ON THE ABSORPTION OF ANTIBODIES FROM THE SUBCUTANEOUS TISSUES AND PERITONEAL CAVITY.

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(*Five Figures.*)

Little attention has hitherto been paid to the rate with which slowly diffusing substances, such as the antibodies in general, are taken into the circulation from the peritoneal cavity or the subcutaneous tissues. The question, however, is of immediate clinical as well as scientific importance. For, if any considerable time elapse after subcutaneous injection before such a body is fully absorbed into the blood, then in urgent cases of disease we lose valuable time by injecting hypodermically the appropriate antisera. The following experiments were undertaken for the purpose of determining the rate at which such absorption occurs. They show that it is exceedingly slow. After intraperitoneal injection the antibody in the blood does not reach its maximum until 25—30 hours later, and after subcutaneous injection the interval is from 2 to 3 days.

The experiments fall into three groups according as the injections were made intravenously, intraperitoneally or subcutaneously. The antibodies used were: 1. coli-agglutinin, derived from goats immunised to the *B. coli*; 2. antitetanolysin, derived from goats immunised to the haemolytic principle in filtered tetanus-cultures; 3. diphtheria antitoxin from horse. The animals used were rabbits, goats and man. Most of the work was done by injecting rabbits with the agglutinin. The results obtained after intravenous and intraperitoneal injections with agglutinin were confirmed by injecting rabbits with the antilysin, and the subcutaneous results were confirmed by injecting rabbits with antilysin, goats with agglutinin, and man with diphtheria antitoxin.

The general method employed was the same in all cases. At varying intervals of time after the injection blood was withdrawn from a vein. The serum was allowed to separate and removed from the clot 18—24 hours after the venesection. (Asepsis as strict as possible was in all cases observed.) The amount of antibody in the different samples of serum was then determined.

In the agglutinin experiments this was done by the method of Madsen and Jörgensen (1902) in the following way. Each sample of serum, as it was obtained, was stored in the cold chamber (2°—6° C.) until the last sample was ready. Then all were examined at the same time, along with some of the agglutinating serum used for the injection. For each sample of serum a series of test-tubes was prepared. Into the first tube of each series was carefully measured a comparatively large dose of the appropriate sample of serum; into the next tube a smaller dose, into the third a still smaller—and so on to the end of the series, the dose in each tube being about $\frac{4}{5}$ths of the dose in the tube preceding it in the series. A similar series of tubes was made of the agglutinin injected. The volume of fluid in all the tubes was then equalised by the addition of 0·9 % NaCl solution, and control tubes were prepared. To every tube was then added 1·5 c.c. of a 12 hours' bouillon-culture of *B. coli*, in which further growth was checked by the addition of a little formalin (0·05 % pure formaldehyde). All the tubes were then rapidly shaken, and placed in an Ostwald water bath at 37° C. After 2 hours the tubes were removed. In each series a scale of agglutination was found, varying from complete or nearly complete precipitation in the tubes with large amounts of serum to hardly perceptible clumping of the bacilli in the lowest tubes. In the series containing the agglutinin injected, a tube was selected which showed a moderate degree of agglutination; and then in each of the other series was found the tube which showed the same degree of agglutination. By this means was determined for each sample of serum the dose which produced the same amount of agglutination, *i.e.* which contained the same amount of agglutinin. Taking this amount of agglutinin as unity, the number of units contained in 1 c.c. can be readily calculated for each sample of serum examined.

With a little practice this method gives excellent results (cf. Dreyer and Jex-Blake, 1906). A similar method was employed for the estimation of the amount of antilysin—the sera being measured against a definite dose of tetanolysin acting upon the washed red blood-corpuscles of the horse. The antitoxin holding sera were examined in the manner explained below.

I. *Intravenous Injections.* The results obtained here confirm the work of other investigators, Madsen and Jörgensen (1902), Knorr (1900), Behring (1900), etc. The antibody introduced into the circulation at one point is rapidly distributed through the blood as a whole. It at once begins to disappear, the fall in the amount in the blood occurring with great rapidity at first but with increasing slowness as time goes on, until eventually it can no longer be detected. This is shown in

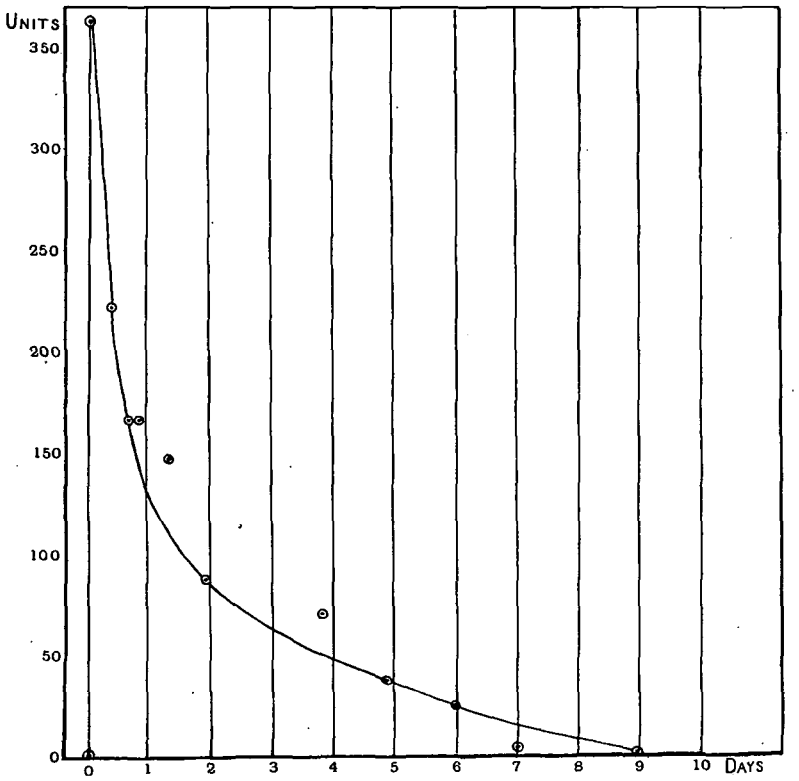

Fig. 1. Curve expressing the amount of antibody in the blood after Intravenous Injection. See this page, and Table I.

Figure 1. A control sample of blood having been first withdrawn, 15 c.c. of agglutinin were injected into the ear-vein of a fresh rabbit of 2600 grammes weight. After 15 minutes, and at each of the subsequent times stated, a small sample of blood was withdrawn. The results of the final examination are given in the following Table I. 1 c.c. of the serum before the injection produced no agglutination.

TABLE I.

The standard degree of agglutination was produced by

0·00275	c.c.	of serum:	15 minutes after injection.
0·0045	,,	,,	9½ hours ,, ,,
0·006	,,	,,	16½ ,, ,, ,,
0·006	,,	,,	21 ,, ,, ,,
0·0068	,,	,,	1 day 8 hours after injection.
0·0115	,,	,,	1 ,, 22 ,, ,, ,,
0·014	,,	,,	3 days 20 ,, ,, ,,
0·026	,,	,,	5 days after injection.
0·0375	,,	,,	6 ,, ,, ,,
0·4	,,	,,	7 ,, ,, ,,

After 9 days, 1 c.c. serum produced only the slightest trace of agglutination. The inverse values of the doses found are used in plotting the curve in Figure 1.

Similar curves were obtained after all the intravenous injections, whether with agglutinin or antilysin. The points, with which we are concerned here, are firstly that the maximum amount of antibody is obtained in the blood at once; and, secondly, that the maximum is a high one.

II. *Intraperitoneal Injections.* The form of the curve obtained after intraperitoneal injection is different from the intravenous curve; see Figure 2. There is first a rise, corresponding to the excess of the amount absorbed from the site of injection over the amount disappearing from the blood after absorption. The rise continues for about 30 hours, and then is followed by a fall similar to that seen in the intravenous curves. The shortest time required to reach the maximum in any of the experiments was 26 hours, the longest 42 hours.

Further, the maximum reached is low. The experiments recorded in Figures 2 and 3 were all performed at the same time on rabbits of equal weights (3000, 2950, and 2900 grammes) injected with equal amounts of agglutinin. The examination of the sera was also made at the one time. The unit is the same in all, and the maxima obtained in the three cases are comparable with one another. After the intravenous injection the maximum obtained was 540 units; after intraperitoneal injection the maximum was 250, or less than one-half after subcutaneous injection the maximum was 143, or less than one-third; see Tables II. and III.

Similar results were obtained with the antilysin experiments.

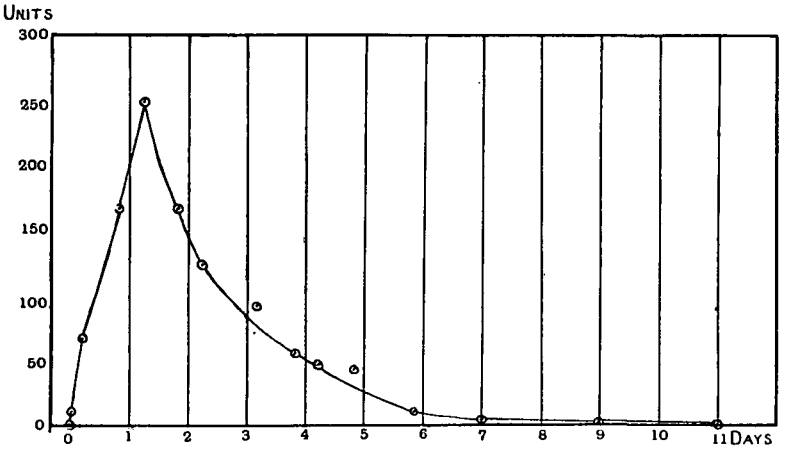

Fig. 2. Curve after Intraperitoneal Injection. See p. 208 and Table II.

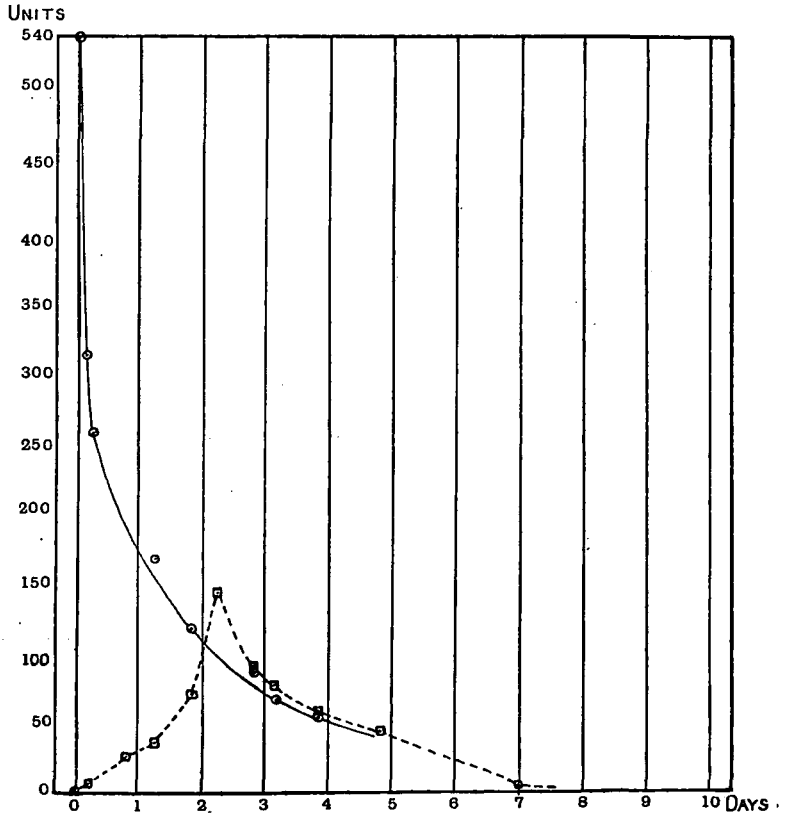

Fig. 3. The curve of Subcutaneous Injection is represented by the broken line; the curve of Intravenous Injection by the whole line. See p. 210 and Table III.

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TABLE II.

Fresh rabbit, 2950 grammes, injected intraperitoneally 15 c.c. agglutinin. Before injection, 0·7 c.c. serum gave no agglutination. The standard degree of agglutination was produced by

0·08	c.c.	serum:	5 minutes after injection.
0·015	,,	,,	5¼ hours ,, ,,
0·006	,,	,,	20 ,, ,, ,,
0·004	,,	,,	30 ,, ,, ,,
0·006	,,	,,	1 day 20 hours after injection.
0·008	,,	,,	2 days 6 ,, ,, ,,
0·011	,,	,,	3 ,, 4 ,, ,, ,,
0·018	,,	,,	3 ,, 20 ,, ,, ,,
0·021	,,	,,	4 ,, 6 ,, ,, ,,
0·0225	,,	,,	4 ,, 20 ,, ,, ,,
0·085	,,	,,	5 ,, 20 ,, ,, ,,
0·225	,,	,,	7 days after injection.
0·5	,,	,,	9 ,, ,, ,,

TABLE III.

Fresh rabbit, 2900 grammes, injected subcutaneously dorsum 15 c.c. agglutinin (at the same time as the experiment of Table II.). Before injection, 0·7 c.c. serum gave the required degree of agglutination. The standard degree of agglutination was produced by

0·45	c.c.	serum:	5 minutes after injection.
0·4	,,	,,	3 hours ,, ,,
0·3	,,	,,	5 hours 10 minutes after injection.
0·037	,,	,,	20 ,, after injection.
0·0275	,,	,,	1 day 5 hours after injection.
0·014	,,	,,	1 ,, 20 ,, ,, ,,
0·007	,,	,,	2 days 5 ,, ,, ,,
0·0115	,,	,,	2 ,, 19 ,, ,, ,,
0·013	,,	,,	3 ,, 3 ,, ,, ,,
0·017	,,	,,	3 ,, 19 ,, ,, ,,
0·0225	,,	,,	4 ,, 19 ,, ,, ,,
0·5	,,	,,	7 days after injection.

III. *Subcutaneous Injections.* The injections in the lower animals were made into the loose tissue under the skin of the back, a little to one side of the middle line. The curve of absorption is of a similar form to that obtained with intraperitoneal injections, but the delay in reaching the maximum is still more pronounced. In Figure 3 are shown two curves plotted together to illustrate the difference between subcutaneous and intravenous injection. The maximum in the subcutaneous curve is delayed until (in this case) 2 days 5 hours have elapsed. The longest interval observed in any of the lower animals, before the maximum is obtained, was 2 days 20 hours. As a rule,

it is a little over 2 days. The shortest time observed was 1 day 15 hours, in the goat whose curve is given in Figure 4. In this case it is evident from the shape of the curve, that a bleeding was not made at the time when the antibody was at its maximum, and that the true maximal period was somewhat later than that actually observed.

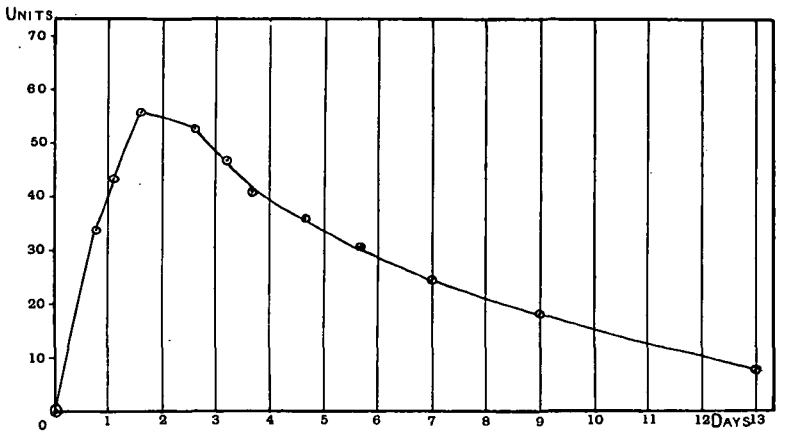

Fig. 4. Curve of Subcutaneous Injection in Goat. See this page, above, and Table IV.

TABLE IV.

Fresh goat of 13 kilos. 80 c.c. agglutinin injected subcutaneously. Before injection, 1 c.c. serum produced no agglutination. Standard agglutination was produced by

0·03 c.c.	serum:	18 hours after injection.
0·023 ,,	,,	26 ,, ,, ,,
0·018 ,,	,,	1 day 15 hours after injection.
0·019 ,,	,,	2 days 15 ,, ,, ,,
0·021 ,,	,,	3 ,, 4 ,, ,, ,,
0·024 ,,	,,	3 ,, 16 ,, ,, ,,
0·028 ,,	,,	4 ,, 16 ,, ,, ,,
0·032 ,,	,,	5 ,, 17 ,, ,, ,,
0·04 ,,	,,	7 days after injection.
0·055 ,,	,,	9 ,, ,, ,,
0·09 ,,	,,	13 ,, ,, ,,
0·115 ,,	,,	15 ,, ,, ,,

Scattered through the literature of immunity are to be found a few incidental observances of this phenomenon of slow absorption. Thus Pfeiffer and Friedberger (1904) noted its occurrence in rabbits with cholera-serum, and Bulloch (x. 1898) in a donkey injected with diphtheria antitoxin. In nearly all of these cases the observers' attention was directed to the end of the curve, and the earlier stages

of the process were not systematically examined. The only instance I have been able to find, where the blood was examined at frequent short intervals after subcutaneous injection, is recorded by Behring (1897). It is the case of a goat injected with diphtheria antitoxin, and the slow rise in the blood is well-marked.

Although these observations are so few and unsatisfactory, they serve to confirm the experiments recorded here and to show that the very slow absorption of antibodies into the blood is the rule in the lower animals. The subcutaneous tissue, however, is so different in the case of man that it was desirable to study the absorption in the human subject. An attempt was made with antitetanolysin, but when the sera were examined it was found that normal human serum is in itself very strongly antilytic to tetanolysin, and no reliance could be placed on the results obtained.

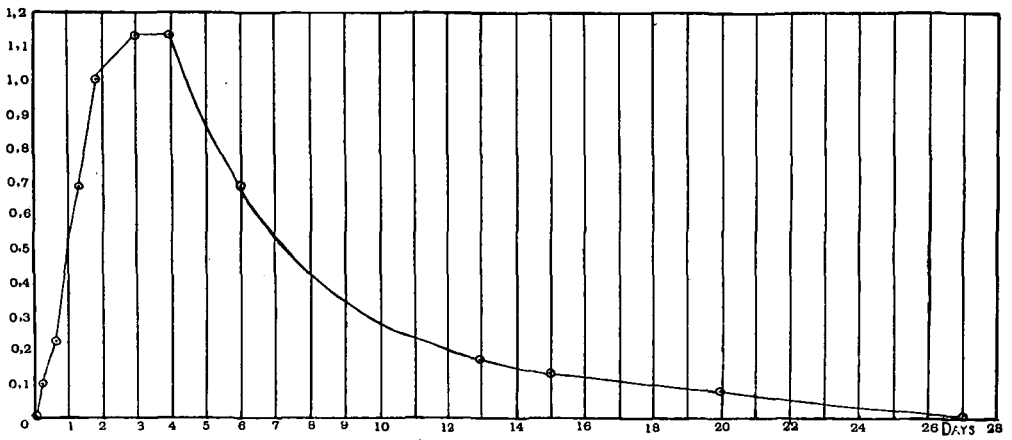

Fig. 5. Curve of Subcutaneous Injection in Man: Diphtheria Antitoxin. See this page, below, and Table V.

An experiment was therefore made with diphtheria antitoxin. 9000 units were injected into the subcutaneous tissue of the abdomen of a normal healthy man, weighing 72 kilos: and at successive intervals thereafter blood was withdrawn from the veins of the forearm. In 18—24 hours the serum was taken off the clot, and shortly afterwards injected together with toxin into guinea-pigs. The animals used nearly all weighed 250 grammes: in no case were they less than 240 or more than 270 grammes at the time of injection. The toxin used was the Test Toxin of the Statens Serum-Institut at Copenhagen, and was standardised in terms of the Ehrlich antitoxin unit. Of the toxin, 0·02 c.c. was

neutralised by 0·00015 c.c. of the serum injected, and this amount of serum contained 0·0675 units. The quantity of serum obtained from each bleeding, which just sufficed to neutralise 0·02 c.c. of the toxin, was determined. The amount of serum containing 0·0675 units was thus ascertained for each bleeding, and from this the number of units per 1 c.c. of serum was obtained. The results are shown in Table V.

TABLE V.

1 c.c. of the patient's serum contained :

Before injection :			no demonstrable antitoxin.	
5 hours	after	injection :	0·1 units	antitoxin.
14 ,,	,,	,,	0·225 ,,	,,
32 ,,	,,	,,	0·68 ,,	,,
44 ,,	,,	,,	1·0 ,,	,,
3 days	,,	,,	1·13 ,,	,,
4 ,,	,,	,,	1·13 ,,	,,
6 ,,	,,	,,	0·68 ,,	,,
13 ,,	,,	,,	0·17 ,,	,,
15 ,,	,,	,,	0·14 ,,	,,
20 ,,	,,	,,	0·08 ,,	,,
27 ,,	,,	,,	no demonstrable antitoxin.	

These figures are plotted in Figure 5. The curve shows that the maximum is not reached till the end of the third day, *i.e.* 72 hours after the injection. During that period the amount of antitoxin in the blood increases steadily. It is still at the same level 24 hours later, and may have risen and fallen again in the interval. Three weeks after the injection antitoxin is still demonstrable in the blood, but it has disappeared after 4 weeks. Cf. Behring (1897), Bulloch (x. 1898), Bomstein (1897), Müller (1897).

The maximum content reached was only 1·13 units per 1 c.c. On the view that the amount of serum in man is $\frac{1}{43}$rd of the body weight (Haldane, 1900), the patient in this case contained approximately 1674 c.c. of serum. The maximum amount, then, that the serum would have contained, if all the antitoxin injected had been present in the blood at one time, is $9000 \div 1674$, or about $5\frac{1}{3}$ units per 1 c.c. The amount actually obtained was less than one-fourth of this total.

The clinical bearing of these results may be briefly pointed out. It is generally admitted that in advanced cases of diphtheria, or other diseases where antisera are commonly employed, it is desirable to introduce into the system as quickly as possible as much as possible of the appropriate antiserum. Any, even the shortest, delay is to

be avoided. If we inject hypodermically we are losing time. Two or three days elapse before the injection is fully absorbed, and even then the amount actually circulating at one time is very considerably less than we may obtain by other means. It seems clearly indicated that in all urgent cases intravenous injection should be performed. It would be well to follow this up by a subcutaneous injection also, in order that the antitoxin slowly absorbed from the tissues may counteract the rapid loss after the intravenous injection. The amount in the circulation could thus be maintained at a higher level for a longer time.

The results of the subcutaneous experiments have an interest in a different connection. Ransom (1901) has shown that antitoxin injected into the subcutaneous tissues is removed by the lymphatics and not by the blood-vessels. Now, it is commonly thought that the lymph-vessels open into the connective-tissue spaces by direct communication. If this be so, it is difficult to account for the extreme slowness with which the absorption occurs. This difficulty disappears, if we suppose with Ranvier that the lymph-system is closed as the blood-vascular system is closed, and that transfusion of fluids takes place through the walls of this system. Rapidly diffusing substances, such as strychnine, will pass readily through these walls, and be absorbed with great rapidity. On the other hand, slowly diffusing bodies, such as the antibodies, will diffuse with difficulty through such membranes, and considerable time will be required for their complete absorption into the general circulation. These experiments thus lend support to the view that the lymphatic system is a closed one.

Summary.

1. Antibodies in general are absorbed very slowly from the peritoneal cavity in lower animals, and from the subcutaneous tissues in man and animals. Absorption from the latter is not complete until at least 2—3 days have elapsed.

2. The amount of antibody present at any one time in the general circulation after intraperitoneal or subcutaneous injection is very much less than the amount injected.

3. Clinically, in urgent cases of disease, to inject antibodies subcutaneously is not only to lose 2—3 days' time before the full action can be obtained but to reduce the amount of action that the dose injected can have.

4. By intravenous injection the maximum amount of action is obtained at once.

The whole of the experiments recorded in this paper were made in the Statens Serum-Institut at Copenhagen, where every possible facility was given me for the carrying out of this research. To Dr Thorvald Madsen I desire to express my thanks not only for his unvarying kindliness and courtesy, but also for suggestions and assistance constantly given. I am pleased to have this opportunity of expressing my indebtedness to him.

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