



# Basic Study of Cernilton

### *Immuno-Serological Findings*

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## 1. Introduction

CERNILTON is a pollen preparation extracted from a mixture of 8 different pollens and contains as its chief ingredient some 20 kinds of amino acids. It also contains small amounts of sugar and glycoside but no sedimentary proteins.

Judging from its method of extraction and the results of analysis, there seems little danger of the preparation having an antigenic or allergenic property. Nevertheless, immunoserological studies are carried out here for the sake of safety.

## 2. Materials

Materials used were Cernitin T-60 and Cernitin GBX which are extracted and purified from crude pollen. The former contains mainly amino acids (no proteins) while the latter mainly lipid. Both were placed at the authors' disposal by Tobishi Pharmaceutical Co., Ltd. after chemical analysis.

## 3. Methods

### *1) Antibody-producing Properties of Cernitin T-60 and Cernitin GBX*

Animals used were male rabbits weighing about 2.5 kg, which were immunized according to the dosage schedules below. Ten days after the last injection the blood was drawn and serum separated for determination of the antibody titre.

#### *Group A (Cernitin T-60 Administration Group)*

1. 2,400 g Rabbit 2 ml/kg of Cernitin T-60 (30 mg/ml) injected intravenously 3 times weekly for 2 weeks (total dosage 360 mg/kg).

2. 2,500 g Rabbit    ||                    ||
3. 2,500 g Rabbit 0.05 ml (15 mg) of Cernitin T-60 solution (300 mg/ml) injected into foot pad at 5 sites (2 times weekly, 4 times all).
4. 2,400 g Rabbit    ||                    ||

*Group B (Cernitin GBX Administration Group)*

1. 2,600 g Rabbit 0.05 ml (36 mg) of Cernitin GBX original liquid injected into food pad at 5 sites (2 times weekly, 4 times all).
2. 2,500 g Rabbit    ||                    ||
3. 2,400 g Rabbit    ||                    ||

*2) Sensitogenicity of Cernitin T-60 and Cernitin GBX (Anaphylactic Shock: Guinea Pigs)*

Cernitin T-60 and Cernitin GBX were injected subcutaneously into the groin in 10 male guinea pigs weighing about 250 g, 2 times weekly 4 times all (each time 15 mg). Two weeks after the last injection the antigens (15 mg/ml) were injected intravenously for observation of anaphylactic shock.

*3) Sensitogenicity of Cernitin T-60 and Cernitin GBX Arthus' Phenomenon: Rabbits*

To the rabbits immunized according to method 1) 0.1 ml of the antigens (15 mg/ml, 3 mg/ml, 0.6 mg/ml) was injected subcutaneously to the back (after shaving off the hairs) 10 days after the last injection, and the presence of reddening and induration was examined.

*4) Determination of Antibody Titre*

*A. Precipitin Reaction:* Immunized rabbit-serums obtained under method 1) were activated (56°C, 30 mins) and diluted serially with 5% gum Arabic physiological saline. The precipitin reactions of Cernitin T-60 and Cernitin GBX were then examined routinely.

*B. Haemagglutination Reaction:* Immunized antisera obtained under method 1) were studied routinely according to the agglutination reaction test for sensitized corpuscles, using sheep corpuscles sensitized with Cernitin T-60 and Cernitin GBX, as follows: Antisera (inactivated) were made subject to adsorption by sheep-corpuscles washed beforehand. Sheep-corpuscles were first treated with tannic acid, sensitized with the antigens (1 mg/ml in case of Cernitin T-60 and 0.1 mg/ml in case of Cernitin GBX, dissolved or suspended in phosphate buffer solution with a pH of 6.2), and then added to the diluted series of antisera at a dose of 0.05 ml. Agglutination values were determined after storing the antisera so obtained at a temperature of 37° C for 2 hours and at room temperature for 20 hours.

*C. Gel-Precipitin Reaction:* The test was carried out in a routine manner according to Ouchterlony's method. Antiserums obtained under method 1) were used as testing samples while Cernitin T-60 and Cernitin GBX as antigens.

## Results

### *A) Serological Study of Rabbit-Serums Immunized with Cernitin T-60 and Cernitin GBX*

Serological study was made of the serums (7 rabbits) immunized according to method 1).

#### *A) Precipitin Reaction (double layer method):*

Table 1

		Precipitin Reaction (diluted antiserums)			
		Undiluted Antiserum	X2	X4	X8
Rabbits Group A (T-60 Administration Group)	No. 1	–	–	–	–
	2	–	–	–	–
	3	–	–	–	–
	4	–	–	–	–
Rabbits Group B (GBX Administration Group)	No. 1	–	–	–	–
	2	–	–	–	–
	3	–	–	–	–

Notes: Cernitin T-60 (30 mg/ml) was used as precipinogen for antiserums of Group A (Nos. 1-4). For antiserums of Group B (Nos. 1-3) Cernitin GBX (1.5 mg/ml) was used.

As may be noted from the Table, results were negative in all cases, revealing no antibodies at all.

#### *B. Haemagglutination Reaction:*

Results of haemagglutination reaction test carried out according to method 4) are as given in Table 2.

Table 2

		X10	X20	X40	X80	X160	X320	X640
Rabbits Group A (T-60 Administration Group)	No. 1 a	+++	+++	++	+	+	-	-
	b	+++	+++	++	+	-	-	-
	No. 2 a	+++	+++	++	+	+	-	-
	b	+++	+++	+	-	-	-	-
	No. 3 a	+++	+++	+++	++	+	+	-
	b	+++	++	++	+	-	-	-
	No. 4 a	+++	+++	++	+	+	-	-
	b	+++	+++	++	+	-	-	-
Rabbits Group B (GBX Administration Group)	No. 1 a	-	-	-	-	-	-	-
	b	-	-	-	-	-	-	-
	No. 2 a	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-
	No. 3 a	-	-	-	-	-	-	-
	b	-	-	-	-	-	-	-

Notes: a...Corpuscles sensitized with Cernitin T-60  
b...Corpuscles sensitized with Cernitin GBX

The agglutination values of the rabbit-serums of Group A (immunized with Cernitin T-60) were 160-320 with Cernitin T-60 sensitized corpuscles. Even with Cernitin GBX sensitized corpuscles the values were as high as 40-80. The rabbit-serums of Group B (immunized with Cernitin GBX) showed no agglutination at all with Cernitin T-60 or Cernitin GBX sensitized corpuscles.

#### C. Gel-Precipitin Reaction (Ouchterlony's Method):

Precipitin reaction test as carried out according to Ouchterlony's method, with the antiserums (Group A 4 cases, Group B 3 cases) placed in the center and the antigens in the peripheral areas, as shown in the left chart, revealed negative results in all cases with no appearance of precipitation lines.

#### B) Sensitigenocyt of Cernitin T-60 and Cernitin GBX

A. *Anaphylactic Shock (Guinea Pigs)*: Antigens (15 mg/ml) were administered intravenously at a dose of 1 ml to guinea pigs sensitized according to method 2) and observation was made as to the presence of anaphylactic shock.

Table 3

	Shock Injection	Guinea Pigs	Symptoms
Cernitin T-60 Sensitized Group	Cernitin T-60 (30 mg/ml) 1 ml i.v.inj.	1. 240 g	– (Survived)
		2. 260 g	– ( II )
		3. 250 g	– ( II )
		4. 280 g	– ( II )
		5. 260 g	– ( II )
Cernitin GBX Sensitized Group	Cernitin GBX (15 mg/ml) 1 ml i.v.inj.	6. 250 g	– ( II )
		7. 270 g	– ( II )
		8. 240 g	– ( II )
		9. 250 g	– ( II )
		10. 260 g	– ( II )

As may be seen from the Table, no cases showed anaphylactic shock and all cases survived.

*B. Arthurs' Phenomenon (Rabbits):* Rabbits were immunized according to method 1). After shaving off the hairs, the antigens (0.1 ml) were administered subcutaneously to the animals at 6 sites and observation was made as to the presence of the symptoms of reddening and induration. Results are given in Table 4.

**Table 4**

		Cernitin T-60			Cernitin GBX		
		15 mg/ml	3 mg/ml	0.6 mg/ml	15 mg/ml	3 mg/ml	0.6 mg/ml
Rabbits Group A Immunized with T-60	No. 1	1.2 X 1.1 cm	–	–	–	–	–
	2	1.4 X 1.2 cm	–	–	–	–	–
	3	1.0 X 0.8 m	–	–	–	–	–
	4	0.7 X 0.8 cm	–	–	–	–	–
Rabbits Group B Immunized with GBX	No. 1	–	–	–	–	–	–
	2	–	–	–	–	–	–
	3	–	–	–	–	–	–

Note: Figures indicates sizes of reddening (in diameters).

As the results would show, there was observed a slight degree of reddening when Cernitin T-60 was injected subcutaneously at a concentration of 15 mg/ml in rabbits immunized with Cernitin T-60. No bleeding, necrosis or induration was noted, however.

#### 4. Summary and Conclusion

Pollen extracts Cernitin T-60 and Cernitin GBX were studied immunoserologically to examine their antigenicity and sensitinogenicity, with results as summarized below.

1) Examination was made as to the antibody-producing properties of Cernitin T-60 and Cernitin GBX using the serums of immunized rabbits. Results were negative in all cases by means of the precipitin

reaction (double layer method) and gel-precipitin reaction (Ouchterlony's method) tests. By means of haemagglutination test the agglutination value was slightly elevated in Cernitin T-60 immunized rabbit-serums but not in Cernitin GBX immunized serums.

2) Observation was made as to anaphylactic shock in guinea pigs strongly sensitized with Cernitin T-60 and Cernitin GBX, but the results were negative in all cases.

3) Observation was also made as to Arthus' phenomenon using rabbits strongly sensitized with Cernitin T-60 and Cernitin GBX. When Cernitin T-60 was used as the antigen and given at a concentration of 15 mg/ml, there was observed a slight degree of reddening in rabbits immunized with Cernitin T-60. At lower concentrations no symptoms were revealed at all. Results were negative in all rabbits immunized with Cernitin GBX.

It may be said in conclusion that both Cernitin T-60 and Cernitin GBX have either no or, if any, an extremely slight degree of antigenicity or sensitinogenicity.