

A study on the effect of digested pollen extract* on the frequency of spontaneous lung infections in rats.

Introduction:

In a study carried out at the Norwegian Institute of Work Science, Department of Occupational Health, it was found that addition of a specially digested pollen extract to the food of rats, was preferred, when the test animals were given free choice between three different food mixtures. All of these food mixtures presumably being fully satisfactory combinations of necessary nutritious elements (protein, carbohydrates and fat, vitamins, minerals and trace elements) (1).

There was no change in the original three mixtures during the experimental except for the addition of 1 % of Cernilton to one of the food mixtures. It is well-established that rats (as also many other animals) have a pronounced ability to choose a food mixture containing substances, which may prove necessary to them (2, 3). We found those results interesting and they gave a rational basis for further studies as to a possible effect of pollen extract in the form of Cernilton. If being a general roborating substance, it might be possible to explain at least to some degree the results reported on the good results obtained by giving Cernilton tablets to patients with chronic prostatitis and also to patients with infectious diseases e.g. in the upper respiratory tract.

Macroscopic pathological-anatomical lung infections.

In the same report there was by the macroscopical pathological-anatomical examination observed a marked difference as to the frequency of infected lungs in the control animals compared with those which had been given Cernilton in their food. An english edition of the above mentioned report were published in an almost identical form as the first mentioned report in Norwegian (4). Here the author is concluding: "in the tests carried out using self-selection cages, a tendency was detected, which might be interpreted to mean that the Cernilton diet may contain one or more substances that are useful to the living organism, although it is not possible to offer any explanation for the action mechanism of such an assumed effect".

As to the pathological findings on autopsy of the lungs they were concentrated on the difference in the frequency of findings of gross macroscopical-pathological changes between the two groups: Controls and Cernilton-treated rats.

The following definitions were given as "marked pathological findings" (5):

- a) Definite enlargement of the lungs with slow and uncomplete retraction. Muco-purulent excrete from the trachea either spontaneously or by slight pressure on the lung for further examination.
- b) Distinct palpable nodules which by section contained large amounts of purulent secret, "infected bronchiectasis".
- c) Well-defined dark red or greyish-red atelectasis which by section contained purulent secretion.

These findings which according to many authors who have studied the lung pathology in rats (5,6,7,8) are remarkably common and they are of decisive importance when using rats for studying experimentally, affections in their lungs.

Pathological-anatomical changes of the kind mentioned are very frequent when rats reach an age of 18 - 24 months or more. Mostly one will find such lung pathological changes in 50-75 per cent when carefully examining the lungs by autopsy, even though these rats very seldom show obvious clinical symptoms or signs of advanced lung infection before being killed and subjected to autopsy.

By carefully standardizing of all controllable factors, the author has succeeded in keeping the number of such changes at a level at about 10 - 20 per cent, when using rats in lung experiments. This has partly been obtained by using antibiotic treatment (given intraperitoneally once a week during the observation period).

In this first experiment the autopsy was carried out as a routine and there had been no original intention to study especially the lung changes. The four groups of animals used in functional tests, studying the possible influence of Cernitin on spontaneous motoric activity were kept on the diet on 1% Cernilton for a total period of 6 - 7 months. In this Cernilton-treated group only one animal out of 12(6 males and 6 females) showed a macroscopic lung change as defined above. In the control group one rat died before end of the experiment, nothing definite was mentioned by the physician, carrying out the routine autopsy, which thus did not indicate lung infection. Of the remainder 11(5 males and 6 females) there was altogether 6 with macroscopical-pathological lung changes of the kind described above. Because of the often varying findings as to lung infections and the small number of animals in these tests, the author stated: "In the opinion of the author, it is not possible to present any definite conclusions on the basis of the above. These findings may possibly indicate a certain effect, but if a comparison is made with the often widely varying results obtained from otherwise untreated control animals in the same age

the results do not permit any definite conclusions to be drawn even though they may be interesting per se and can be considered to motivate continued investigations on the lines proposed here".

In the above mentioned experiment the difference as to macroscopic lung infections between the control group and the Cernilton-treated group may be calculated statistically (Table 1).

Table 1.

Effect on pathological lung changes in preliminary tests with 1 per cent Cernilton in the food.

Treatment	Total No. of animals	No. of rats with macr. pathol. anat. lung changes		S.E. of the percentage
		No.	Per cent	
Standard food without Cernitin	12	6	50	15.1
Standard food with Cernitin	12	1	8.3	8.2

Difference between Cernilton-treated and standard food-treated animals in per cent:

$$41.3 \pm 17.2$$

$$t = 2.42$$

$$0.05 > P > 0.02$$

This indicated a statistically, probable significance that the results might not have arisen by chance. If everything being equal except for the addition of Cernilton to the food, there is presumed to be less than 5 per cent but more than 2 per cent probability for an accidental result of this kind. Nevertheless the small number of animals and the somewhat lower frequency in the treated group than usual and, more important, the higher frequency in the control group, kept on our standard food, convinced the author that it was impossible to draw any other conclusions from these experiments than what was stated above.

Experimental conditions.

As a consequence of this first experiment it was therefore carried out two supplementary experiments, one during the winter 1968 - 69, and one during the winter 1969 - 70. These experiments were carried out in an other animal stable, where the basic conditions were not so good and carefully controlled as in the animal stable in the first experiment. The first mentioned experiments were carried out where the functional tests took place. This makes it of primary importance to keep all controllable conditions as optimal and constant as possible.

The author holds the view that it might be of interest to find out whether the difference (if it should occur again) would be more or less pronounced when the conditions in the stable as draught, temperature, humidity, quality and care of cages etc. was not kept at the same optimal level as in the first experiment.

Two test series comprising originally altogether 40 animals in each group were carried out. Before starting the differentiation in food-supply (at about 4 - 5 months of age) there died two animals in the intended control-group and one in the intended Cernilton-group.

Cernitin (given as Cernilton) was added to the food in an amount of one per cent. The experiment was started as mentioned above some time after dividing animals comparing each other as to sex and weight and as mentioned above at an age of 4 - 5 months and it was continued for 6 months.

Results:

During the 6 months of experiment there died 3 animals, all after more than three and a half months: one in the Cernitin-treated group and two in the control group. By autopsy of these rats that was marked lung infections in the one animal belonging to the Cernitin-treated group. One of them dying spontaneously in the control group had a big tumor, in the control group had distinct pathological infections in the lungs.

At the end of the experiment a careful macroscopical pathological-anatomical examination was carried out of all remaining animals, making at that time 38 rats in the Cernitin-treated group and 36 rats in the control group. All rats were of course presented with blind numbers to the examiner (the author). By this autopsy there was found 13 animals with lung changes of the kind defined above in the Cernitin-group, and 21 in the control group.

Table 2

Frequency of macroscopical pathological-anatomical lung infections at autopsy at the end of experiment in Cernilton-treated and control rats.

Treatment	No. of rats at onset	Died spontaneously during experiment	No. of rats at end of experiment	Macr. pathol. anat. lung infections		
				No.	Per cent	S.E.
Standard food	33	2	36	21	58.3	+8.19
Standard food with Cernilton	39	1	38	13	34.2	+7.91

Difference between controls and Cernitin-treated animals in per cent:

$$23.9 \pm 11.39$$

$$t = 2.098$$

$$0.05 \quad P \quad 0.02$$

This may seem to indicate a probable statistical significance in favour of the group being given Cernilton-containing food compared with the standard food mixture. It may, however, be more conclusive when taking into consideration also the animals dying during the latter part of the experiment.

Table 3.

Frequency of pathological-anatomical lung infections in animals dying after more than three and a half months (14 weeks) of the experiments or at the autopsy at the end of experiments (6 months).

Treatment	No. of animals at onset of experiment	Total No. of rats with macr. pathol. anat. lung infections		
		No.	Per cent	S.E.
Standard food	38	22	57.9	± 7.69
Standard food with Cernitin	39	14	35.9	± 8.01

Difference between controls and Cernitin-treated animals in per cent:

$$22 \pm 11.10$$

$$t = 1.982$$

$$P \approx 0.05$$

When making these animals into consideration there are hardly a 5 per cent significance any longer.

Comments:

The results of these experiments may be of interest when compared with the first one (Table 1). The less pronounced favourable results in the last studied groups (Table 2 and 3) may indicate that an eventual effect of Cernilton, which most probably may be due to a general roborating effect of the preparation, are unable to prevent the deleterious effect of less satisfactory conditions. This seems according to the author's opinion to strengthen the view that there may possibly be a positive effect because of a general roborating influence e.g. due to the supply of a balanced combination of trace elements, vitamins, and a small amount of essential amino acids which may in itself give the type of effect which we in lack of a more precise expression are mentioning: general roborating effect. That there also is a certain streptolysin inhibitory effect of a hitherto not

(9, 10) but whether this factor has any well-defined effect as to infections in the respiratory tract in rats is yet an open question.

Comparing the results obtained on the frequency of spontaneous lung infections in rats by adding Cernilton to the food, with observation reported as to the positive effect of Cernilton on infectious diseases in man, it also seems to suggest the view that the general roborating effect is the most probable explanation of a positive effect. The effect on chronic prostatitis (11, 12, 13, 14, 15, 16, 17, 18) and on infections in the upper respiratory tract (19, 20, 21, 22).

The statistical evaluation of the results seems to indicate a tendency in the direction that there may be a beneficial effect of Cernilton in these cases of infectious diseases, but the effect is seldom so pronounced that they are given a satisfactory statistical result by evaluation. This of course may be due to the mostly very small groups examined, but it may also be explained because of the very difficult differential diagnostic problems in many of these infectious diseases.

It seems to the author that the general roborating effect yields a rational explanation for presuming an effect in cases where either the amount of or the balance between the substance which is unsatisfactory in the daily diet. In acute cases or cases where antibiotic- or chemotherapeutic treatment may be indicated, Cernilton is by no means an alternative and may be contraindicated when running the danger that it may be used not as a complement but as a substitute to well-defined indications for antibiotic- and chemotherapeutic treatment. The effect of Cernilton may be to support the natural resources of the organism to counteract infections. This will mostly be actual for longterm treatment or as prophylaxis against exacerbations e.g. in chronic prostatitis or as prophylactic agent in very frequently recidiving infections in the upper respiratory tract.

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