# IMMUNOLOGIC ASSESSMENT OF A MODEL OF EXPERIMENTAL BERYLLIOSIS

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The development of specific morphological changes of berylliosis in rats is accompanied by the development of a positive cutaneous allergic reaction to soluble beryllium compounds and also by the formation of both humoral and sessile autoantibodies against lung nucleoproteins.

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For a long time it has proved impossible to reproduce in animals a chronic form of berylliosis, a condition in the pathogenesis of which considerable attention is directed toward immune mechanisms [5, 10, 11]. The intratracheal injection of large doses of beryllium caused the development of beryllium pneumonia [9, 10]. It was only after the injected dose of beryllium oxide had been reduced to 20-5 mg that a lesion of the lungs morphologically resembling the clinical form of chronic berylliosis was obtained in rats [2, 3, 6]. However, because of differences in immunologic reactivity of man and animals, morphological similarity may be the result of different processes and need not reflect immunologic changes in the body. No information could be found in the literature concerning the immunologic similarity between experimental berylliosis produced in rats and chronic berylliosis in man.

The object of the present investigation was to study the immunologic indices in rats with experimental berylliosis and compare them with the results of clinical investigations in chronic berylliosis.

## EXPERIMENTAL METHOD AND RESULTS

Inbred August rats weighing 100-120 g were used in the experiments. Experimental berylliosis was produced by the intratracheal injection of 2.5 mg finely dispersed beryllium oxide dust in 0.5 ml physiological saline.

Chronic berylliosis in man is characterized by a highly specific cutaneous allergic reaction to soluble beryllium compounds (the Curtis test [8]). For this reason, in the experiments of series I the Curtis test was carried out 25-60 days after treatment of the experimental rats, i.e., at the stage of morphologically developed berylliosis. Intact rats (control I) and animals 1-2 months after intratracheal injection of 25 mg quartz (control II) were used as controls. Rats with developed silicosis were used as controls of specificity, because the existence of a pathomorphological process in the lungs of the experimental rats could have a nonspecific activating action on the Curtis skin test.

In the first variant of the experiment beryllium chloride was injected intradermally in a dose of 2.5-5 $\mu$g in 0.02 ml physiological saline into 54 rats. The skin reaction was observed for several days. The intensity of the reaction was assessed by the diameter of the zone of infiltration and of hyperemia surrounding it (Table 1).

The presence of a reaction, although it was less marked, in the intact animals can be explained by the considerable primary toxic action of beryllium chloride.

The difference between the reactions of the experimental and control rats to injection of beryllium chloride were seen most clearly 48 h after performance of the skin test: the mean diameter of the zone of infiltration and hyperemia in the experimental rats was 4.73±0.68 mm, in the intact rats 1.84±0.40 mm, and in the rats with silicosis 2.43±0.72 mm ($P < 0.01$ and $P = 0.02$ respectively).

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TABLE 1. Distribution of Rats by Groups Depending on Intensity of Reaction to Intradermal Injection of 2.5-5 μg Beryllium Chloride

| Intensity of reaction (in mm) | 24 h after injection of $BeCl_2$ | | | 48 h after injection of $BeCl_2$ | | |
|---|---|---|---|---|---|---|
| | expt. | control I | control II | expt. | control I | control II |
| 0 | – | 5 | 1 | 2 | 7 | 2 |
| 1–3 | 10 | 9 | 3 | 7 | 10 | 3 |
| 4–5 | 10 | 6 | 2 | 7 | – | 2 |
| 5 | 7 | – | 1 | 8 | – | – |

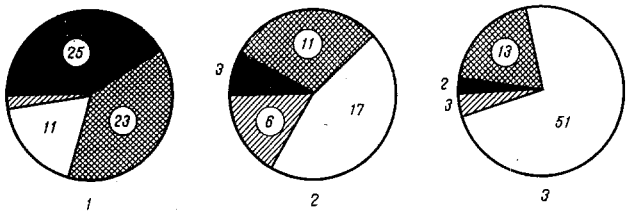


Fig. 1. Intensity of scarification skin test with 0.05-0.2% $BeCl_2$ solution. 1) In rats with berylliosis; 2) with silicosis; 3) intact rats. Black sectors show strongly positive, cross-hatched areas weakly positive, obliquely shaded areas nonspecific, unshaded areas negative reactions; numbers in sectors show number of rats in group.

Intradermal tests were used in order to ensure a more accurate dosage of beryllium chloride injected. However, they were complicated to perform and were not justified in use because it was impossible to inject the desired dose accurately. For this reason, to confirm the results obtained, a scarification skin test was carried out on 166 rats with 0.05-0.2% beryllium chloride solution. Two tests were carried out side by side on each animal: one with physiological saline, the other with beryllium chloride. If a reaction was obtained to physiological saline, the reaction with beryllium chloride was regarded as nonspecific (Fig. 1).

It will be clear from Fig. 1 that positive reactions were distinctly predominant in rats with berylliosis and virtually absent in intact animals. The reaction of rats with silicosis of one month's duration was weaker than the reaction of intact animals, in agreement with data indicating the depressed reactivity to various allergens of patients with silicosis [7]. However, the intensity of the scarification test in the animals with silicosis of two months' duration was somewhat higher than in the intact animals; a higher percentage of nonspecific reactions also was observed.

In the experiments of series II, in view of results showing the appearance of humoral antibodies against the lung nucleoproteins of rats with experimental berylliosis in patients with berylliosis, the precipitating antibodies against the analogous antigen were studied in the experimental rats.

Using the ring precipitation reaction with an initial antigen concentration of 2 mg protein/ml, precipitating antibodies against lung nucleoproteins* were found in the blood serum of the animals tested (Table 2).

The antibody titer in the experimental and control rats was maintained at the level 1:5-1:20. These groups were therefore compared on the principle of the presence or absence of antibodies. The appearance of antibodies in the blood serum of intact rats may be associated with previous inflammatory diseases of the respiratory organs.

These results are in complete agreement with the results of investigation of the sera of patients with berylliosis by Hoigne's method [1].

In the experiments of series III tests were carried out for cell-attached antibodies against nucleoproteins isolated from the lungs of rats with experimental berylliosis. Preliminary experiments showed that the dose of nucleoprotein for intradermal injections should not exceed 20-40 μg protein because of its toxicity. After injection of nucleoprotein in this dose (in a volume of 0.02 ml), the reaction at the point of injection reached its greatest extent in the intact animals after 24 h, and in rats with disease of one month's duration after 48 h. In 17 of the 20 rats with developed berylliosis, a zone of infiltration sometimes accompanied by hyperemia developed, its mean diameter being 2.65±0.44 mm. No hyperemia developed in

*The nucleoprotein fraction was isolated from the lungs of intact August rats or rats with berylliosis by Belozerskii's method [4].

TABLE 2. Presence of Precipitins Against Nucleoproteins of Rat Lungs in Experimental and Control Animals

| Antigen used for test | Group | Days after beryllium administration | No. of rats | No. of sera with antibodies | % of total no. | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|
| Lung nucleoproteins of rats with experimental berylliosis | Exptl. | 7–30 | 56 | 17 | 31 | 1.45 | 0.23 |
| | | 40–90 | 51 | 31 | 61 | 25.2 | < 0.01 |
| | Cont. I | – | 58 | 12 | 21 | – | – |
| Lung nucleoprotein of intact rats | Exptl. | 30 | 17 | 9 | 53 | 0.79 | 0.4 |
| | Cont. I | – | 28 | 11 | 39 | – | – |

20 intact animals, infiltration was observed 3.4 times less frequently, and its mean diameter was 0.75± 0.37 mm ($P < 0.01$).

These investigations thus showed that the development of specific morphological changes of berylliosis in the lungs after intratracheal injection of beryllium oxide is accompanied by the development of a positive cutaneous allergic reaction to soluble beryllium compounds and also, evidently, by the formation of both humoral and sessile autoantibodies against lung nucleoproteins. This indicates that the immunologic reactivity of these rats is similar to that of patients with chronic berylliosis, and that this type of lesion can be used as an experimental model of chronic berylliosis.

## LITERATURE CITED


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