Genetic control of adjuvant-induced arthritis in rats

A widely used animal model of chronic inflammatory disease used for screening of anti-inflammatory or immunosuppressive effects of drugs, or both, is adjuvant-induced arthritis in rats (Pearson, 1956; Newbould, 1963; Andersen, 1970). However, the difficulty in evaluating these effects arises owing to the variation of manifest symptoms of the disease. Some experimental data suggest that the disease frequency might in part be dependent on genetic factors (Glen & Gray, 1965; Swingle, Jaques & Kvam, 1969). As this influence has not been studied systematically, we began an investigation of this aspect.

Several inbred strains of rats were tested (Table 1). Two of them (LEW and AVN) were chosen for further genetic analysis as there was a sharp difference in the incidence of the disease between them. A classical genetic method of analysis using both inbred strains, their first (F_1), second (F_2), and both backcross generations (B_1 and B_2), was employed. An estimate of the number of genes involved was based on testing the significance of differences between observed and expected disease frequency in all generations used, by use of a modified minimum χ^2-test (Elston, 1966).

Adjuvant arthritis was induced in 7–10 weeks old animals by an intradermal injection of 0·1 ml of complete Freund's adjuvant (5 mg of *Mycobacterium tuberculosis* vaccine/1 ml of mineral oil) into the left hind paw. Swelling of non-injected paws as the most prominent feature of the disease, was evaluated on the 21st day after the adjuvant administration.

Since there was no difference between females and males of either inbred strain (number of responding/total number: LEW: 9/9 and 10/10; AVN: 0/10 and 1/20, respectively), the pooled data of both sexes were analysed. The number of animals in

Table 1. *Incidence of adjuvant-induced arthritis in different inbred strains.*

	LEW	BP	Wistar	BN	AVN
Number of examined	19	5	10	5	30
Number of responding	19	2	3	1	1

Table 2. *Incidence of adjuvant-induced arthritis in different generations derived.*

	P_1 (LEW)	B_1 ($P_1 \times F_1$)	F_1 ($P_1 \times P_2$)	F_2 ($F_1 \times F_1$)	B_2 ($P_2 \times F_1$)	P_2 (AVN)
Number of examined ..	19	30	34	28	30	30
Number of responding ..	19	20	6	10	6	1
Probability of incidence ..	0·9794	0·6003	0·2212	0·3655	0·1306	0·0400
Observed incidence ..	100%	67%	18%	36%	20%	3%

all generations together with observed percentage of disease incidence and its probability, are given in Table 2. As the value of the F_1 generation shows, a higher resistance to induction of adjuvant disease is a dominant character. The measure of dominance, D, estimated according to Bruell (1962), reached the value of 0·65 indicating a partial dominance. The value of χ^2-test of deviations of observed frequencies of arthritis from expected ones was found to be 2·05 which is not statistically significant ($P > 0{\cdot}50$, d.f. = 3). Therefore, comparing this result with the frequency of the disease in other inbred strains, which suggests a polygenic control,

it is obvious that the polygenic system involved is of an additive character. The variation of the disease incidence should be, therefore, according to Grüneberg (1952) considered as a "quasi-continuous" one.

We reported here a so far not described case of nearly zero frequency of adjuvant-induced arthritis in the AVN strain of rats. On the other hand, there exists a 100% incidence of the disease in the LEW strain. These strains could be a suitable material for investigation of detailed pathogenesis of the disease.

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An apparatus for facilitating the measurement of tracer movements in a large number of muscle strips

Studies of the efflux of radioactive tracers from muscle have assisted the understanding of processes involved in contraction (Langer, 1968; Shanes & Bianchi, 1960; Van Breeman, 1969). The time course of tracer release is usually measured at convenient time intervals by assay of the medium in which the tissue has been suspended (Burgen & Spero, 1968). Should the specific activity of the tracer be low, the radioactivity released may very rapidly fall below the limits of detection, and it becomes necessary to assay the tracer remaining in the muscle strips as a function of time. Since it is frequently desirable to study tracer efflux under conditions similar to those under which contractility is measured, the strips should be set up under tension. The apparatus described below enables up to twenty-five tissues to be suspended under a tension of approximately 0·5 g in a single organ bath. The tissues may be removed rapidly for assay.

The tissues are suspended between small stainless-steel hooks which fit over wire projections from the base of a support, and stainless-steel springs attached to small pegs, which slot into the upper platform of the support, where they are retained by a low raised lip. The apparatus, (Fig. 1A) is placed in a 600 ml beaker the bath volume of which is effectively 400 ml and the physiological solutions are admitted to, and drained from, the bath under pressure. The solution is gassed down the central column of the tissue support, and the temperature is maintained by allowing the organ bath to stand in a temperature-controlled water bath.

In a typical experiment equilibrium is attained after 1 h in a suitable physiological solution. The bath is then drained and filled with medium containing the radioactive tracer, and after the required pre-incubation period, the bath is again drained, and the tissues are washed thereafter at frequent intervals, with fresh physiological solution. At convenient intervals one or more tissues are removed from the support and assayed