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### **Opinion on the action of Cernitin.**

We have been requested to investigate the preparations designated as Cernitin T 60 and Cernitin GBX with respect to their therapeutic effects.

Clinical observations indicate that these preparations are capable of effecting a cure of infections caused by bacteria or virus.

The mode of action of these preparations appears to be highly complex. Nevertheless, it seemed worthwhile to elucidate whether the subjective improvement could be verified by objectively measurable effects.

The effect on bacterial toxins, on enzymes and on antibodies can be satisfactorily demonstrated experimentally in vitro and in vivo.

#### **1. Effect of Cernitin T 60 and Cernitin GBX on streptolysin.**

##### *Method:*

A streptolysin-0 solution in physiological saline solution containing 40 streptolysin-0 units per ml was prepared. Cernitin T 60 was added to this streptolysin solution to give final concentrations in the toxin solution of 0.1 %, 0.5 %, 1 % and 10 %.

Cernitin GBX was dissolved in distilled water containing 20 % polyethylene glycol 400, giving a solution containing 1 % Cernitin GBX. This 1 % solution was treated with lyophilized streptolysin-0, so that a final concentration of 40 units per ml was obtained.

Owing to the sensitivity of streptolysin to heat, the mixture of toxin and Cernitin was stored in a cooler at + 4° C. The reaction was permitted to proceed for various intervals of time, after which the activity of the streptolysin was determined. A streptolysin-0 solution which did not contain Cernitin was stored under identical conditions and served as a reference standard.

Loss of streptolysin activity was determined by treating the streptolysin test-sample and the streptolysin control solution with an equal part of sodium thioglycolate (which served as reducing agent) and three parts of a physiological saline solution which had been

diluted by 1:5. After the mixing the streptolysin test-sample and the streptolysin control solution remained 10 minutes at room temperature and the toxin activity was measured as follows:

Tube No.	Reduced streptolysin solution	Physiological saline solution	Buffer solution	2 % Blood cell suspension
1	0.5 ml	—	0.5 ml	0.25 ml
2	0.45 ml	0.05 ml	0.5 ml	0.25 ml
3	0.4 ml	0.1 ml	0.5 ml	0.25 ml
4	0.35 ml	0.15 ml	0.5 ml	0.25 ml
5	0.3 ml	0.2 ml	0.5 ml	0.25 ml
6	0.25 ml	0.25 ml	0.5 ml	0.25 ml
7	0.2 ml	0.3 ml	0.5 ml	0.25 ml
8	0.15 ml	0.35 ml	0.5 ml	0.25 ml
9	0.1 ml	0.4 ml	0.5 ml	0.25 ml
10	0.05 ml	0.45 ml	0.5 ml	0.25 ml

As seen from the Table, a series of test tubes was prepared into which portions of toxin solution were pipetted, the volume of each portion decreasing progressively by 0.05 ml and amounts of physiological saline solution then being added to each tube to make up a total volume of 0.5 ml. To this solution was added 0.5 ml of a buffer solution (composition: 1.45 g  $\text{KH}_2\text{PO}_4$ ; 7.6 g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ; 4.8 g NaCl; aqua dist. 1 lit.) and 0.25 ml of a 2 % blood cell suspension.

Using this experimental equipment, it was possible to test, both qualitatively and quantitatively, the deleterious effect of the residual amounts of toxic substances by means of various concentrations of active material, the time of action of the detoxicating substances being taken into account. In addition, the preparation of a series of dilutions of toxin and Cernitin permitted detection of even partial detoxication of the streptolysin-O, this being of great value to the full exploitation of the detoxicating effect of Cernitin T 60 and Cernitin GBX.

The haemolysis was noted after immersion for 45 minutes in a water-bath at 37° C.

*Result:*

a. *Cernitin T 60*

Forty units of streptolysin-0 in 1 ml of 10 % or 1 % solution of Cernitin T 60 were completely inactivated within 30 minutes at + 4° C.

In a 0.5 % Cernitin T 60 solution, the destruction of the blood cells by streptolysin was almost completely arrested after 2 hours at + 4° C (see Table 1). On the other hand, a 0.1 % Cernitin T 60 solution could not suppress the effect of the toxin even after 10 days at + 4° C (see Table 2).

b. *Cernitin GBX*

Inactivation of streptolysin-0 commenced in a 1 % Cernitin GBX solution only after two days of exposure at + 4° C. On the ninth day, the streptolysin (40 units per ml) was completely inactivated (see Table 3.)

**II. Effect of Cernitin T 60 and Cernitin GBX on staphylolysin.**

*Method:*

Staphylolysin was introduced into a 1 % Cernitin T 60 solution and into a 1 % Cernitin GBX solution in amounts which gave final concentrations of 1 unit per ml.

Both Cernitin T 60 and Cernitin GBX were diluted with a 1 % glucose broth and 20 % polyethylene glycol 400 was also added to the latter substance. Our choice of glucose broth for the staphylolysin experiments was dictated by the fact that the toxin remains active for a longer time in this medium than in physiological saline solution. Measurements of the toxic activity of this staphylolysin preparation at 37° C were made at various time intervals.

A staphylolysin solution of the same concentration, treated in an identical manner, served as a reference standard.

The toxic activity of the solutions was determined as follows:

A series of 10 reagent tubes was prepared, each containing 1 ml of a 1 % glucose broth. One ml of the mixture of toxin and Cernitin (or of the toxin control solution) was introduced into the first tube.

After thorough mixing, 1 ml of this solution was transferred to the second tube. This procedure was then repeated throughout the entire series until a row of geometrically decreasing concentrations of the toxin and Cernitin preparation and of the control solution was obtained. One ml of solution from the last tube was discarded. One ml of a 2 % rabbit erythrocyte suspension was pipetted into each tube. The tubes were then immersed in a water-bath at 37° C and the haemolysis was recorded after two hours.

*Result:*

No inactivation of the staphylolysin by 1 % Cernitin T 60 or by 1 % Cernitin GBX solution could be observed even after 10 days' exposure at 37° C.

**III. Determination of the influence of Cernitin T 60 and Cernitin GBX on enzymes.**

*A. Urease:*

One g of Cernitin T 60 was added to 100 ml of a urease solution containing 1 mg urease per ml. (This procedure was repeated with Cernitin GBX). These solutions, as well as a control solution formulated in an identical manner but without addition of Cernitin, were stored at room temperature.

After 1, 2, 5 and 24 hours, the activities of the urease preparations were determined according to the method recommended by the firm of C. F. Boehringer and Sons, Mannheim, for their reagents. The test procedure is appended to the Tables.

The sera used had the following urea concentrations, expressed in mg %:

26	37	67	170	308 and
27	40	68	220	320.
35	65	160	256	

*Result:*

Neither Cernitin T 60 nor Cernitin GBX were capable of affecting the activity of urease.

The results obtained in two experiments are presented in Table 4.

B. *Acid phosphatase:*

To test the influence of Cernitin on acid phosphatase, preparations of serum maintaining the following activities, expressed as mMoles of acid phosphatase, were prepared:

1.95	3.0	5.2	11.5
2.4	4.5	6.0	24.0 and
2.6	5.1	7.1	36.0

Cernitin T 60 or Cernitin GBX was added to the sera so that the final concentrations in the serum amounted to 1 ‰. The preparations were then incubated at 37° C and the activities of the acid phosphatase were determined after 1, 2, 4 and 6 hours.

Serum prepared and treated in an identical manner, but without the addition of Cernitin, was used as a reference standard.

The acid phosphatase was assayed according to the method recommended by the firm of C. F. Boehringer and Sons, Mannheim, for their reagents. The test procedure is appended to the Tables.

*Result:*

Cernitin T 60 and Cernitin GBX were added to solutions of acid phosphatase of various activities to give final concentrations of 1 ‰. This concentration of Cernitin T 60 or of Cernitin GBX was incapable of influencing the activity of the acid phosphatase even after 6 hours of exposure at 37° C.

C. *Glutamate pyruvate transaminase:*

To test the influence of Cernitin on glutamate pyruvate transaminase, preparations of serum maintaining the following activities of the enzyme, expressed as mU, were prepared:

14	40	93	185
22	62	123	227 and
31	85	156	333.

Cernitin T 60 and Cernitin GBX was added to the sera to give final concentrations of 1 ‰. The serum-Cernitin-mixing was then incubated at 37° C and the activities of the enzymes were determined after 1, 2, 4 and 6 hours.

Serum prepared and treated in an identical manner but without the addition of Cernitin was used as a reference standard.

The glutamate pyruvate transaminase activity was determined according to the method recommended for their reagents by the firm of C. F. Boehringer and Sons, Mannheim. The test procedure is appended to the Tables.

*Result:*

Cernitin T 60 and Cernitin GBX were added to sera with different glutamate pyruvate transaminase activity to give final concentrations of 1 ‰. This concentration was not capable of affecting the glutamate pyruvate transaminase activity even after 6 hours of exposure at 37° C.

*Summary:*

Clinical observations indicate that cases of inflammation caused by bacteria or virus could be favourably influenced by Cernitin.

Although the action of these preparations seems to be highly complex, attempts were made to achieve an objective appraisal of their effect on bacterial toxins and on enzymes occurring in the human organism.

It was found that the destruction of blood cells caused by streptococcal toxins could be arrested by both Cernitin T 60 and Cernitin GBX. Cernitin T 60 was especially active, a 1 ‰ solution leading to inactivation of a very high streptolysin concentration within half an hour. Even a 0.5 ‰ solution of Cernitin T 60 could inactivate large doses of the toxin after 4 hours at 4° C.

Neither a 1 ‰ solution of Cernitin T 60 nor a 1 ‰ solution of Cernitin GBX had any effect on the staphylococcal toxins which caused the destruction of blood cells.

Neither Cernitin T 60 nor Cernitin GBX appeared to have any effect on the enzymes: urease, acid phosphatase or glutamate pyruvate transaminase.

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**Table 1.**  
**Inactivation of streptolysin-0 in a 0.5 % solution of Cernitin T 60 at 4° C.**

For dilution of the streptolysin-0 solution, see p. 3.

Tube numbers

Time of effect	1	2	3	4	5	6	7	8	9	10
Control soln. 24 hours	++++	++++	++++	++++	++++	++++	++++	++++	∅	∅
30 min.	++++	++++	++++	+	(+)	∅	∅	∅	∅	∅
1 hour	++++	++++	++++	++	++	∅	∅	∅	∅	∅
2 hours	+	++	∅	∅	∅	∅	∅	∅	∅	∅
4 hours	+	+	∅	∅	∅	∅	∅	∅	∅	∅
24 hours	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅

++++ = complete lysis of rabbit erythrocytes (fully active toxin).  
 +++ = 75 % of erythrocytes are haemolyzed (toxic activity reduced by 25 %/∅).  
 ++ = 50 % of erythrocytes are haemolyzed (toxic activity reduced by 50 %/∅).  
 + = 25 % of erythrocytes are haemolyzed (toxic activity reduced by 75 %/∅).  
 ∅ = No haemolysis (toxin is inactive).

**Table 2.**  
**Inactivation of streptolysin-0 in a 0.1 % solution of Cernitin T 60 at 4° C.**

For dilution of the streptolysin-0 solution, see p. 3.

Tube numbers.

Time of effect	1	2	3	4	5	6	7	8	9	10
Control soln. 10 days	++++	++++	++++	++++	++++	++++	++++	++	∅	∅
10 days	++++	++++	++++	++++	++++	++++	+	∅	∅	∅

- ++++ = Complete lysis of rabbit erythrocytes (fully active toxin).
- +++ = 75 % of erythrocytes are haemolyzed (toxic activity reduced by 25 %/u).
- ++ = 50 % of erythrocytes are haemolyzed (toxic activity reduced by 50 %/u).
- +
- ∅ = 25 % of erythrocytes are haemolyzed (toxic activity reduced by 75 %/u).
- ∅ = No haemolysis (toxin is inactive).

**Table 3.**  
**Inactivation of streptolysin-0 in a 1 % solution of Cernitin GBX at 4° C.**

For dilution of the streptolysin-0 solution, see p. 3.

Tube numbers.

Time of effect	1	2	3	4	5	6	7	8	9	10
Control soln. 9 days	++++	++++	++++	++++	++++	++++	++++	++++	∅	∅
1 days	++++	++++	++++	++++	++++	++++	++++	∅	∅	∅
2 days	++++	++++	++++	++++	++++	++++	∅	∅	∅	∅
5 days	++++	++++	++++	++++	++++	++++	∅	∅	∅	∅
7 days	++++	++++	++++	++++	++++	∅	∅	∅	∅	∅
9 days	∅	∅	∅	∅	∅	∅	∅	∅	∅	∅

- ++++ = Complete lysis of rabbit erythrocytes (fully active toxin).
- +++ = 75 % of erythrocytes are haemolyzed (toxic activity reduced by 25 %).
- ++ = 50 % of erythrocytes are haemolyzed (toxic activity reduced by 50 %).
- + = 25 % of erythrocytes are haemolyzed (toxic activity reduced by 75 %).
- ∅ = No haemolysis (toxin is inactive).

**Table 4.**  
**Determination of urea with urease preparations containing**  
**1 % of Cernitin T 60 or of Cernitin GBX.**

The urea content is given in mg %.

Time of effect of Cernitin on urease at room temperature.	Urease + Cernitin T 60	Urease + Cernitin GBX	Urease without Cernitin (control)
1 hour	26	25	24
2 hours	25	26	25
5 hours	25	24	24
24 hours	23	24	24
1 hour	308	309	311
2 hours	310	311	309
5 hours	309	311	312
24 hours	292	295	293