SERUM SICKNESS IN RABBITS

I. MANIFESTATIONS OF SERUM SICKNESS

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PLATE 54

(Received for publication, June 19, 1931)

The occurrence in man of reactions subsequent to a first injection of certain foreign proteins has long been recognized and, following the general use of antisera of various types, these reactions were thoroughly studied by von Pirquet and Schick (1) who gave the name serum sickness or serum disease to this phenomenon. It was possible to study it quite extensively as it occurs in man. However, the occurrence or production of an analogous condition in laboratory animals has not to our knowledge been reported and it has, therefore, been impossible to study this condition experimentally.¹

It appeared improbable that man should be the only species in which serum sickness might occur and our effort to produce this disease was based upon the assumption that some laboratory animals should be susceptible under certain conditions. The literature concerning serum sickness in man shows quite definitely that the occurrence rate of this disease is greater in proportion to the amount of antiserum injected. It seemed, therefore, that experimental work would have to be carried out with injections of relatively large doses of serum. The animals used in these experiments, rabbits, were chosen because in some respects at least they seem to stand closer to man in reactions of hypersensitiveness than do guinea pigs.

¹ Bécère, Chambon and Ménard (2) noted in cattle the appearance of varied forms of eruptions, rise of body temperature and evidences of disability associated with locomotion appearing approximately 4 days after the injection of large quantities of horse serum.
It was, of course, impossible to assume in advance what phenomena might be expected as evidences of serum sickness in the rabbit following the administration of a single large dose of horse serum. The animals were, therefore, kept under close observation subsequent to injection. They were observed as a routine at about 9 a.m. and about 4 p.m.; particularly in the earlier experiments several observations were made at night during the fifth, sixth, seventh and eighth 24 hour period. It became evident, however, that the morning and afternoon observations were adequate for noting the important reactions. Fortunately the reaction was quite an obvious one. At about the 5th or 6th day after injection of a single large dose of normal horse serum, it was evident in some cases that the rabbit's ears were distinctly reddened. On closer examination there was an erythema involving a large portion of the ear, especially intense in the basal portion but involving the lower two-thirds. In addition, pressure made evident that this erythema was accompanied by edema which varied considerably in degree but which also involved all or part of the lower two-thirds of the ear.

Our first successful results were obtained with animals which had received 5 cc. pro kilo.

Previously and also later, we injected animals with 3 cc. pro kilo but in no case was a positive result obtained; only a total of eight rabbits received this smaller dose but in view of the per cent occurrence when we used larger doses we should have expected at least one to five of these animals to give evidence of a reaction. Only one animal showed a minimal and limited erythema on the 8th day after injection; this erythema might be considered as suggestive but the reaction was rather of the type which we have not accepted as being actually positive in other groups of animals. We are inclined to believe, therefore, that an injection of 3 cc. pro kilo is too small a dose to produce reactions and that in all probability 5 cc. pro kilo are practically the minimal amounts required to cause the appearance of this phenomenon in rabbits. We also increased the amounts injected, using 7.5 cc., 8 cc. or 10 cc. pro kilo with essentially the same results as when the 5 cc. doses were injected; the comparative results using the various quantities are analyzed below.

The serum in our first experiments was injected into the marginal vein of the ear. Since the reactions were evident on the ears the question naturally arose as to whether they were not dependent upon extravasation of serum into the tissues of the ear or possibly to
trauma associated with the manipulation of the ear. It appeared that this was not the case in view of the fact that the reactions occurred both in the ear used in the injection and in the other ear. However, we used other routes for introduction of the serum; at first, because the reactions had followed intravenous injection, we injected a number of animals either intracardially or intrajugularly (this latter method necessitating operative procedure), and it became evident in view of the positive results in these latter groups that the site of intravenous injection had no influence on the result. Rabbits were also injected intramuscularly and a few subcutaneously and in these also the reactions appeared in the ears. In the majority of later experiments the serum was injected into the subcapular tissues since this route of administration was essentially one of the easiest and since the results compared favorably with any of the other modes of administration which were tried. The serum was in each animal injected half under the right scapula and half under the left one.

In the majority of our experiments we used white rabbits since they proved to be more satisfactory than colored ones. The reactions were more evident on their ears than on the ears of rabbits having darker hair. However, marked reactions were observed in brown and even black haired rabbits and there was no difficulty in recognizing the reactions in these animals. Our impression however was that the occurrence of the reaction was somewhat less frequent in the colored animals, but the number of these does not permit a final statement. It is possible also that certain conditions associated with the experiments carried out with these colored animals were responsible for the smaller percentage of positive reactions.

It may be noted that the occurrence of hemorrhages, localized erythema and localized edema is more frequently seen in ears of normal white rabbits than in the ears of normal colored animals.

As previously stated the reactions were quite evident but they must and can readily be differentiated from the reactions occurring in the normal rabbit. The anatomical distribution, apart from the intensity

\footnote{This route of administration was suggested by Dr. John Auer on the assumption that the massaging due to the constant movement of the forelegs would assist in more rapid absorption such as might take place if the serum were injected intramuscularly.}
and extent of the reactions, was a cardinal point in this connection; it therefore becomes necessary to consider various areas of the rabbit's ear.

It is preferable to examine the ear by daylight and by both diffuse reflected light and transmitted light (preferably therefore in a well lighted room with the observer facing the window). Artificial light can be used but when viewing the ear by transmitted light, the source must not be too bright; indirect reflection of the artificial light is preferable. Direct sunlight is not satisfactory since the intense light gives a pink color to the normal ear due to the light passing through the vascular bed and the cartilage. It is of advantage to examine both the posterior and anterior aspect of the ear.

Roughly one may divide the ear into two vertical divisions lying external and internal to the central vessels and horizontally into thirds which are spoken of as lower, middle and upper; occasionally the division may be made on the basis of quarters.

One area of the ear must be especially noted; this is an area which for the sake of a descriptive term will be referred to as the triangle. Actually this area may include nearly all of the middle third and possibly a portion of the upper third of the external half of the ear; it is referred to as a triangle however because reactions here tend to have a somewhat triangular form with the base toward the marginal vein and the apex pointing downward and inward towards the central vessels. This area usually appears more vascularized than other portions of the ear.

Attention may also be called to the frequent presence of a cartilaginous fold well towards the base of the ear and several millimeters (3 to 7) inside the external margin of the ear.

We have observed the ears of 147 normal rabbits for periods varying from 2 to 7 weeks in order to determine what reactions occur under normal conditions. One observation a day was made upon these animals. The normal animals were kept under various conditions; from six to twelve together in runways or one to three rabbits in smaller cages. The reactions in normal rabbits do not depend upon factors influenced by manner of caging: all types of reactions were observed regardless of the manner of housing the animals.

Since certain reactions did appear in normal animals the possibility of food influencing the occurrence of these was studied. Normal animals were fed on oats, hay, (alfalfa or prairie alternating) and vegetables (carrots and cabbage); or this same diet with the addition of a commercial rabbit "chow;" or third, on oats, "chow" and vegetables. Regardless of the diet the reactions were essentially the same.

Hemorrhages, fairly extensive or petechial in character, are frequently, in fact
almost constantly, noted on the ears of normal rabbits. They may appear in
almost any portion of the ears. Frequently they are associated with trauma such
as scratches either on the posterior or more frequently on the anterior surface of
the ear. At times the hemorrhagic areas suggest definitely by their arrangement
that they are due to scratches but it may be impossible to observe any abrasions
associated with these. It may be noted that hemorrhages of varying degree
appear both in rabbits which were caged with others and in those which were
alone in a cage. Hemorrhages in the ear may at times result in thickening of
the ear at such areas. Hemorrhages are to be considered as normal appearances
and not in any way characteristic of the reactions associated with serum sickness
in rabbits.

Definite vascular changes of various types may be noted. A dilatation of the
larger vessels occurs more or less frequently; somewhat less frequent in occurrence
is a widespread dilatation of the entire capillary (or arteriolar and venular) bed of
the ear. Particularly this latter type of appearance may rise and subside as the
ears are watched for a minute or more. These reactions are to be considered
essentially as of normal occurrence. However, it has frequently been noted that
in injected animals from 6 to 24 hours before the appearance of the typical reac-
tions the alternation of dilatation and contraction of the small vessels may be
seen; this is not a constant occurrence however. It must be recognized that
while these reactions of vascular instability may give to the ears the appearance
of marked redness, this appearance comes and goes as one observes the ears, and
has no permanency.

Often about the larger vessels and less frequently about the smaller ones a
slight redness extending for 1 or 2 mm. on either side may be noted, giving the
vessels a fuzzy appearance. This is not due to hemorrhage since it disappears on
pressure. This reaction like those discussed above is to be considered as one
occurring in animals which had not received horse serum.

Areas of duskeness may often be noted on the ears. A limited area may appear
darker than the remaining ear surface; most frequently this occurs in the outer
median area (triangle) of the ear. Frequently it appears associated with the
fading of scattered petechial hemorrhages in this portion, and may then have a
brownish dusky appearance. These reactions again are essentially different
from those appearing in the ear after injection of serum.

Limited and scattered areas of erythema appear from time to time in a very
large per cent of the normal rabbits examined. These areas are usually discrete
and relatively small and may occur anywhere in the upper two-thirds of the ear,
again more often in the limited portion within the middle outer third (triangle) of
the ear. Frequently also such areas are seen well towards the median margin
in the upper two-thirds of the ear. Occasionally such areas are noted in the lower
portion but it must be kept in mind that in the normal animal they involve only
limited areas and do not simulate the widespread erythema seen in injected
animals. Most frequently one small erythematous area from 0.5 to 1 cm. in
diameter may be seen; less frequently but by no means exceptionally two or three
and occasionally more such areas may be noted but they are usually quite widely
separated. In only three of the 147 normal rabbits was a widespread distribution
of the erythema noted; one of these cases was associated with petechial hemorrhages
involving a large part of the ear; in the other two the erythema involved chiefly
the two portions mentioned above—the outer middle third and the inner upper
two-thirds of the ear. It is essential in examining the ears that the presence of
scratches associated with the erythema be carefully searched for since frequently
these are the presumptive causal factors underlying the limited erythematous
areas.

Edema may at times occur associated with the erythematous or hemorrhagic
areas. Actually this has been noted at one time or other in 35 of the 147 normal
animals, that is in approximately 23 per cent of animals. It must be clear from
what has been said regarding the location of the erythematous areas that edema
since it usually occurs with erythema is noted chiefly in the upper two-thirds of
the ear, or in the so called triangle or along the inner margin. Occasionally edema

<table>
<thead>
<tr>
<th>Edema</th>
<th>?</th>
<th>±</th>
<th>+</th>
<th>++</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rabbits</td>
<td>2</td>
<td>16</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

is also noted along the line of the median vessels. In only three normal animals
has edema been noted in the lower third of the ear. The presence of the edema
is usually determined by pressing the ear between the fingers and then palpating
for the ridge which should be present. The degree of edema varies widely; at
times the ridge about the pitted area is just palpable, or at the other extreme the
pitting resulting from the pressure may be visible to the eye. We have attempted
to express the degree of edema by a scale running from very slight to marked,
using as symbols; ?; ±; + or ++. The occurrence of various degrees of edema as
noted in the ears of normal rabbits appears in Table I. It is doubtful, however,
whether the degree of edema is of any importance in differentiating between the
reactions in normal and in injected animals. It is rather the location and the
associated reaction which set apart the reaction in the injected animals.

After the above description of the reactions which may be noted in
normal rabbits it is possible rather briefly to point out the character-
istics of the reactions occurring in injected animals. Only two of the
above mentioned phenomena are to be considered as characterizing
the reaction of serum sickness in the rabbit; these are erythema and
edema. The general picture of the reactions in the injected animal is
sharply different from that seen in the normal animal, and a few observations of the positive reactions actually serve best to fix their distinctive appearance.

In all 136 injected animals are included in the observations here discussed; 103 of these were injected with normal sera from various horses, while 33 were injected with various antisera.

While the erythematous reactions in normal animals are discrete and limited in the areas involved, in the injected animal the greater part of the ear is reddened and is permanently red (that is we are not dealing with a transient vascular or capillary flushing). There is more or less of a pattern in the involvement; the central upper portion of the ear is more likely to be uninvolved leaving a wedge-shaped area relatively white and pale; at times the point of the wedge extends downward along either or both sides of the central vessels, reaching occasionally to the lower third of the ear. The outer upper quarter

| TABLE II |
| Degree of Edema in Ears of Rabbits Subsequent to Injection of Serum |
| Edema | ? | ± | + | ++ |
| No. of rabbits | 7 | 11 | 29 | 17 |

may also be unaffected. The character of the erythema is different from that seen in normal animals. It is diffuse, not discrete, often fairly uniform in intensity or at times darker patches appear against the lighter flushed background. Often the erythematous ears appear distinctly mottled. The intensity varies and one may see reactions varying from a definite pink to an intense red. At times the erythema is morbilliform or at others it may be scarlatinal. Usually both ears of the rabbits are affected but occasionally only one ear shows the erythema; it is, however, exceptional that erythema occurs in only one ear.

Erythema is more frequently noted than is the edema, and it may occur without the presence of edema. The edema may affect only one ear although the involvement of both ears is frequent. The edema associated with the reaction in rabbits varies in degree much as does the edema in normal animals. In Table II we have shown the number
of rabbits showing varying degrees of edema subsequent to injection of serum and have noted in each animal only the most marked degree of edema. It can be seen that there is a slight tendency for more animals to show marked reactions in the injected group than in the normal animals. However, the intensity of the reaction is in no way to be considered as differentiating the typical reactions in injected animals. The location and extent of the edema are the characterizing qualities. Edema occurs in serum sickness in rabbits in the lower third of the ear which portion as noted above is rarely affected in the normal animal; it may appear either lateral or medial to the central vessel or on both sides, or occasionally it affects the small area outside the cartilaginous thickening mentioned above. The edema often involves not only the lower portion of the ear but extends upwards and affects the entire lower half or two-thirds of the ear; in such cases there is a tendency for the edema to be more marked in the basal portion and to become less intense in the upper portion. Almost without exception the upper third or quarter has been found free of edema. Both ears may be affected or only one and it is not infrequent to find only a limited basal area on one ear edematous. The edema is then far less constant in occurrence and less widespread in distribution than the characteristic erythema. In a number of animals the edema may appear and disappear only to reappear on a subsequent day. More frequently however the edema after once diminishing does not reappear or increase in intensity. Usually the edema is associated with the erythema, that is, they are both present; as stated above however erythema may appear without edema, and in a few cases (three) edema persisted even though the general erythema had disappeared. In one animal only did basal edema appear without previous or subsequent generalized erythema.

It is evident then that the reactions subsequent to serum injections were strikingly different from those seen in normal animals and these latter cannot be mistaken for the typical reactions. It is probable that mild reactions not as definitely characteristic as those just described above occur in injected rabbits, manifested usually by irregular and relatively limited erythematous reactions on the ears, since frequently about the 5th, 6th or 7th day after injection such evidences were noted; we have, however, not listed such doubtful reactions as
positive. In all about thirteen animals in our series showed such
doubtful reactions but in order to remain on a safe basis we have con-
sidered animals presenting such reactions as non-reacting. It seemed
to us preferable to set a distinctive and definite picture to be considered
as the positive reaction.

The number of animals observed is apparently adequate to serve
for statistical presentation and therefore there are given below tabula-
tions of various observations relating to the reactions in the rabbits.
In this analysis only the rabbits injected with normal horse serum
have been considered since it appears advisable for various reasons to
consider separately at some later time the group of animals injected
with the antisera. It is evident from Table III that approximately

<table>
<thead>
<tr>
<th>General erythema and edema, both definite</th>
<th>General erythema, positive; edema questionable</th>
<th>General erythema, positive</th>
<th>Edema, positive</th>
<th>Total considered positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>71 (68.9%)</td>
</tr>
<tr>
<td>General erythema, questionable</td>
<td>Edema, questionable</td>
<td>No reaction</td>
<td></td>
<td>Total considered negative</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>19</td>
<td></td>
<td>32 (31.1%)</td>
</tr>
</tbody>
</table>

70 per cent of the injected animals showed what we have con-
sidered a positive reaction. The percentage occurrence of serum sick-
ness in this series of animals compares relatively well with the
percentage reported as occurring in man following the injection of
large doses of serum.

Since the sex was not noted in ten of the animals used in some earlier
experiments the number of animals in Table IV showing the occurrence
of reactions in the two sexes is smaller than the total used and listed
in Table III. While it appears from Table IV that the reactions show
a lower incidence in females than in males we hesitate to assume that
this would be a correct statement of the fact. We have noted fre-
quently that pregnancy seems to cause a suppression of the reaction
and a number of the female rabbits proved to have been pregnant
during the period of the experiment. We may therefore possibly assume that existence of pregnancy may have been a factor in the lower occurrence of reactions in the females.

The body weight of the animals had apparently no influence upon the occurrence of the reactions as shown in Table V. We may use

### TABLE IV

**Occurrence of Reactions in Ears of Rabbits of Various Sex**

<table>
<thead>
<tr>
<th>Sex</th>
<th>General erythema and edema, both definite</th>
<th>General erythema, positive; edema questionable</th>
<th>General erythema, positive</th>
<th>Edema, positive</th>
<th>Total positive</th>
<th>Total considered negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of males</td>
<td>30</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>No. of females</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>27</td>
<td>20</td>
</tr>
</tbody>
</table>

### TABLE V

**Occurrence of Reactions in Rabbits' Ears in Relation to Body Weight**

<table>
<thead>
<tr>
<th>Body Weight</th>
<th>1600-2000 gm.</th>
<th>2001-2500 gm.</th>
<th>2501-3000 gm.</th>
<th>3001 gm. upwards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>12</td>
<td>36</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>No. positive</td>
<td>6</td>
<td>29</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>No. negative</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

### TABLE VI

**Occurrence of Reaction in Rabbits' Ears in Relation to Route of Injection of Serum**

<table>
<thead>
<tr>
<th>Route of Injection</th>
<th>Ear vein</th>
<th>Intra-cardial</th>
<th>Intra-jugular</th>
<th>Intra-muscular</th>
<th>Subcutaneous</th>
<th>Into sub-occipital region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>33</td>
<td>6</td>
<td>13</td>
<td>4</td>
<td>5</td>
<td>42</td>
</tr>
<tr>
<td>No. positive</td>
<td>25</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>No. negative</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

the weight as a rough measure of age and, therefore, age apparently has no bearing on the occurrence of the reaction. The only group in which the percentage occurrence is distinctly lower is in the smaller or younger animals but it is doubtful whether the numbers used in the group justify a final conclusion.

The influence of the route of introduction of the serum is shown in
Table VI. The figures are presented regardless of the quantities of serum injected. Irrespective of the route of administration of the serum, positive reactions were obtained. It is also evident that the reactions on the ears were not in any way dependent upon extravasation of horse serum into the tissues of the ear during the course of injection, which might have been the case when injections were made into the ear vein. Certainly introduction of the serum by any of the routes used, excepting through the ear vein, should result in no abnormal or special localization of serum in the tissues which later showed the reaction. In the small series of animals given subcutaneous injections the percentage occurrence of positive reactions is relatively low; in addition to the uncertainty introduced by the small number of animals used, it should be noted that all of the non-reacting animals in this series were sick during the course of the experiment. It appears that

<table>
<thead>
<tr>
<th>Serum injected pro kilo</th>
<th>3 cc.</th>
<th>5 cc.</th>
<th>7-8 cc.</th>
<th>10 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>8</td>
<td>31</td>
<td>51</td>
<td>21</td>
</tr>
<tr>
<td>No. positive</td>
<td>0</td>
<td>22</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>No. negative</td>
<td>8</td>
<td>9</td>
<td>15</td>
<td>8</td>
</tr>
</tbody>
</table>

when the animals are not in good health during the experiment the reactions tend either to be questionable or negative. Many of the animals which are noted as showing questionable erythematous reactions were sick, and as already stated such reactions have been listed as negative ones. It does not appear that the introduction of the serum directly into the vascular system tends to increase the occurrence of the reactions, when compared with other routes of introduction.

In Table VII is shown the number of rabbits developing a positive reaction subsequent to the injection of varying quantities of horse serum. As stated previously the quantity injected was based upon the weight of the animal and we used varying amounts pro kilo. The animals injected with 3 cc. pro kilo showed no positive reactions. There is certainly no evidence that injections of increasing amounts of serum tend to increase the occurrence of the reaction; possibly
rather the reverse appears from the figures but whether this would hold in a larger series of animals is questionable. Certainly if a minimal adequate amount (accepting 5 cc. pro kilo as such) is injected the occurrence of reactions is practically maximal.

The first appearance of the reactions was usually manifested by the erythematous reactions, at times however edema appeared alone, or frequently the erythema and edema were noticed simultaneously. Therefore, in Table VIII in which we have shown the day after the single injection of serum on which positive reactions first appeared, in most cases this represents the appearance of erythema, often of erythema and edema combined and only occasionally the appearance of edema alone.

It will be noted from Table VIII that very few reactions occurred before the 5th day and also relatively few after the 6th day. The vast majority of the reactions were first noted on either the 5th or 6th days. These reactions varied markedly in duration, in some cases lasting for as long as 4 or even 5 days. It must be made clear that both erythema and edema did not always last equally long in the same animal. In Table IX the duration of the reaction is given and it is evident here that the two types of reaction were not of equal duration.

### TABLE VIII

**Number of Rabbits Showing Reactions on Various Days after Injection of Serum**

<table>
<thead>
<tr>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rabbits</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>27</td>
<td>27</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### TABLE IX

**Duration of the Reaction on the Rabbits' Ears**

<table>
<thead>
<tr>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema</td>
<td>27</td>
<td>27</td>
<td>12</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Edema</td>
<td>30</td>
<td>25</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Erythema and/or edema</td>
<td>20</td>
<td>32</td>
<td>13</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
During the periods that the reactions were present there might occur variations in the degree of erythema, or of the edema, or one might disappear and the other persist. It can then be stated that the reactions showed at times periods of exacerbation and remission without at any time during their duration showing complete disappearance. We have never noticed what might definitely be considered a recurrence subsequent to disappearance of the reaction. In a few animals (20 in all) suspicious reactions of erythema and edema were noted on the 9th to 16th day after injection of the horse serum. However, these reactions cannot be considered as typical on the basis of the criteria which have been outlined above; the location, extent and general appearance left doubt as to their being typical and for these reasons we have not considered them as recurrent reactions. It may be that the criteria are too strictly drawn, but until considerable additional evidence is available it seems preferable to exclude these reactions noted at or after the 9th day subsequent to the injection, from the classification of reactions due to the injection of the serum.

It was stated above that a series of rabbits were injected with various antisera. In this series of 33 rabbits typical reactions were noted in eleven animals, or in 33 per cent. A larger proportion of these reactions occurred in a group injected with the concentrated antistreptococcus serum in contrast with a group injected with the antidiphtheritic serum. Since, however, in this latter group there were many rabbits with colored ears (brown) and since there is the definite suggestion that such animals do not react as well as do rabbits with white ears, we hesitate to lay stress upon the percentage difference noted when comparing the group injected with normal horse serum with the group injected with the two antisera. We are planning a further study of reactions subsequent to the injection of various antisera.

The important fact is, however, that the reactions occur subsequent to a single injection of various antisera as well as subsequent to the single injection of normal horse serum.

We wish to express our appreciation of the kindness of Sharp and Dohme in supplying us with diphtheria antitoxin as well as normal horse serum and of the kindness of Eli Lilly and Company in supplying us with concentrated antistreptococcus serum and normal horse serum.
DISCUSSION

The reactions described above have repeatedly been referred to as reactions of serum sickness in rabbits. Naturally the justification for assuming that these reactions are analogous to those which characterize serum sickness in man must be made clear. One may set down briefly the following characteristics of the disease in man. (1) Serum sickness appears subsequent to even a first injection of the foreign protein into man. (2) Serum sickness appears after an incubation period of from 4 to 12 days or possibly even a longer period. (3) The cardinal symptoms of serum sickness in man are urticarial reactions, erythematous reactions which vary considerably in intensity and area of involvement. (4) Associated with these cardinal symptoms but occurring less constantly there occur a rise of body temperature, arthralgia associated with swelling of the joints, swelling of lymph nodes, edema involving often the face and usually the looser tissue and certain other less obvious reactions. (5) The reactions may show variations from day to day, even to a disappearance and subsequent reappearance. (6) After the reactions have disappeared the individual is apparently restored to a normal state. (7) Serum sickness may appear in the immediate or accelerated form (within 8 hours or within 24 to 48 hours respectively) when the protein is injected for a second time into man, provided there has been a lapse of time between the first and second injections.

In the experiments reported here the reaction of the rabbits was subsequent to a first injection of a single large dose of horse serum. In a number of experiments serum was taken from the rabbits before the injections of horse serum were given and tested for the presence of precipitins; in no animals were precipitins demonstrable. It seems reasonably certain that we were in the case of the reacting rabbits not dealing with reactions of preexistent hypersensitiveness but with a reaction essentially analogous to that appearing in man after a first injection of serum.

The symptoms of serum sickness in man appear after an incubation period of several days. According to Longcope (3) the majority of the reactions occur on the 6th to 10th day after injection. Coca (4), states that 24 to 48 per cent of reactions in man appear less than 8
days after injection and only 14 per cent later than 12 days after. Sturtevant (5) in his study of 500 patients treated with diphtheria antitoxin noted serum sickness as occurring as early as the 1st day after treatment; the greater number of cases appeared from the 5th to the 9th day. Weaver (6) in a large series of patients given diphtheria antitoxin also reported reactions in the first 5 days but the majority of reactions appeared on the 6th to 10th days. It is evident that in the rabbits injected with horse serum a very large number showed reactions on the 5th and 6th days and only a few on either the 3rd, 4th, 7th or 8th days. The interval between injection and appearance of the reaction is therefore not exactly alike in man and rabbit, but there is a marked similarity in the incubation period in the two species.

The reactions appearing on the rabbits' ears subsequent to the injection of horse serum are characterized by erythema and edema. It is true that in man urticarial rashes are probably the most constant phenomena. We have never noticed in rabbits any reactions which might be considered as responses to the itching associated with urticaria; that is, excessive scratching of any parts of the body. However, the erythema on the rabbits' ears was as has been stated previously often of a morbilliform or scarlatiniform type and certainly may be considered as similar to the erythema noted in man. Whether the edema noted in the rabbits' ears should be considered as analogous to the urticarial edema in man or to the edema seen so often about the face is not determinable, but it might in view of the frequency with which it occurs seem more like the former. It is true that in rabbits the edematous areas were not sharply outlined and were not pale and therefore differ distinctly from urticarial wheals. It is probable, however, that the edema of the rabbits' ears may be considered as similar to one or another of the reactions occurring in man.

Symptoms suggesting arthralgia, or evidence of swollen joints, were not noted in any of the animals, although careful observations were made in order to note any such reactions. It was not possible to be assured that in any of the injected or reacting rabbits the lymph nodes were enlarged; the difficulty of palpating the axillary or inguinal lymph nodes made it impossible to reach a conclusion in this regard. However, in one rabbit which before injection showed a chain of enlarged lymph nodes running from the axilla to the groin, subsequent
to injection and at a time when the reaction appeared on the ears these lymph nodes were distinctly enlarged, were softer than they had been previous to the injection, giving distinctly the feeling that inflammatory reactions were occurring about them. After the disappearance of the reactions on the ears the nodes again seemed to diminish in size and become hard as they had been previously. This isolated observation does not justify the conclusion that the lymph nodes are affected in rabbits as in man following the injection of serum. Possibly however it may seem to raise the question as to whether the enlargement of lymph nodes associated with serum sickness in man occurs in normal nodes or in abnormal ones; we should keep in mind the fact that serum sickness is usually produced in individuals suffering from an infection or an intoxication.

The temperature reactions of the injected rabbits have been studied and will be reported in a later communication.

It is apparent from the discussion above that the reactions occurring in rabbits are at least in part very similar to the cardinal reactions in man. The variations from day to day of the extent and intensity of the reactions of serum sickness as noted in man are distinctly more marked than the variations noted in rabbits. However, in some of the rabbits, on second observation some 8 to 10 hours after first being noted, the intense erythema would become distinctly less marked, only to return to its original intensity about 24 hours after it was first noted. In a similar fashion, but less frequently, the edema showed variations in degree from one observation to another, waxing and waning to an evident extent. After the reactions in the rabbits' ears had disappeared there was no evident residual injury, just as in man.

In a small number of rabbits which received a second injection of horse serum about 3 to 7 weeks after the first one, reactions in all respects identical to those which we have described above appeared either within 8 hours after injection or within 24 to 72 hours. These reactions are certainly analogous to the immediate and accelerated reactions of serum sickness in man.

In view of the similarities which have been pointed out above between serum sickness in man and the reactions noted on the rabbits' ears it appears certain that these phenomena can be considered as manifestations of serum sickness in rabbits.
CONCLUSIONS

1. The injection of a single large dose of normal horse serum into rabbits results in the appearance 3 to 8 days later of erythematous and edematous reactions on the ears in 68.9 per cent of the animals.
2. The injections may be given by any of several routes and reactions appear when the site of injection is definitely distant from the ears.
3. Injections of various antisera into rabbits cause the appearance of similar reactions.
4. These reactions can be considered as manifestations of serum sickness in rabbits.

REFERENCES

4. Coca, A. H., in Tice, Practice of medicine, 1924, 1, 162.

EXPLANATION OF PLATE 54

Fig. 1, a and b. a, left ear of normal rabbit. b, left ear of injected rabbit showing erythema of serum sickness; scarlatinal in basal third; mottled to morbilliform in two middle quarters.
Fig. 1

(Fleisher and Jones: Serum sickness. I)