LIPOIDS AS INHIBITORS OF ANAPHYLACTIC SHOCK.

STUDIES ON FERMENT ACTION. XVIII.*

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The refractory period following anaphylactic shock, noted early in the study of anaphylaxis, has been variously explained. Among immunologists the idea seems to be generally accepted that the condition is due to an exhaustion of the specific immune substance which together with complement is supposed to act upon the introduced antigen.

Rusznjak (1) first noted that immediately following anaphylactic shock, especially if protracted or following an extended latent period, a definite rise in the antitryptic titer of the animal's serum occurred. Rusznjak, assuming that the hypothesis of Rosenthal (2) in regard to the nature of serum antitrypsin was correct, interpreted his experiment as a demonstration of the splitting of proteins during anaphylactic shock. He furthermore advanced the idea that the period of resistance following shock was due to this increase in the antiferment property of the blood. Seligmann (3) could not confirm these findings, but Pfeiffer and Jarisch (4) later found the observations to be correct, and furthermore showed that a similar rise in antitrypsin occurred following various protein intoxications,—hemolysins, protein split products, etc. Zinsser (5) has recently shown that an increased resistance is found to anaphylatoxins following a first sublethal injection, and we have noted a similar condition with serotoxin (6). Following a sublethal serotoxin injection a well marked rise in the antitryptic titer is observed.

The observation of Rusznjak is therefore true not only for anaphylaxis but probably for every intoxication accompanied by cellular destruction. His conclusions, however, being based on the erroneous theory of Rosenthal as to the nature of serum antitrypsin, are incorrect. We have recently demonstrated that the unsaturated lipoids of the serum are the substances upon which the antitryptic property depends (7). The increase in antitrypsin observed in these cases cannot be due directly to the protein split products, but is to be explained by

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the liberation of lipoids following cellular destruction. The observations of Rusznjak and of Pfeiffer and Jarisch offer then no direct evidence that protein splitting occurs during anaphylactic shock, but merely indicate that a general cellular intoxication has occurred.

The increase in antiferment might nevertheless explain the increased resistance to a second injection of antigen in a sensitized animal. This question can find a solution only if it is possible to increase the antiferment in the blood serum by some means other than a protein intoxication, for following such injury the objection can be made that there is a reduction or exhaustion of both specific and non-specific proteases or antibodies responsible for the shock, together with a destruction of complement. That complement, however, can have no relation to the phenomena, if we regard the intoxication as purely protein, is evident from the fact that the complementary action is not proteolytic but probably lipolytic, a subject which we have briefly discussed in a previous paper (8). Serum antitrypsin being lipoidal and capable of isolation from the serum by means of lipoidal extractives should on reinjection into experimental animals cause an increase in the antiferment property of the blood serum. Such an experiment can be made as follows.

METHODS OF INCREASING THE ANTIFERMENT IN THE BLOOD.

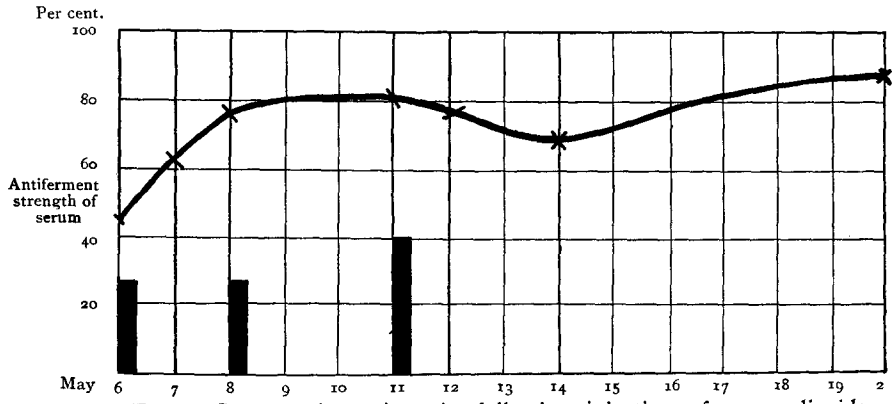
About 300 milligrams of serum lipoids extracted at various times from human and guinea pig serum were dissolved in six cubic centimeters of olive oil. Of this solution a large guinea pig received subcutaneous injections as follows:

May 6, 1914. 1.5 c.c.
May 8, 1914. 1.5 c.c.
May 11, 1914. 2.0 c.c.

The antiferment index, determined by the method discussed in our former papers, is shown in text-figure 1, in which the inhibition of tryptic digestion by 0.05 of a cubic centimeter of serum both before and after the injections is outlined. It will be noted that there is a distinct and prolonged rise from the original 45 per cent. inhibition to more than 80 per cent. as determined on May 11.

Olive oil injected subcutaneously in equal amounts had no effect on the antitryptic index.

Inasmuch as it is difficult to secure large amounts of serum lipoids we have substituted the fats prepared from egg yolk which contain large quantities of unsaturated lipoids. These were obtained as follows: The yolk was extracted twice with alcohol and twice with ether. The extracts were combined and evaporated to dryness. The mass was then extracted with ether and precipitated with acetone. Only the acetone-soluble lipoids were used. As so obtained the lipoids are semifluid and can be injected without the



TEXT-FIG. 1. Increase in antitrypsin following injection of serum lipoids subcutaneously.

use of a solvent. The increase in antitrypsin following the subcutaneous injection of one cubic centimeter in a guinea pig is shown as follows:

	Inhibition by serum before injection. June 29, 1914.	After injection. July 3, 1914.
0.10 c.c. serum	69 per cent. inhibition	79 per cent.
0.075 c.c. serum	44 per cent. inhibition	70 per cent.
0.05 c.c. serum	10 per cent. inhibition	70 per cent.
0.025 c.c. serum	0 per cent. inhibition	0 per cent.

The guinea pig was of medium weight. It was bled from the heart (two cubic centimeters) on June 29, and the egg fat injected the same day. The rise in antiferment is marked.

THE INHIBITION OF ANAPHYLACTIC SHOCK.

Having established the fact that these lipoids will, when subcutaneously absorbed, cause an increase in antiferment, we investigated the effect of such injections on the reaction of sensitized guinea pigs when the specific antigen was injected intravenously.

Six guinea pigs were sensitized on May 16, 1914, with 0.1 c.c. of horse serum intraperitoneally. Three of the animals received subcutaneous injections of serum antitrypsin dissolved in olive oil. The injections, 1 c.c. each, were made on May 21, May 26, and June 5. Purified horse serum albumen solution (1 per cent.) was used for reinjection. The toxic dose of this was determined on the three control animals and resulted as follows:

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
1	240 gm.	0.8 c.c.	0.0033 c.c.	Convulsions, respiratory spasms. Final recovery in 10 min.
2	280 gm.	1.4 c.c.	0.005 c.c.	Death in 2 min. Typical.
3	300 gm.	1.5 c.c.	0.005 c.c.	Same.

The lethal dose, 0.005 c.c. per gram weight, was now injected into the antitrypsin guinea pigs.

Animal No.	Weight.	Dose.	Dose per gm. weight.	Remarks.
4	210 gm.	1.0 c.c.	0.0047 c.c.	Scratches. No convulsions, no respiratory spasms. Remained well.
5	300 gm.	1.5 c.c.	0.005 c.c.	Death in 3 min. Typical.
6	220 gm.	1.1 c.c.	0.005 c.c.	No symptoms except restlessness. Remained well.

In two of these guinea pigs the acute shock had been completely averted. A similar and even more striking effect was obtained with guinea pigs treated with egg fats.

Ten guinea pigs were sensitized with horse serum on July 1, 1914. Six of the animals were injected previously with 1 c.c. of egg fat, on June 29. One of the animals was killed on July 3 and the antitryptic index determined, the result being shown on page 470. They received further injections of 1 c.c. each on July 10 and 16. The effect of the reinjection of a 1 per cent. horse serum albumen solution is shown in the following table.

CONTROL ANIMALS.

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
1	170 gm.	0.425 c.c.	0.0025 c.c.	No effect.
2	230 gm.	1.15 c.c.	0.005 c.c.	Marked respiratory convulsions and spasms. Recovered in 30 min.
3	295 gm.	2.95 c.c.	0.01 c.c.	Death in 2 min. Typical.
4	275 gm.	2.75 c.c.	0.01 c.c.	Death in 2 min. Typical.

The minimum lethal dose was therefore 0.01 of a cubic centimeter per gram weight of guinea pig. The injection into the animals treated with egg fat resulted as follows:

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
5	360 gm.	3.6 c.c.	0.01 c.c.	Immediate respiratory spasms; gradual recovery. Well after 10 min.
6	290 gm.	2.9 c.c.	0.01 c.c.	Scratched; no other symptoms.
7	235 gm.	2.35 c.c.	0.01 c.c.	No symptoms.
8	190 gm.	2.37 c.c.	0.0125 c.c.	No symptoms.
9	240 gm.	4.8 c.c.	0.02 c.c.	Marked respiratory spasms, with dyspnea for 20 min. Complete recovery.

It will be observed that all the animals receiving from one to two times the lethal dose made a complete recovery, and in three of the guinea pigs no symptoms of note were observed. The animals remained well during the next two weeks while under observation. There can then be no question but that the increase in antiferment is able to protect the animal from at least twice the minimum lethal dose of antigen and that this increase in antiferment following a protein shock must have a large share in the resistance to a second injection.

THE INFLUENCE OF LIPOIDS PRESENT IN THE ANTIGEN ON ANAPHYLACTIC SHOCK.

It has been found difficult to induce acute anaphylactic shock by means of whole bacteria, and differences have been noted in the period of time following a second injection of various antigens before symptoms of shock would be elicited, the latent period varying from one to fifteen minutes. A long latent period is common with egg albumen; indeed, Rusznjak employed egg albumen for this reason in his experiments. Inasmuch as we have recently demonstrated (9) that bacteria contain unsaturated lipoids which represent the antiferment and that they resist digestion in a degree proportional to the amount of the lipoids present; and since egg albumen resists digestion by means of the ordinary tryptic ferment unless first acted upon by pepsin in an acid medium, whereby the antiferment is destroyed because of alteration in the colloidal dis-

persion brought about by the change in reaction, we next examined the relative toxicity of an antigen before and after chloroform extraction. For this purpose we used the same preparation of purified horse serum albumen, the method of preparation of which we have discussed previously (6).

Fifty cubic centimeters of a 1 per cent. solution were prepared, of which twenty-five cubic centimeters were thoroughly shaken with chloroform and incubated for forty-eight hours, the flask being shaken at intervals. No autolysis occurred during this time. Before use the chloroform was freed from the serum albumen solution by centrifugation and filtration through a coarse paper filter. Guinea pigs were sensitized with horse serum intraperitoneally on April 13, 1914. The minimum lethal dose of the original horse serum albumen solution was determined as follows:

May 20, 1914.

Animal No.	Weight.	Dose.	Dose per gm. weight.	Result.
1	300 gm.	3.0 c.c.	0.01 c.c.	Typical anaphylactic shock. Death in 2 min.
2	300 gm.	1.5 c.c.	0.005 c.c.	Same.
3	360 gm.	0.9 c.c.	0.0025 c.c.	Recovered.
4	310 gm.	0.77 c.c.	0.0025 c.c.	Typical death in 3 min.
5	270 gm.	0.32 c.c.	0.0012 c.c.	Scratched; no other symptoms.

The minimum lethal dose was therefore about 0.0025 of a cubic centimeter per gram weight. The extracted serum albumen solution was toxic in a much smaller dose, as will be seen from the following table.

Animal No	Weight.	Dose.	Dose per gm. weight.	Result.
6	290 gm.	0.72 c.c.	0.0025 c.c.	Death immediate and typical.
7	290 gm.	0.36 c.c.	0.0012 c.c.	Death immediate and typical.
8	320 gm.	0.2 c.c.	0.0006 c.c.	Death immediate and typical.
9	300 gm.	0.1 c.c.	0.0003 c.c.	Marked convulsions; respiratory spasms. Final recovery.

This experiment would indicate that the lipoids combined with the protein antigen may exert a considerable influence on the relative toxicity of the anaphylactic antigen, and probably explains

the difficulty encountered in obtaining uniform results with such antigens as bacteria which are relatively well protected by their lipoidal components.

THE PREVENTION OF ANAPHYLACTIC SHOCK BY THE SIMULTANEOUS INJECTION OF SOAP SOLUTIONS.

If by extraction of a lipoid containing protein antigen it becomes more toxic, it would seem reasonable that by adding the extracted lipoids to the same the toxicity should be decreased, or, if added in sufficient amount, completely neutralized. We have shown that this can actually be done with serotoxin (6), and we discussed the reasons for the use of soaps in place of lipoid suspensions. A difficulty is encountered, though, in the use of soaps, in that they will in themselves on injection cause a shock similar to that observed in anaphylaxis, so that great care must be used in working with doses that are sublethal (10). While such soaps, oleates for example, are highly antitryptic, we have noted (7) that when incubated with serum, instead of increasing the antiferment index as might be expected, they actually cause a lowering of the antitryptic titer, possibly because of solution of the serum antiferment in the soap solution. Nevertheless, when added to the anaphylactic antigen, soap solutions are able to render the reinjection harmless within certain limits, as will be observed in the following protocols. We may state, however, that we have not been able to secure these results when whole serum was used as an antigen; the results have been obtained only when a solution of horse serum albumen was used. Whether this depends on the greater stability of the albumens as contrasted with the globulins, as might be indicated in the recent work of de Waele (11), or whether it is due to the fact that the soaps lower the antiferment index when added to the whole serum, in which case the injected antigen would have less protection than before, we cannot state.

Sensitized animals and antigen solutions, the toxic doses of which have been described on page 473, were used. 1.5 cubic centimeters of the original serum albumen solution were mixed with one cubic centimeter of a 1 per cent. solution of sodium oleate and incubated

for ten minutes. The surely toxic dose of the antigen was 0.005 of a cubic centimeter per gram weight.

Weight of animal.	Dose.	Dose per gm. weight.	Result.
300 gm.	1.5 c.c. + 1.0 c.c. soap solution	0.005 c.c.	No symptoms.

Similar mixtures were made with the lipoid-extracted serum albumen solution, the lethal dose of which was 0.0006 of a cubic centimeter per gram weight.

Weight of animal.	Dose.	Dose per gm. weight.	Result.
280 gm.	0.2 c.c. + 1.0 c.c. soap solution	0.0007 c.c.	No effect.
260 gm.	0.4 c.c. + 1.0 c.c. soap solution	0.0015 c.c.	No effect.
260 gm.	1.0 c.c. + 1.0 c.c. soap solution	0.0038 c.c.	No effect.

These experiments are simply isolated examples from numerous trials which have always shown the same result. In the last protocol the protection is observed to be ample against five times the minimum lethal dose.

Whether or not this protection by the soaps is a mechanical one, due to the formation of a thin soap membrane about the aggregates of the antigen, whether it is due to the actual antiferment property of the soap, or whether its action depends on changes induced in the cellular membranes of the animal so injected, rendering the cells less permeable to the toxic substances responsible for the symptom-complex, can, of course, not be decided from these experiments. We are, however, inclined to assume that the latter is the explanation, especially in view of the work of Schultz (12), Dale (13), Weil (14), and Coca (15), showing that the origin of the shock is probably cellular and not humoral.

CONCLUSIONS.

1. The antitryptic titer of the serum can be increased by subcutaneous injections of serum lipoids (antitrypsin) and of the lipoids from egg yolk.

2. Animals so injected show a relative immunity to acute anaphylactic shock (two minimum lethal doses).

3. Extraction of lipoids contained in antigens increases the toxicity of the antigen when injected into a sensitized animal.

4. Sublethal doses of soap solutions injected simultaneously with the antigen (purified horse serum albumen) prevent anaphylactic shock.

5. The refractory state following anaphylactic shock is related in part to an increase in the antitryptic titer of the serum.

BIBLIOGRAPHY.

1. Rusznjak, S., *Deutsch. med. Wchnschr.*, 1912, xxxviii, 168.
2. Rosenthal, E., *Folia Serolog.*, 1910, vi, 285.
3. Seligmann, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1912, xiv, 419.
4. Pfeiffer, H., and Jarisch, A., *Ztschr. f. Immunitätsforsch., Orig.*, 1912–13, xvi, 38.
5. Zinsser, H., and Dwyer, J. G., *Jour. Exper. Med.*, 1914, xx, 387.
6. Jobling, J. W., and Petersen, W., *Jour. Exper. Med.*, 1914, xix, 480.
7. Jobling and Petersen, *ibid.*, p. 459.
8. Jobling and Petersen, *idem*, xx, 321.
9. Jobling and Petersen, *ibid.*, p. 452.
10. Jobling and Petersen, *Ztschr. f. Immunitätsforsch.*, 1914, xxii (in press).
11. de Waele, H., *Ztschr. f. Immunitätsforsch., Orig.*, 1914, xxii, 170.
12. Schultz, W. H., *Jour. Pharmacol. and Exper. Therap.*, 1909–10, i, 549.
13. Dale, H. H., *Jour. Pharmacol. and Exper. Therap.*, 1912–13, iv, 167.
14. Weil, R., *Jour. Med. Research*, 1912–13, xxvii, 497.
15. Coca, A. F., *Ztschr. f. Immunitätsforsch., Orig.*, 1913–14, xx, 622.