

STUDY ON TUBERCULOSTATIC ACTIVITIES OF BODY FLUIDS
OF RABBITS GIVEN TRITON WR 1339 WITH SPECIAL
REFERENCE TO THE ROLE OF LIPEMIA

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During the course of studies on the antituberculous activities of body fluids of animals, it was occasionally noted by the authors that the growth of tubercle bacilli *in vitro* was somewhat inhibited when cloudy, instead of clear, serum was added to the culture media. This led us to assume that lipemic serum had a certain inhibiting effect on the growth of tubercle bacilli *in vitro* and perhaps also *in vivo*.

In 1951, Cornforth et al.¹⁾ reported that Triton WR 1339, a commercial non-ionic surface-active agent, was capable of preventing the development of disease, when administered to mice experimentally infected with virulent tubercle bacilli. Solotorovsky²⁾ confirmed this fact, and showed evidence of the synergistic effect of this drug with dihydrostreptomycin in experimental tuberculosis.

Triton was known to be an agent which produced marked lipemia in animals when administered in large doses. So, it seemed likely to us that the antituberculous activity of Triton might be due to its lipemia producing activity.

Cornforth et al. also showed that Triton did not affect virulent tubercle bacilli *in vitro*, and Rees³⁾ demonstrated that serum from mice or guinea pigs treated with therapeutic doses of this drug had no tuberculostatic effect *in vitro*.

In addition, Mackaness⁴⁾ demonstrated by the tissue culture method that monocytes of rabbits or guinea pigs treated with Triton were able to inhibit intracellular growth of tubercle bacilli *in vitro*, although the serum of these animals or Triton itself had no effect.

Thus, the generally accepted theory is that the antituberculous activity of Triton is due solely to changes of cellular activity and not at all to increased antituberculous activity of body fluid.

Cornforth and Rees also reported that animal tuberculosis was markedly suppressed by doses of Triton too small to produce lipemia.

Since the relationship between lipemia and inhibition of growth of tubercle bacilli is still unclear, an attempt was made to elucidate the role of lipemia in the resistance to tuberculosis not only to understand the effect of Triton, but also to contribute to our knowledge of antituberculous agents.

Materials and Methods

Animal: Male white rabbits, weighing approximately 2 kg, were used in all experiments.

Triton: A 12.5% solution of Triton was prepared with physiological saline. Doses of Triton will be described in each section.

Bacilli: Seven day cultures of H37Rv strain of tubercle bacilli in Kirchner's medium were used.

The chamber method: The chambers O and K, described by Tsuji et al.⁵⁾, were used.

With chamber O, it is possible to cultivate tubercle bacilli by a type of slide culture method within the living animal body, especially in a medium which contains no cells.

With chamber K, bacilli are cultivated in a medium containing low molecular substances of body fluid but neither protein of body fluid nor cells.

By this method it is possible to analyse the effect of body fluids on the growth of tubercle bacilli *in vivo* without any interference by cells.

Triton was given intravenously at 3 day intervals until the end of the experiment, and the same volume of physiologic saline was injected intravenously in control animals.

Seven days after the beginning of the injection, three O and three K chambers were implanted into the peritoneal cavities of Triton-treated and control rabbits. 1, 2, and 3 weeks after the implantation of chambers one O and one K chamber was taken out, and bacillary growth in the chambers was examined.

Experimental Results

1. Experiments in rabbits which showed apparent lipemia after injections of Triton.

300 mg of Triton per Kg of body weight was given at 3 day intervals. The contents of the chambers were markedly cloudy in all instances, and there were no differences between chambers O and K. The fluid in the chambers from control animals was quite clear. It was certain that marked lipemia was induced by Triton treatment.

The pH, as measured by B.T.B. test papers, was 6.8-7.0 in all chambers both treated and control.

Results are shown in Table 1.

Table 1. Results Obtained by the Chamber Method in Rabbits with Obvious Lipemia following Injection with Triton.

	Chamber O			Chamber K		
	7 days	14 days	21 days	7 days	14 days	21 days
Triton treated rabbits	—	—	—	—	—	—
Control rabbits	—	++	+++	—	÷	÷

In the O chambers of control rabbits, tubercle bacilli showed apparent multiplication at the end of the second week, and vigorous growth at the end of third week.

In the O chambers of treated rabbits, no bacillary growth was noted at the end of the second or the third week. In the K chambers, though very weak multiplication was seen in the controls, no growth was seen in the treated animals.

These results clearly indicate that in treated animals body fluid completely separated from cells has a powerful inhibiting activity on the growth of tubercle bacilli *in vivo*, and that this inhibiting effect is mainly attributable to the action of low molecular substances in body fluid.

2. Experiments in rabbits which did not develop lipemia in spite of the treatment with Triton.

Nine rabbits were divided into 3 groups of 3. The first group received intravenous injections of 100 mg of Triton per Kg of body weight at 3 day intervals.

The second group was injected with 50 mg (in 6.25% solution) Triton at 3 day intervals.

The third group, controls, received intravenous saline.

On the eighth day after the beginning of injections two O and K chambers were implanted. After two and three weeks the chambers were removed and examined for bacillary growth.

Blood, removed before and after the implantation, showed no lipemia in any instance. The contents of all chambers were also very clear.

The pH of the contents of all chambers was 6.8–7.0.

Results are shown in Table 2.

Table 2. Results Obtained by the Chamber Method in Rabbits with no Lipemia in spite of Treatment with Triton.

	Chamber O		Chamber K	
	14 days	21 days	14 days	21 days
Triton 100 mg/kg treated rabbits	—	—	—	—
Triton 50 mg/kg treated rabbits	—	—	—	—
Control rabbits	++	+++	÷	÷

In the O chambers of control animals multiplication of bacilli was noted after 2 weeks, and vigorous growth after 3 weeks. There was no bacillary growth in any of the chambers from treated rabbits.

In the K chambers of control animals, slight multiplication of tubercle bacilli was seen, but absolutely no growth was seen in those from treated animals. These results indicate that rather small doses of Triton can inhibit the growth of tubercle bacilli in the body fluids of treated rabbits; the main factors in this effects are low molecular substances, and macroscopically definite lipemia is not essential.

3. Experiments in rabbits with lipemia induced by a high cholesterol diet.

It is known that a high degree of lipemia can be induced by feeding rabbits with cholesterol.

Rabbits weighing 2 Kg were fed every day with 500 mg of cholesterol per Kg mixed in "Okara" (bean-curd refuse: usual diet of laboratory rabbits in Japan). Care was taken to insure that the animals ingested all the cholesterol. Body weights and other conditions were excellent during this feeding, better than those of control rabbits fed Okara without cholesterol.

Five weeks after the start of this diet lipemia was apparent to the naked eye, and chambers were implanted.

The high-cholesterol diet was continued to the end of the experiment. 1, 2, and 3 weeks after the implantation, the chambers were taken out and examined for bacillary growth.

The contents of the chambers in all rabbits fed with cholesterol were cloudy, but very clear in all control animals. The pH of the contents of all chambers was 6.8-7.0.

Results are shown in Table 3.

Table 3. Results Obtained by the Chamber Method in Rabbits Given Cholesterol or Fat Emulsion.

	Chamber O			Chamber K		
	7 days	14 days	21 days	7 days	14 days	21 days
Rabbits fed cholesterol	—	++	+++	—	÷	÷
Rabbits given injections of fat emulsion	—	++	+++	—	÷	÷
Control rabbits	—	++	+++	—	÷	÷

No difference in bacillary growth was seen between treated and non-treated control animals.

These results show that lipemia induced by a high cholesterol diet does not increase the inhibiting activity of the body fluid.

4. Experiments in rabbits given a commercial neutral fat emulsion.

Hikasa⁶⁾ succeeded in making a neutral fat emulsion for intravenous use, and commercial preparation of it are in clinical use. Hayashi⁷⁾ reported that this emulsion was useful in the treatment of experimental tuberculosis in rabbits.

10 ml of 15 per cent emulsion were injected twice daily (morning and evening) into 2 Kg rabbits. Injections were continued every day to the end of the experiment. Control rabbits were injected with 10 ml physiological saline daily.

Chambers were implanted one day before the first injection.

1, 2, and 3 weeks after implantation chambers were taken out, and examined for bacillary growth.

No side effects of the injection of fat emulsion were noted.

Slight transient lipemia was seen by the naked eye immediately after the injection and it continued for about 20 minutes, but no typical constant lipemia was induced by this emulsion. The contents of the chambers were clear.

Results are shown in Table 3.

No difference was seen in bacillary growth in the chambers of treated and non-treated animals.

Discussion

Occasional observations that the growth of tubercle bacilli in culture media seemed to be reduced when lipemic serum was used led us to assume that lipemic body fluid might be tuberculoinhibitory not only *in vitro* but also *in vivo*.

Although the present investigations have not thoroughly clarified the question, it may be said that lipemia does not parallel the tuberculoinhibitory activity *in vivo*. Multiplication of virulent tubercle bacilli, H37Rv, was completely suppressed in chambers implanted into the peritoneal cavities of rabbits given large doses of Triton and showing very cloudy serum, but lipemic body fluid from rabbits fed large amounts of cholesterol had no effect. It is certain that the biochemical conditions accompanying the lipemia vary with the cause of the lipemia, and that the tuberculostatic activity has no direct connection with cholesterolemia *per se*. As a matter of fact, even non-lipemic body fluid completely inhibited growth of tubercle bacilli in chambers implanted into the peritoneal cavities of rabbits treated with smaller doses of Triton. The antituberculous effect of Triton seems to have no direct relationship to the lipemia.

Hikasa's neutral fat emulsion neither induced lipemia nor had any detectable effect on the humoral defense of rabbits against tuberculosis.

It may be worthy of note that the body fluid of rabbits treated with Triton acquired an increased ability to restrict multiplication of tubercle bacilli *in vivo*,

although the results obtained by Mackaness and the fact that Triton is not bacteriostatic for virulent tubercle bacilli *in vitro* strongly suggest that the mechanism of the effect of Triton on tuberculosis lies mainly in its ability to alter cellular activity. The role of body fluid cannot be ignored.

It was demonstrated some years ago that the low molecular fraction of body fluid of normal animals and man contained tuberculostatic substances, and chemical analysis showed that these substances were contained mainly in the organic acid and polypeptide fractions. The present investigations show clearly that the body fluid, especially its low molecular fraction, increases in tuberculostatic power during Triton treatment. This leads us to think that surface-active agents like Triton act on lipid metabolism and release increased amounts of low molecular substances, probably fatty acids, into the circulating blood and then into the body fluid.

In addition, the influence on protein metabolism cannot be ignored, because serum protein counteracts the static action of low molecular substances on tubercle bacilli.

Although further investigation is required to explore the mechanism involved in the action of Triton in the defense against tuberculosis, it may be emphasized that the humoral as well as the cellular defensive mechanism is important.

Summary

With the chamber method, it was demonstrated that body fluid completely separated from cells of rabbits treated with either large dose (500 mg per Kg) or small dose (100 mg per Kg) of Triton WR1339 showed increased static activity on the growth of tubercle bacilli *in vivo*.

Cloudiness of serum (lipemia) had no relation to this activity, and lipemia induced by feeding rabbits a high cholesterol diet did not affect the resistance of body fluid to tuberculosis.

Hikasa's neutral fat emulsion did not increase the ability of body fluid to inhibit the growth of tubercle bacilli *in vivo*.

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