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Inactivation of Lethal Toxicity of Salmonella Endotoxin by Chicken Serum and Tissue Extracts

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Summary

Chicken tissue extracts have an ability to inactivate the hemorrhagic lethal toxicity of a salmonella endotoxin for chick embryo. Remarkable detoxication was given by kidney extract. Also extracts of spleen, liver, and sexual glands had a relatively high ability of detoxication though the intestinal tract, heart and serum could hardly detoxicate.

The optimal temperature for the detoxication by the liver extract was from 37°C to 45°C and the optimal pH was from 7 to 8. The detoxicating factor was inactivated by heating to 65°C for 5 minutes. The detoxication was promoted by Ca^{++} or Mg^{++} but suppressed by Mn^{++} or Hg^{++}, though Fe^{++}, Cu^{++}, Co^{++} and Ba^{++} were no effective.

These characteristics seems to be almost the same as those of mammals except for the optimal pH and the dependance on the divalent cations.

It has been wellknown that tissue homogenates, sera and plasmas of many kinds of mammals are able to alternate the toxicity of bacterial endotoxins (1～8), but little is known about fowl. Inoue (9) observed that a chicken serum and a liver extract inactivated the antigenicity of an endotoxin. While, Inoue and Katsuno (10, 11) suggested that the agents inactivating the antigenicity of the endotoxin in mouse and swine tissues were different from those which inactivated the toxicity of the toxin. Therefore, it is uncertain whether chicken tissues and serum are able to inactivate the toxicity of the endotoxin or not. In this paper, the effects of chicken tissue extracts and serum on the lethal toxicity of a salmonella endotoxin for chick embryos were observed.

Materials and Methods

Serum and Tissue Extracts: White Leghorn chicks, 4 to 7 months old, were killed by bleeding. Their organs, liver, spleen, lung, kidney, sexual glands and

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small intestine, were immediately removed, weighted, homogenated with 3 volumes of cold alkaline isotonic KCl solution and centrifuged at 10,000 r.p.m. at 4°C for 30 minutes. The supernatants were used for the test of the detoxication of a salmonella endotoxin. Sera were used for the experiments immediately after segregation. In a few cases, the organs and sera were kept under freezing condition at -25°C till the experiments were performed.

Endotoxin: The endotoxin used was derived from *Salmonella pullorum* L-60131 with trichloroacetic acid according to the method of Boivin. The LD_{80} on 10-day-old chick embryos of the toxin dissolved in phosphate buffer was 120 μg.

Procedure of Detoxicating Reaction: Mixtures containing 1.5 ml of tissue extract or serum, 0.3 ml of endotoxin solution (12 mg/ml) and 1.2 ml of phosphate buffer (pH 6.9) were incubated at 37°C for 1 hour and then heated at 100°C for 5 minutes to stop the reaction. The pH of the reaction mixtures were 6.6 to 6.8. As the control, a mixture containing 1.5 ml of the KCl solution used for homogenization, in place of the tissue extracts or serum, was prepared and treated same as above. Then, these mixtures were used for assaying of the residual toxicity of the endotoxin.

Assay of Toxicity of Endotoxin: The method of Smith and Thomas (12) was used in assaying for toxicity of the endotoxin treated with or without tissue extract and serum. One tenth ml of the treated mixture was respectively dropped onto the chorioalantoic membrane of 10-day-old chick embryos. After 24 hrs of incubation, the mortality of the embryos with hemorrhagie was detected. Reduction of the toxicity was calculated with following eguation.

$$\text{Reduction of toxicity} = \left(1 - \frac{\%\ \text{mortality by tested mixture}}{\%\ \text{mortality by control mixture}}\right) \times 100\%$$

Results and Discussion

Effects of Tissue Extracts and Serum on Toxicity of Endotoxin.

Table 1 shows that the tissue extracts and serum reduced the lethality of the chick embryos by the endotoxin. The highest reduction of the toxicity, 76 per cent, was obtained by kidney extract. The reduction by the sexual glands, spleen and liver extracts were 50 to 65 per cent. Extracts of the lung, heart and digestive tract had low abilities, 20 to 40 per cent, and serum had much less ability. Farrar (6) observed that the kidney in guinea pig was the most effective organ on the lethal action of an endotoxin for chick embryos and the heart possessed an intermediate effect, but the skeltal muscle, blood and lung were much less active. Inoue and Katsuno (10) showed that the spleen, liver and kidney of mice had high effectiveness. Keen (5) demonstrated that the inactivation of the tumor-necrotizing property of an endotoxin was highly occurred by the liver, kidney, heart and spleen of rabbit. From these facts, it is known that the main organs of

chick detoxicating the toxicity of endotoxins are almost the same as those of other animals.

TABLE 1 *Detoxication of Endotoxin by Chicken Tissue Extracts and Serum*

Tissues	Reduction of toxicity	Tissues		Reduction of toxicity	
Liver	53.8± 6.5%	Heart		25.1+8.2%	
Spleen	60.8± 7.2	Small intestine		22.4±4.3	
Kidney	76.3± 5.8	Serum		11.0±9.9	
Lung	40.4±10.3	Sexual gland	Male	65.7	65.2±9.1
			Female	64.5	

Mean±S.D. of 3 males and 2 females.

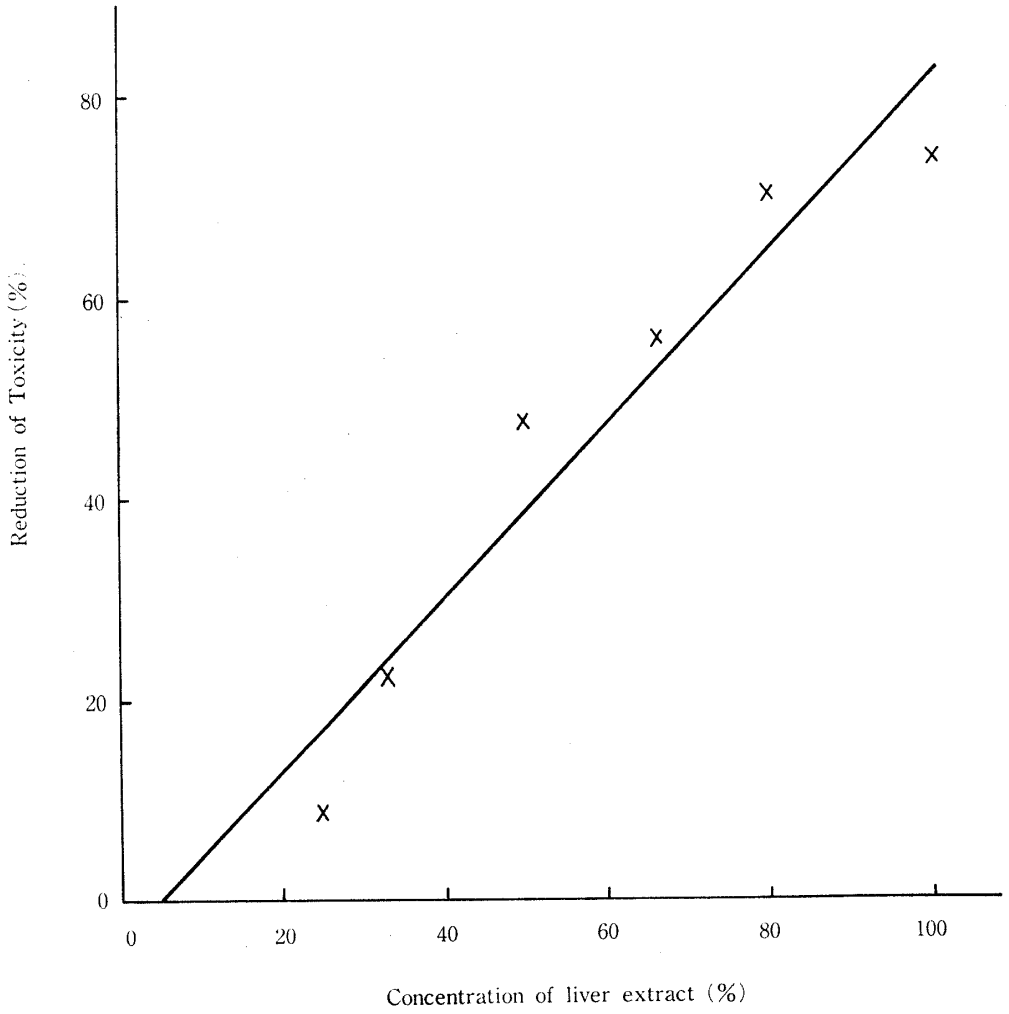

Fig. 1 Relationship between concentrations of chicken liver extract and detoxication of endotoxin.

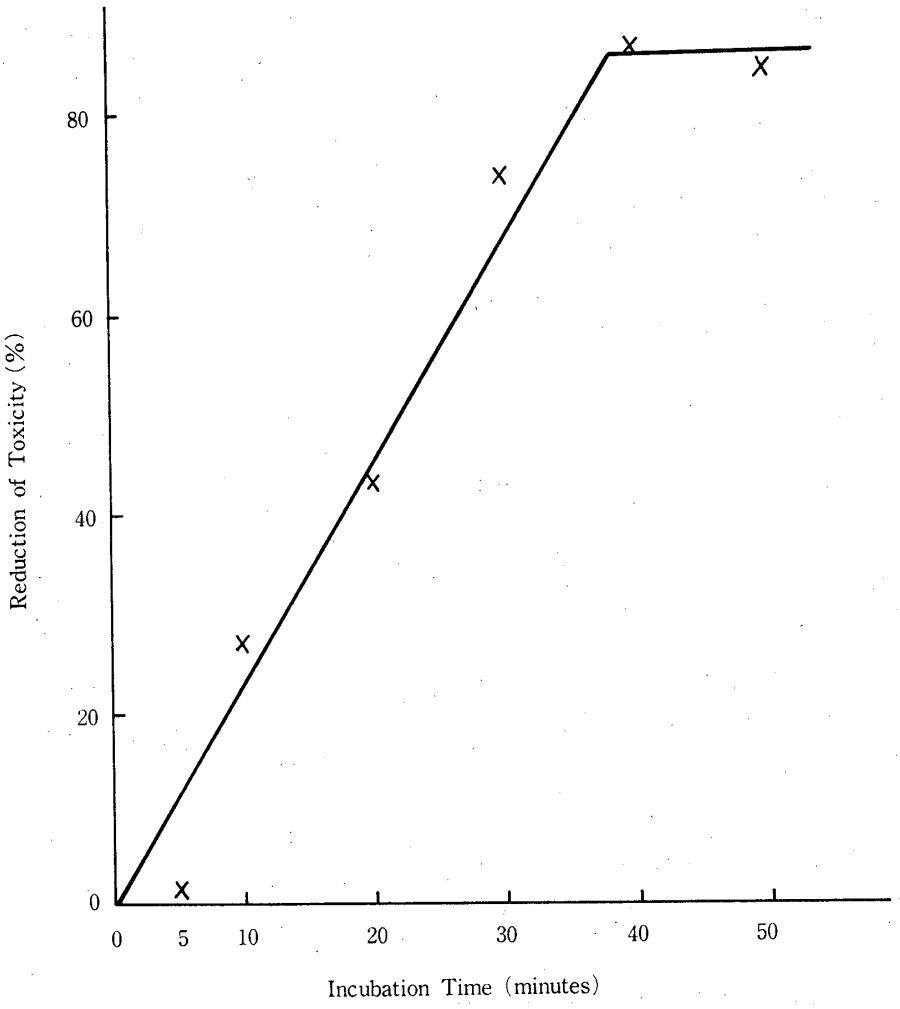

Fig. 2 Relation between incubation time and progress of detoxication of endotoxin by chicken liver extract.

Characteristics of Endotoxin Detoxicating Factor in Chicken Liver.

Using the liver extract having a relatively high ability of the detoxication, as above mentioned, the characteristics of the detoxicating agent were observed. The results are as follows.

1) Relationship between the concentration of liver extract and the rate of detoxication: The original liver extract was diluted to varying concentrations with the alkaline isotonic KCl solution and the respective diluted extracts were used for the detoxicating reaction. As the results, the rates of the detoxication varied with the concentrations of the extract and a significant regression line was obtained (see Fig. 1). Thus, it is found that the rate of the detoxication depends upon the amount of detoxicating agent in the liver extract. Similar results were reproted on the human serum (1, 3) and on the rabbit liver extract (4).

2) Incubation time and proceeding of detoxication: The reaction mixtures were incubated at 37°C during 5 to 50 minutes. Fig. 2 shows that the reduction

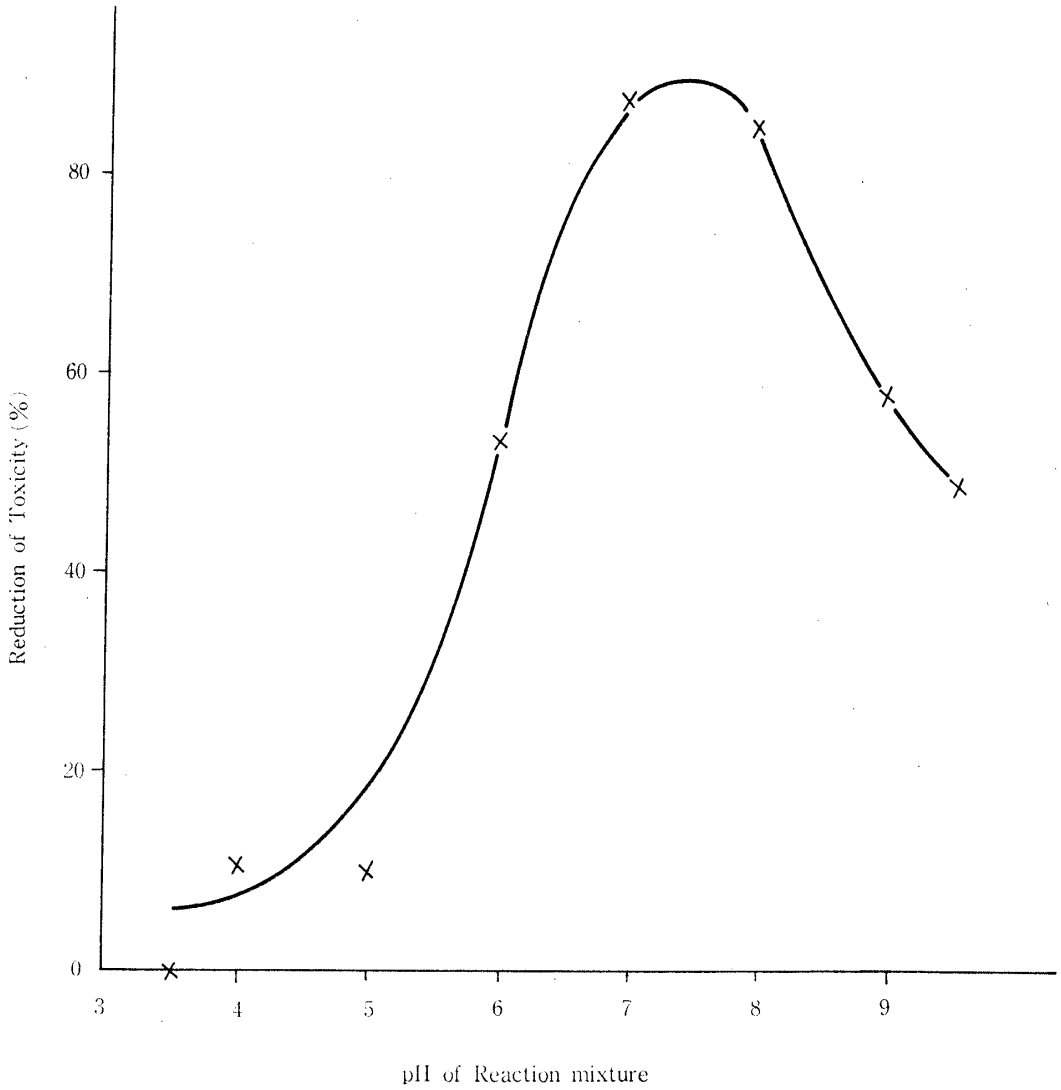

Fig. 3 Effect of pH on detoxicating activity of chicken liver extract.

of the toxicity of the endotoxin by liver extract proceeded in proportion to the incubation time and the reaction reached nearly a plateau at 40 minutes when 80 percent of toxicity was loss. Similar results has been reported in other studies on the inactivation of the tumor hemorrhaging activity or the pyrogenicity of endotoxins by human plasma (1, 3) and rabbit liver (4).

The detoxicating activity of chicken liver extract may be higher than that of dog's spleenic homogenate which achieved nearly the maximum inactivation at 3 hrs, mesured by loss of lethality for mice (8).

3) Optimal pH of the detoxicating reaction: Detoxication was tested using the liver extracts adjusted to varying pH ranged 3.5 to 9.5 with 0.1 N hydrochloric acid or 0.1 N sodium hydroxyde solution prior to addition of the endotoxin. Corresponding to the adjusted pH, 1/50 M acetic buffer (pH 3.5 to 6.0) or 1/50M

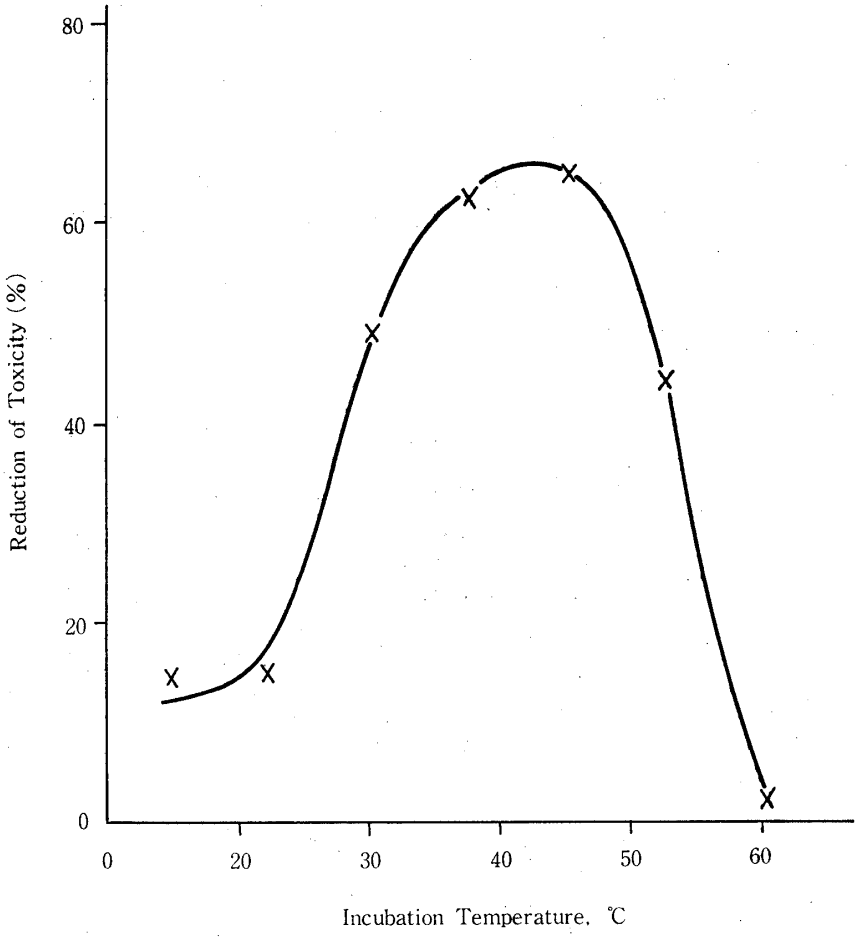

Fig. 4 Effects of incubation temperature on detoxication of endotoxin by chicken liver extracts.

and phosphate buffer solutions (pH 6.0 to 9.5) were used in the reaction mixtures. The results are shown in Fig. 3 in which it is found that the optimal pH for the detoxication was between 7 and 8, and no activity was observed in acid side, lower than pH 4.0. The optimal pH obtained here is similar to that of rabbit kidney (5) but different from that of the rabbit liver extract (4) and human plasma (1, 3) by which the maximum loss of the toxicity obtained even at pH 9 or more. While, a lot of precipitate appeared when pH of the liver extract was adjusted to lower than 5.0 so that the endotoxin detoxicating agent in the chicken liver might be denatured.

4) Effects of incubation temperature on detoxication: The inactivation of the endotoxin by the liver extract was detected at various temperatures from 15°C to 60°C. As shown in Fig. 4, the optimal temperature of the detoxication was between 37°C and 45°, and a moderate detoxication was observed at 30°C and 50°C, though the reaction did not proceed at 15°C and 60°C.

It has been reported that the optimal temperature of a rabbit liver extract

was between 37°C and 70°C (4) and that the inactivation of endotoxin by human plasma proceeded rapidly at higher temperatures, even 90°C (3). So the optimal temperature of the chicken liver seems to be different from those of other animals.

5) Effects of divalent cations on the detoxication: It has been known that the detoxication of endotoxin by human plasma was supressed by some divalent cations (2). So the experiments were done to find out whether the detoxication by the chicken liver extract was affected by divalent cations.

After the liver extract was dialyzed against phosphate buffer solution (pH 7.0), it was centrifuged at 10,000 r.p.m. for 30 minutes to remove the precipitate. The mixture consisting of 1.5 ml of the supernatant, 0.3 ml of the endotoxin solution, 0.3 ml of one of the divalent cation solutions listed in Table 2 and 0.9 ml of the physiological salt solution were incubated to test the detoxicating activity. Hence, the concentration of the cations used was 10^{-3} M in final.

TABLE 2. *Effects of Divalent Cations on Detoxicating Activity of Chicken Liver Extract.*

Divalent cations added	Reduction of toxity	Divalent cations added	Reduction of toxity
$MgCl_2$	61.5%	$CoCl_2$	38.5%
$CaCl_2$	75.0	$CuCl_2$	25.0
$MnCl_2$	13.5	$BaCl_2$	25.0
$FeCl_2$	38.5	$HgCl_2$	13.5
None	38.5		

The detoxication was supressed by Hg^{++}, Mn^{++} and promoted by Ca^{++}, Mg^{++}, while not affected by Fe^{++}, Cu^{++}, Co^{++}, Ba^{++} as shown in Table 2. These results are different from those in the other reports which showed the supression by Ca^{++} in human plasma (2, 3) and in rat plasma (7), though Ca ion did not interfere the activity of rabbit liver extract (4). This fact is also different from a recent report by Von Eschen and Rudbach (13) in which they described that inactivation of endotoxin by sera of chicken and other birds was supressed by Ca^{++}.

6) Effects of heating on the activity of the liver extract: The liver extract was previously heated in water baths kept at 45°C, 55°C and 65°C for varying times from 0 to 40 minutes, and then used for the detoxicating reaction.

As a result, it was found that the detoxication was remarkably diminished by pre-heating for 5 minutes at 65°C and for 10 minutes at 55°C though the diminition at 45°C was relatively small (see Fig. 5). Thus, the endotoxin detoxicating agent in chicken liver seems to be heat labile as same as the factors found in human (1, 3) and rat (7) plasmas and in rabbit liver extract (4, 5). While, as shown in Fig. 4, the detoxication was the hightest at about 45°C of incubation temperature and relatively high at 55°C. The facts suggest that the rate of detoxication was faster than that of diminition by heating under these temperatures.

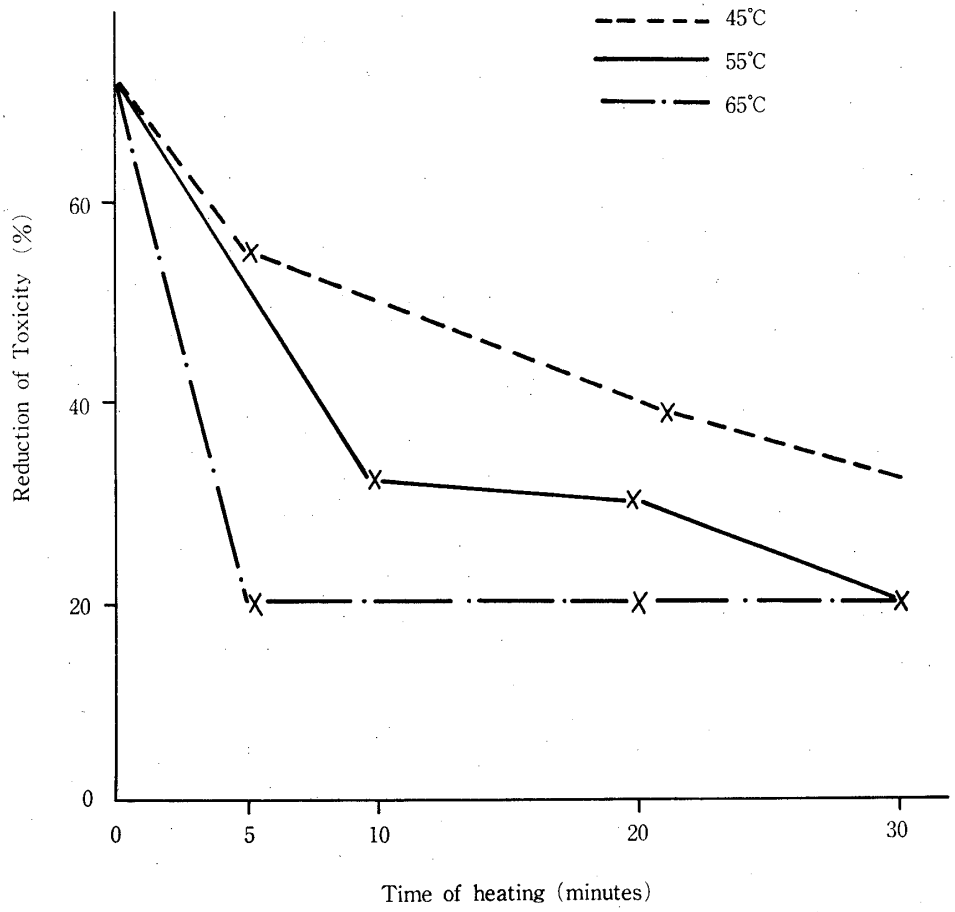

Fig. 5 Effect of heat treatment on detoxication by chicken liver extract.
The mixture whithout endotoxin was pretreated at each temperature for varying times and endotoxin was added, then incubated at 37°C for one hour.

Conclusion: Putting these results together, it has become clear that chicken tissue extracts can alter the toxicity of the endotoxin and that the characteristics of the active factor are almost the same as those of other animals except for the optimal temperature and the dependance on divalent cations.

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