore, that the antibody content of the central nervous system can be estimated by titration of the blood.

It is important to ascertain whether antibodies accumulate in the tissues of the central nervous system at a faster rate when the immune serum is injected into the spinal fluid instead of the peripheral blood stream. This question is being investigated and will be the subject of a subsequent publication.*

In the previous studies it was found that the agglutinin titers of the extracts of the spleen, liver, lung, kidney, uterus and skin are about the same and on the average ten times lower than that of the serum (extract of 1 gm. of organ compared with dilution of 1 cc. of serum). The titer of extracts of the muscles of the leg is lower than those of the other organs examined, varying from 1 to 5 per cent expressed as percentage of the titer of the serum. In contrast to these organs the brain and cord yield extracts that contain antibodies in very low titer, less than 1 per cent of the titer of the serum. (See Chart 1.)

CONCLUSIONS

1. Antibodies can be extracted from the brain and spinal cord of rabbits actively or passively immunized with typhoid bacilli.

2. The titers of the antibodies in the extracts of brain and cord depend upon the titer of the blood serum. In actively immunized rabbits the following numerical relationships exist between the titers of the serum and of these organ extracts: The ratio of the titer of the serum is to the titers of extract of brain and of the spinal cord about as 100 is to 0.8; the titer of the serum is to the titer of the cerebrospinal fluid as 100 is to 0.3. In passively immunized rabbits the titer of the serum is to the titer of brain and spinal-cord extract as 100 is to 0.7.

3. The antibodies recovered from the brain are not due to the presence of blood in it for perfusion of the brain does not reduce its antibody content appreciably.

4. Antibodies penetrate into the spinal fluid from the blood even in the absence of inflammation of the meninges. When the penetration is completed the following numerical relationship exists between the titer of the serum and that of the cerebrospinal fluid: 100 to 0.25.

* More general discussion of the literature will be published in a subsequent paper.

5. The penetration into the cerebrospinal fluid of antibodies injected intravenously proceeds at a slow rate, being completed only several hours after the immune serum has been injected. The penetration of antibodies into the tissue of the brain occurs at a very rapid rate. It is completed within 15 minutes.

6. It is very unlikely that when the immune serum is injected intravenously the antibodies reach the brain tissue by way of the cerebrospinal fluid, for (1) the antibody titer of the cerebrospinal fluid is lower than that of the brain extract, and (2) antibodies penetrate faster into the tissue of the brain than into the cerebrospinal fluid.

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