

## THE INFLUENCE OF DURATION OF SENSITIZATION ON ANAPHYLAXIS IN THE GUINEA-PIG

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It has long been known, from the results of numerous workers, that the amount of protein required, in the reacting injection, to induce anaphylactic shock in the sensitized guinea-pig, is smaller in animals which have been sensitized for a long period, than in those tested soon after they become sensitive. No investigation appears to have been made of whether the amount of antigen needed to desensitize the tissues varies with the duration of sensitization, with the exception of some experiments on serum-sensitized guinea-pigs (Winter, 1944*a*).

In these experiments, it was found that the amount of globulin required to desensitize the uterus *in vivo*, when the reacting dose was injected into the portal blood, was greater in animals sensitized for 50 days than in animals which were examined 15-20 days after sensitization. There was also a greater tendency to shock, after the injection of a small amount of globulin, in the 50-day animals. In the present work this study has been extended, to include globulin-sensitized and albumin-sensitized animals, to maintain the investigation for a longer period, and to determine whether the increase in the amount of antigen required to desensitize the animal, as sensitization advances, is peculiar to serum-sensitized guinea-pigs alone. The point is of some theoretical interest, since it is well known that animals, sensitized by a single protein, which have recovered from anaphylactic shock induced by this protein, cannot be thrown into shock by another, even much larger, injection of the same antigen given shortly after recovery from shock. A similar finding applies to smooth muscle; it was shown by Dale (1912), and by Dale & Hartley (1916), that when a full reaction has been elicited from the isolated uterus of the sensitized guinea-pig, the further introduction of antigen to the bath causes no response from the muscle. It is remarkable, therefore, that in the serum-sensitized guinea-pig the amount of globulin needed to cause anaphylactic shock should decrease with the longer time of sensitization, while the capacity of the serum-antibody in the whole animal to fix globulin should increase.

### METHODS

Three groups of forty-eight virgin guinea-pigs were used in succession, the largest number which it was possible to accommodate at one time. The animals weighed between 200 and 250 g. on the date of sensitization. The groups were sensitized with horse serum, albumin and globulin respectively, the method of sensitization and the preparation of the proteins used being the same as in the previous paper (Winter, 1944*a*). Some animals from each group were tested when they first became sensitive, the serum-sensitized and globulin-sensitized guinea-pigs 15-18 days after injection and the albumin-sensitized animals after 28-30 days. Other animals from each group were tested after 48-51 days and the remainder after 90-93 days. The general principle of all the tests was the same; each animal was anaesthetized with ether, the first uterine horn was

removed and tested in the bath for sensitivity, 0.1 c.c. of horse serum being used in every case. The reacting dose of antigen was injected, into the portal blood unless otherwise stated, and the animal was killed 1-2 hr. later, when the second horn was tested in the bath. When, owing to the susceptibility of the animals to shock, the reacting dose of protein had to be given in more than one injection, the details are given in the protocols, but in all cases the animals were killed 1-2 hr. after the last injection. One preparation of globulin was used as the reacting antigen for the serum-sensitized and globulin-sensitized animals; the albumin-sensitized guinea-pigs were injected with albumin. The albumin and globulin, dissolved in 0.9% NaCl, were used in three concentrations: for injections of 1.0 mg./100 g. and over, 10 mg./c.c.; for injections smaller than 0.05 mg./100 g., 1.0 mg./c.c.; for all other injections, 5 mg./c.c.

## RESULTS

### *Animals sensitized by albumin*

In the earlier work (Winter, 1944a) no symptoms of shock were observed in any albumin-sensitized animal which was given a reacting dose of albumin, but in those experiments the minimum amount of albumin necessary to desensitize the uterus was not exceeded. The present series (Table 1) shows that, with approximately twice the minimum amount of albumin, shock may

TABLE 1. Animals sensitized by albumin

| mg. albumin per 100 g. |       |       | Days sensitized | Reaction of 2nd horn | Remarks                                                                                                                                     |
|------------------------|-------|-------|-----------------|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| 1st                    | 2nd   | Total |                 |                      |                                                                                                                                             |
| .                      | .     | 0.1   | 28-30           | + - + +              | No reaction                                                                                                                                 |
| .                      | .     | 0.25  | 28-30           | - - - +              | No reaction                                                                                                                                 |
| .                      | .     | 0.5   | 28-30           | - -                  | No reaction                                                                                                                                 |
| .                      | .     | 0.5   | 28-30           | .                    | Two animals. Death in shock 4-5 min. after injection. 2nd horns not examined                                                                |
| .                      | .     | 0.25  | 49-50           | + + + +              | No reaction                                                                                                                                 |
| .                      | .     | 0.5   | 49-50           | - - -                | No reaction                                                                                                                                 |
| .                      | .     | 0.5   | 49-50           | .                    | Two animals. Death in shock 4-5 min. after injection. 2nd horns not examined                                                                |
| 0.05                   | 0.15  | 0.5   | 49-50           | - - -                | 1st injection given. 1st horn removed. 2nd injection given. 3rd injection 2½ hr. later                                                      |
| .                      | .     | 0.5   | 91-93           | .                    | Four animals. All died about 5 min. after injection                                                                                         |
| 0.05                   | 0.15  | 0.5   | 91-93           | -                    | 1st injection given. Moderate shock during removal of 1st horn. 2nd injection given. 3rd injection 3 hr. later                              |
| *                      | 0.025 | 0.1   | 91-93           | -                    | Injections as above. No reaction                                                                                                            |
|                        | 0.025 | 0.1   | 91-93           | - -                  | Injections as above. Moderate shock in one animal, severe in the other, after 1st injection                                                 |
| *                      | 0.025 | .     | 91-93           | .                    | Two animals. Death 4-5 min. after injection                                                                                                 |
|                        | 0.025 | 0.1   | 91-93           | .                    | Death 5 min. after 2nd injection                                                                                                            |
| 0.005                  | 0.02  | 0.25  | 91-93           | - - + + +            | 1st injection given. 1st horn removed. 2nd injection given. 3rd injection 3 hr. later. 1st animal suffered severe shock after 2nd injection |

\* Group A (see text).

occur in some animals soon after they become sensitive. The susceptibility of the 50-day albumin animals to shock was no greater than that of those sensitized only for 28 days, but the amount of albumin required to desensitize the uterus had increased. With still longer time of sensitization the 90-day guinea-pigs showed symptoms, or died in shock, after a much smaller amount of albumin than was required to cause shock in the 50-day animals; on the other hand, although the susceptibility of the 90-day animals to shock had increased, there was no increase, or even a slight decrease, in the amount of albumin required to saturate the albumin-antibody in the whole animal.

Results obtained from some of the guinea-pigs sensitized 90 days with albumin bear closely on previous experiments (Winter, 1944*b*), which showed that the first uterine horns were insensitive in a number of animals in which anaphylactic shock occurred. Six animals in Table 1, marked group A, were injected with albumin, 0.025 mg./100 g.; two died in shock. The remainder received 0.1 mg./100 g.; one died, and two others showed moderate or severe symptoms of shock. Only one of the six guinea-pigs showed no symptoms. Four other animals were given 0.025 mg./100 g., followed, after removal of the first uterine horn, by 0.1 mg./100 g. The first uterine horns of these animals proved to be insensitive. No symptoms of shock were observed in these guinea-pigs, although they received the same sensitizing injection at the same time as the others. It is unlikely, therefore, that these insensitive uteri were in fact sensitive, and merely failed to react when tested in the bath, through inability of the antigen, for some reason, to enter the muscle.

#### *Animals sensitized by serum*

No symptoms of shock were observed in any 15-17-day serum-sensitized guinea-pig given a reacting dose of globulin in amount considerably greater than that necessary to desensitize the animal. Sensitiveness to a small dose of globulin was apparent in the 50-day animals, as was also the increase in the amount of protein required to desensitize the uterus. The experiments with 15- and 50-day serum animals in Table 2 are therefore confirmatory of the earlier results. Extension of this series to 90 days showed that there was a further increase in the amount of globulin required to saturate the serum-antibody in the tissues. A few 90-day guinea-pigs were still available, and these were given injections of globulin into the systemic circulation; the results show that the increase in the amount of globulin required to desensitize the animals, when they are kept for a long period, is not entirely to be ascribed to increased fixation of the antigen by cells in the liver. Although it is doubtful whether there was any significant change in the susceptibility of the serum animals to shock between 50 and 90 days, it is noteworthy that none of the 90-day animals which received the globulin into the systemic circulation showed any symptoms.

TABLE 2. Animals sensitized by serum

| mg. globulin<br>per 100 g. |       | Days<br>sensitized | Reaction of |                                                                                                      | Remarks |
|----------------------------|-------|--------------------|-------------|------------------------------------------------------------------------------------------------------|---------|
| 1st                        | Total |                    | 2nd horn    |                                                                                                      |         |
| .                          | 0.05  | 15-17              | - + - +     | No reaction                                                                                          |         |
| .                          | 0.1   | 15-17              | + - -       | No reaction                                                                                          |         |
| .                          | 0.5   | 15-17              | - - - -     | No reaction                                                                                          |         |
| .                          | 0.5   | 50-51              | -           | No reaction                                                                                          |         |
| .                          | 0.5   | 50-51              | +           | Died 3 min. after injection                                                                          |         |
| .                          | 0.5   | 50-51              | +           | Severe shock; recovered                                                                              |         |
| 0.15                       | .     | 50-51              | .           | Died 4 min. after injection. 2nd horn not examined                                                   |         |
| 0.15                       | 0.5   | 50-51              | - + - +     | 1st horn removed. 1st injection given. 2nd injection 3 hr. later                                     |         |
| 0.15                       | 1.5   | 90-93              | - - -       | Injections as above                                                                                  |         |
| 0.15                       | 1.5   | 90-93              | -           | Injections as above. Moderate shock after 2nd injection; recovered                                   |         |
| 0.15                       | .     | 90-93              | .           | Died 4 min. after injection                                                                          |         |
| 0.15                       | 0.5   | 90-93              | + + + +     | 1st horn removed. 1st injection given. 2nd injection 3 hr. later                                     |         |
| 0.15                       | 0.5   | 90-93              | + -         | Injections as above. Severe shock in both animals after 1st injection; recovered                     |         |
| 0.15                       | 0.5   | 90-93              | - - + + + - | 1st horn removed. 1st injection into inferior vena cava. 2nd injection 3 hr. later into jugular vein |         |

*Animals sensitized by globulin*

Unlike serum-sensitized guinea-pigs, when animals sensitized by globulin first become sensitive, they are susceptible to anaphylactic shock after an injection of globulin insufficient to desensitize the uterus. This conclusion receives further support from the experiments recorded in Table 3, in which three of the seven animals given 0.5 mg. globulin per 100 g. died in shock. Animals kept for 50 days were more susceptible to the reacting protein, but no difficulty was experienced in administering the desired dose by graded injections. The amount of globulin necessary to desensitize the animals was not increased by the longer time of sensitization; in this respect, globulin animals differ from 50-day serum-sensitized guinea-pigs, but they resemble them in showing increased susceptibility to shock. The liability of the 90-day globulin animals to shock, following injection of globulin, was very much greater than at 50 days, and it was probably only by chance in the first two experiments (A and B) with the 90-day guinea-pigs, that the animals survived without shock, after the same series of injections which was successful in the last four 50-day animals. The next six guinea-pigs died in shock, although three received even smaller preliminary injections, and it seemed probable that the whole of the remainder would be lost without yielding more than a few isolated results. Experience with the 90-day serum-sensitized guinea-pigs, in which some of the animals suffered from shock after injection into the portal blood, while none of those injected into the jugular vein showed

TABLE 3. Animals sensitized by globulin

| mg. globulin per 100 g. |       |      |      |       | Days sensitized | Reaction of 2nd horn | Remarks                                                                                                                                                                                                                                                                                                                |
|-------------------------|-------|------|------|-------|-----------------|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1st                     | 2nd   | 3rd  | 4th  | Total |                 |                      |                                                                                                                                                                                                                                                                                                                        |
| .                       | .     | .    | .    | 0.25  | 16-18           | +                    | No reaction                                                                                                                                                                                                                                                                                                            |
| .                       | .     | .    | .    | 0.5   | 16-18           | -                    | No reaction                                                                                                                                                                                                                                                                                                            |
| .                       | .     | .    | .    | 0.5   | 16-18           | +                    | Three animals. Died 4-5 min. after injection. 2nd horns not examined                                                                                                                                                                                                                                                   |
| 0.05                    | 0.1   | .    | .    | 1.0   | 16-18           | +                    | 1st injection given. 1st horn removed. 2nd injection given. 3rd injection 3 hr. later                                                                                                                                                                                                                                  |
| 0.05                    | 0.1   | .    | .    | 1.5   | 16-18           | -                    | Injections as above                                                                                                                                                                                                                                                                                                    |
| 0.05                    | 0.1   | .    | .    | .     | 48-50           | -                    | Death 5 min. after 2nd injection. 2nd horn not examined                                                                                                                                                                                                                                                                |
| 0.05                    | 0.05  | .    | .    | 1.5   | 48-50           | -                    | 1st injection given. Moderate shock during removal of 1st horn. 2nd injection given. 3rd injection 3 hr. later                                                                                                                                                                                                         |
| 0.05                    | 0.05  | .    | .    | 1.5   | 48-50           | +                    | Injections as above. Animals died 5 min. after last injection                                                                                                                                                                                                                                                          |
| 0.05                    | 0.075 | 0.25 | .    | 1.5   | 48-50           | -                    | 1st injection given. 1st horn removed. 2nd injection given. 3 hr. later, 3rd injection given. Intestines replaced in abdomen for 5 min. Final injection given. Severe shock in one animal 6 min. after 2nd injection                                                                                                   |
| 0.05                    | 0.075 | 0.25 | .    | 1.5   | 90-93           | -                    | Experiments A, B. Injections as above                                                                                                                                                                                                                                                                                  |
| 0.05                    | 0.075 | .    | .    | .     | 90-93           | .                    | Experiments C, D, E. Three animals. Slight shock in two after 1st injection. All died 5-7 min. after 2nd injection                                                                                                                                                                                                     |
| 0.01                    | 0.04  | 0.05 | .    | .     | 90-93           | .                    | Experiment J. 1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. Animal died 5 min. later                                                                                                                                                                               |
| 0.01                    | 0.04  | .    | .    | .     | 90-93           | .                    | Experiments K, L. Two animals. Died 3-4 min. after 2nd injection                                                                                                                                                                                                                                                       |
| 0.01                    | 0.04  | 0.05 | 0.25 | 1.5   | 90-93           | -                    | Experiments N, O. 1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. 3 hr. later, 4th injection given. 5 min. interval. Final injection given. Moderate shock in one animal after 2nd, in other after 3rd injection. 1st three injections given into inferior vena cava |
| 0.05                    | 0.075 | .    | 0.1  | 0.5   | 90-93           | +                    | Experiments F, G. 1st injection given. 1st horn removed. 2nd injection given. 2 hr. later, 3rd injection given. 5 min. interval. Final injection given. 1st two injections given into inferior vena cava                                                                                                               |
| 0.05                    | 0.075 | .    | .    | .     | 90-93           | .                    | Experiment H. Animal died 8 min. after 2nd injection. Injections into inferior vena cava                                                                                                                                                                                                                               |
| 0.05                    | .     | .    | .    | .     | 90-93           | .                    | Experiment I. Injection into inferior vena cava. Death 5 min. later                                                                                                                                                                                                                                                    |
| 0.01                    | 0.02  | 0.05 | 0.1  | 0.5   | 90-93           | -                    | 1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. 2½ hr. later, 4th injection given. 5 min. interval. Final injection given. 3rd injection given into inferior vena cava                                                                                               |
| 0.01                    | 0.04  | 0.05 | .    | 0.5   | 90-93           | +                    | Experiment M. 1st injection given. 1st horn removed. 2nd injection given. 5 min. interval. 3rd injection given. 2 hr. later, final injection given. 1st three injections into inferior vena cava                                                                                                                       |

any reaction, suggested that the administration of some of the protein by the systemic blood might aid desensitization of the highly sensitive globulin animals. All the remaining 90-day globulin animals received some of the antigen into the inferior vena cava, and the series was successfully completed. The results show that there was no increase in the amount of globulin required to desensitize the animals after 90 days, but even a slight decrease. The variations in the susceptibility to shock in the three types of animal studied, at the different periods, and the amount of protein necessary for desensitization, are summarized in Table 4.

TABLE 4. Variations in susceptibility of animals sensitized with different antigens

|                                                 | Duration of sensitization (days) |               |                    |
|-------------------------------------------------|----------------------------------|---------------|--------------------|
|                                                 | 17<br>(28 for albumin)           | 50            | 90                 |
| Serum-sensitized animals                        |                                  |               |                    |
| mg. globulin per 100 g. required to desensitize | Below 0.5                        | Increased     | Increased          |
| Susceptibility to shock                         | None found                       | Moderate      | Moderate           |
| Globulin-sensitized animals                     |                                  |               |                    |
| mg. globulin per 100 g. required to desensitize | 1.5                              | Not increased | Slightly decreased |
| Susceptibility to shock                         | Moderate                         | High          | Very high          |
| Albumin-sensitized animals                      |                                  |               |                    |
| mg. albumin per 100 g. required to desensitize  | 0.25                             | Increased     | Slightly decreased |
| Susceptibility to shock                         | Moderate                         | Moderate      | High               |

It is possible that the greater ease of desensitization, when some of the reacting protein was injected into the systemic blood, may be explained by the presence in the liver of sensitized cells which fix the antigen. It has already been suggested (Winter, 1944 *a, b*) that there is a differential distribution of the sensitizing antibody in the tissues of the guinea-pig, and in some animals the primary event in anaphylactic shock may occur in the liver. If this view is correct, there will be, when a very small amount of antigen is given, a greater tendency to shock when injection is made into the portal than into the systemic blood, but no difference will be observed when a larger quantity of protein is used, since, in the latter case, there will still be a sufficient concentration of antigen in the blood to affect the liver cells, should these be more highly sensitized than those in the smooth muscle of the lungs. The results obtained from the 90-day globulin animals support this view. If the five experiments A-E in Table 3 are compared with the four F-I, it will be seen that, with the larger dose of globulin, there was no significant difference between the susceptibility of the animals to shock. With the smaller dose, however, the three animals J-L, given the protein into the portal blood, all died; the three animals M-O, receiving it in the systemic circulation, all survived.

*Shock in albumin animals after sensitization for 90 days*

All the albumin-sensitized guinea-pigs recorded in Table 1 received the reacting dose of antigen in the portal blood. The results obtained from the highly sensitized serum and globulin animals, which showed a greater susceptibility to shock when a very small amount of antigen was injected into the portal blood, than when it was given into the systemic circulation, made it of interest to determine whether the same effect was shown in 90-day albumin-sensitized guinea-pigs. Twenty-four animals were given the usual preliminary injection of albumin in order to test this point. After 90-92 days, twelve were examined: all were sensitive, and the results were so convincing that the remainder were used for another purpose. In the first six experiments the very small dose of 0.025 mg. albumin/100 g. was injected, into the mesenteric vein or the inferior vena cava, in alternate animals: the first uterine horn was then removed and tested for sensitivity. The three guinea-pigs which received an injection into the portal blood all died in shock 3-4 min. later; of the three animals which received the antigen into the systemic blood, one died in shock, the other two showing no symptoms. An even smaller dose, of 0.015 mg./100 g. was used for the next six animals. Two of the three guinea-pigs given the injection into the portal blood died in shock; none of those which were given the albumin into the inferior vena cava showed any reaction. When, however, the five animals which survived the small injection of albumin into the inferior vena cava without symptoms, received, by the same route, 5 min. after the first injection, a further injection of albumin, 0.05 mg./100 g., three died in shock 3-4 min. later. There is, therefore, as was found in the case of the globulin-sensitized animals, no significant difference between the results obtained on injection by either route, when the dose of antigen is sufficiently large.

These experiments, which supplement the corresponding results obtained with the 90-day serum-sensitized and globulin-sensitized guinea-pigs, may be considered to be conclusive in showing the presence of sensitized elements in the livers of these animals, and shock must be initiated, in many cases, by the interaction of antigen and antibody in the liver. If the sensitizing antigen had not affected any cells in the liver, then, owing to the slow circulation of the portal blood through this organ, the susceptibility of the animals to shock would always have been greater when the reacting antigen was injected into the systemic blood, whereas the opposite was actually discovered. The experiments of Falls (1918) cannot be accepted in this connexion. Falls found that the amount of serum required to cause anaphylactic shock was less when injected into the portal blood than into the systemic circulation, but large doses of serum were given to the animals, and some died, not in acute shock,

but 20–30 min. after the reacting dose was administered. Moreover, the amount of serum injected often approached that which caused symptoms or death in the non-sensitized controls.

*Globulin-sensitized and serum-sensitized animals given  
a reacting injection of serum*

As will be mentioned in the discussion, the distinction between serum-sensitized and globulin-sensitized animals, especially at 15–20 days, plays an important part in the argument already put forward (Winter, 1944*a*) that serum contains a complex protein. It has been shown that, when they first become sensitized, the serum animals may be desensitized by globulin without shock, and require less of this protein to effect desensitization than is needed by globulin-sensitized guinea-pigs; the latter, moreover, are more susceptible to shock, after an injection of globulin, than are serum animals. It is well known that the sensitization of guinea-pigs to proteins is influenced by many factors, among these being the quantity of protein injected. The protein content of horse serum is variable, but there was probably always more protein in the 0.1 c.c. used for the sensitizing injections than was present in the 2 mg. of globulin, containing some ammonium sulphate, by which the globulin animals were sensitized. Certain earlier observations make it unlikely that the differences in the amounts of protein in the sensitizing injections were sufficiently large to have an appreciable influence on the characteristics which have been found, in serum-sensitized and globulin-sensitized animals, given the reacting injection of globulin directly into the circulation.

It was shown (Winter, 1944*a*, Tables 19, 20) that when serum-sensitized and globulin-sensitized guinea-pigs were desensitized by serum injected into the duodenum, the former animals required three times more serum than the latter. Similar indications were obtained in experiments recorded in Tables 10 and 11 of the same paper, when the reacting dose of serum was injected into the portal blood; the serum-sensitized animals required more serum for desensitization than did those sensitized by globulin. While the serum-sensitized guinea-pig requires less globulin for desensitization than the globulin animal, the position is reversed when serum is the reacting antigen; this suggests that the characteristics of the serum-sensitized and globulin-sensitized guinea-pigs are determined only by the nature of the protein used in the initial injection, and are independent of small differences in the amount of the antigen. When the experiments in Tables 10 and 11 of the previous paper were performed, only a few were carried out at the minimum level of serum required to desensitize the animals, and no stress was laid upon these, since at that time they seemed to be of no special interest. Owing to the very small quantity of serum required, the difficulty of determining the minimum amount needed for desensitization was pointed out.

In the experiments about to be recorded, the same technique was employed; large animals were used, and all were given a reacting injection of 0.01 c.c. of serum. The same sample of serum (kept at +3°C.) was used for all the reacting injections, and the experiments were completed in 3 days. Animals of uniform size were chosen, and the average weights of the serum-

sensitized and globulin-sensitized series at the time of operation were almost identical, being 414 and 413 g. respectively. Previous findings (Winter, 1944*c*) made it advisable to give the injections into the portal blood in some animals of each type, and in others into the systemic circulation. In one group, the first horn was removed, serum was injected into the portal blood, and each animal was killed  $3\frac{1}{2}$  hr. later. In the other group, the first horn was removed and tested for sensitivity; 3 hr. later, the anaesthetic was given, serum was injected into the jugular vein, and the animal was killed 15 min. later. The results are given in Table 5, and show a marked difference between the animals sensitized by the two proteins. As was to be expected (Winter, 1944*c*), the animals used in Table 5 were also differentiated by the occurrence of shock. No symptoms were noted in any serum-sensitized guinea-pig: four of those sensitized by globulin suffered from shock; one died, the others recovered.

TABLE 5. Globulin-sensitized and serum-sensitized animals (18–20 days)  
given an injection of 0.01 c.c. serum

|                             | Reaction of 2nd horn |               |
|-----------------------------|----------------------|---------------|
|                             | Globulin animals     | Serum animals |
| Injection into portal blood | - - - -              | - + - + +     |
| Injection into jugular vein | - - - + -            | + - + +       |

### DISCUSSION

Comparison of earlier results (Winter, 1944*a*, Tables 4, 5) with Table 3 of the present paper, shows that, in the present experiments, the amount of globulin required to desensitize animals which were sensitized by this protein, was very much smaller. Denaturation of the globulin used in the first experiments must have occurred to a considerable extent during the preparation. I am at a loss to account for such a pronounced variation in activity, when the same process of isolation was followed. The responsible factor is not likely to be the grinding of the dry protein, since the albumin used in the present work was also more active than that previously prepared (cf. Table 1 of this, with Table 3 of the original paper), and the albumin requires no grinding in the true sense, the crystalline mass breaking up almost at a touch. It is possible that the antigenic properties of proteins are always impaired to some extent by isolation, and it is clearly essential that when comparison is made between the amount of protein which is required to desensitize different animals, the same sample of antigen should be used, a procedure which has been followed in the present work. When a new preparation is made, this must be tested for activity against previous samples.

In the first paper (Winter, 1944*a*), one sample of globulin was used for the experiments in Tables 1–7 inclusive; a second for Tables 8–24, and a third for the remainder of the experiments in the first, and all involving the use of globulin in the second paper (Winter, 1944*c*). One sample

of albumin was used for the experiments of Tables 2-22. Two more preparations of albumin were then made. One was used for the remaining injection experiments of the first, and for those of the second paper, but both were used at different times for the tests on isolated uteri of serum-sensitized and albumin-sensitized animals reported in the last part of the first paper (1944*a*).

The use of different protein preparations has diminished the difference between the amounts of globulin required to desensitize serum-sensitized and globulin-sensitized guinea-pigs, but this difference is still marked, as may be seen by reference to Tables 2 and 3 of the present work. Two other series of experiments in the original paper need to be repeated, for the same reason. The first, showing the effect of albumin in enhancing the desensitizing power of globulin on the globulin-sensitized animal (Winter, 1944*a*, Table 14), has already been repeated, using the same preparations of globulin and albumin as for other experiments in the present paper. Eight experiments were performed, and the amount of globulin needed was found to be reduced to one-thirtieth of that required when globulin alone was injected. The second series, whose validity is in doubt, is that in which animals were sensitized by globulin, followed at 3 weeks' interval by albumin (G-A animals); it is my intention to repeat this series at the first opportunity.

Until it can be said with certainty that no significant impairment of the antigenic properties of the albumin and globulin has occurred during the process of isolation, the evidence that the desensitizing action of serum is due to a protein complex, and not to free globulin and free albumin, must rest on the action of serum when absorbed from the intestine. Attention has been drawn to this question in the first paper, in which it was stated: 'Appreciable denaturation would reduce the value of conclusions drawn from the difference found between serum and the proteins derived from it, when injected into the circulation. On the other hand, denaturation would enhance this difference when the intestinal route was being used; under these conditions serum was shown to be much less active than albumin and globulin.'

That the sensitizing action of serum is due to a complex protein is probable from the evidence which has already been presented for the influence of the 'masked' albumin in the isolated uteri of animals which have been sensitized by serum for 28 days or longer. There was no sensitiveness to albumin in the uteri of the 28-day serum-sensitized animals in these experiments; like those of globulin-sensitized guinea-pigs, the uteri responded strongly to globulin and were qualitatively identical. The essence of the problem is whether, if free albumin is present in serum, the albumin is antigenically inert and takes no part in the sensitization, in which case serum-sensitized and globulin-sensitized animals should be identical; or whether the albumin influences the globulin so that serum-sensitized animals differ from those sensitized only by globulin, although no difference can be shown by the response of the

isolated muscle. It is highly probable, from the evidence presented in this and earlier papers, that serum-sensitized and globulin-sensitized guinea-pigs are not identical. The reason for the discrepancy between these results and those of Dale & Hartley (1916) has not yet been found, but it is important to note, by reference to Tables 2, 3 and 5, that serum-sensitized and globulin-sensitized animals differ, both in their sensitiveness to the reacting protein, and in the amount of this protein required to desensitize the tissues, when they have been sensitized less than 21 days, i.e. at a time when all observers are agreed that sensitiveness to albumin cannot appear in the uterus. It is difficult to explain these differences otherwise than as indicating that the albumin is combined with the globulin of serum to form a complex protein.

The changes in the sensitization mechanism in the guinea-pig which have been found to occur as the time of sensitization increases, present a problem of unusual complexity. In the three types of animal studied, there was in two, viz. in the serum and in the globulin animals, an increase in the sensitivity to the reacting antigen at 50 days, shown by the reduced amount of protein necessary to cause anaphylactic shock. At 90 days, there was a further increase in sensitivity, with the possible exception of the serum-sensitized guinea-pig. As regards sensitivity, therefore, the results were generally consistent in the three groups. The changes in the amount of antigen, however, which was required to desensitize the animals, showed no such consistency. In the serum-sensitized guinea-pigs, the amount of globulin which was needed to abolish the reaction of the uterus increased at each period; in the globulin-sensitized and albumin-sensitized animals, the amount of antigen which was required to desensitize the tissues appeared to vary independently of the amount which was necessary to cause shock. It has already been remarked (Winter, 1944*a*) that the reaction of the sensitized uterus is of little use as a guide to the response of the whole animal, when the reacting dose of antigen is injected, since some animals may respond by shock, while others, of apparently equal uterine sensitivity, show no reaction. It is clear that, after the injection of the reacting dose of protein into the sensitized animal, fixation of antigen may occur in all, but causation of shock only in some cases. Probably two factors are involved; it is the shock-producing factor alone which has been extensively studied. In guinea-pigs which have been sensitized only for a few weeks, the shock-producing mechanism is but slightly developed; at this time many animals may be given the reacting injection without shock; in the case of the serum-sensitized guinea-pig, given globulin, no animal has shown symptoms. After some months, many animals suffer from shock, but even at this time, some may be desensitized without any reaction.

From the results obtained in the present work, we may conclude that, in animals sensitized for some months, when sufficient antigen is injected to

desensitize the uterus, only a very small fraction is needed to set in motion the shock-producing mechanism. This mechanism is believed to be the release of preformed toxic substances from sensitized cells in the animal, following the antigen-antibody reaction (for a recent review, see Dragstedt, 1941). Schild (1937) has shown that the anaphylaxis reaction in the guinea-pig leads to the release of histamine-like substances from many tissues. The means by which toxic substances are released by the antigen-antibody reaction is still unknown, and it may well be that the release of these substances in amount necessary to cause symptoms does not occur in every animal, nor always from the same tissue. It is remarkable that the greatest release of histamine, in Schild's experiments, occurred in the aorta, while the liver was inactive.

It is difficult to accept the view (Dragstedt, 1941) that the toxic substances released by the antigen in anaphylactic shock in the guinea-pig are 'normally resident' in various tissue cells, without some qualification. Many more guinea-pigs which have been sensitized for some months suffer from anaphylactic shock, than those which have been sensitized only for a few weeks. Two possibilities must therefore be borne in mind. It may be assumed that, in the shock-producing mechanism, the first event is cell damage induced by the reaction between antigen and antibody, and this allows the release of toxic substances into the blood. The toxic substances, if present in the cells in the same amount in both types of animal, must be released with greater speed, after more extensive cell damage, in the guinea-pigs which have been sensitized for 90 days; the rate of release in the 15-28-day animals must be, in many cases, too small for the toxic substances to cause symptoms. If the rate of release in the two types of animal is approximately equal, on account of a similar extent of cell damage, the toxic substances must be present in greater amount in the cells of the 90-day guinea-pig, and one must therefore conclude that the sensitizing antigen increases the formation of these substances in the cells, as the duration of sensitization advances.

#### SUMMARY

1. Guinea-pigs have been sensitized by horse serum, albumin, and globulin respectively. A study has been made at different intervals after sensitization, of the susceptibility of the animals to shock, and of the amount of antigen required to desensitize the tissues *in vivo*. Only the changes in susceptibility to shock were consistent in the three types of animal.

2. Further evidence is adduced which indicates that serum contains a complex protein, and it is shown that the characteristics of serum-sensitized and globulin-sensitized guinea-pigs are independent of small differences in the amount of protein in the sensitizing antigens.

3. When a very small amount of protein is given in the reacting injection to highly sensitized animals, shock is more frequently caused when the in-

jection is made into the portal blood, as compared with injection into the systemic circulation. It is concluded that, in the three types of animal studied, sensitization of cells occurs in the liver.

4. It has been found that albumin and globulin, when prepared from horse serum by the same method, may vary in antigenic activity. The bearing of this finding on the author's previous results is discussed.

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