Variation in Epiphyseal Maturation of Medial Epicondyle of Humerus in Inbred Strains of Mice. (25569)

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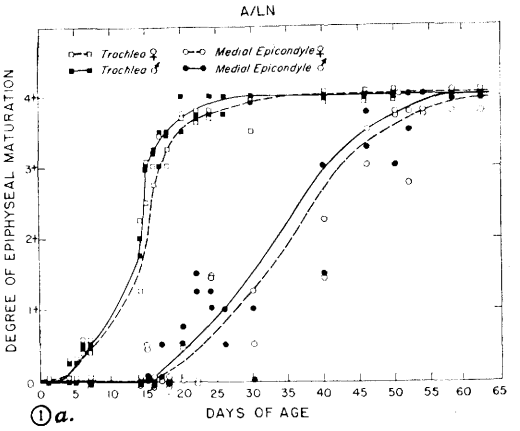
In previous studies, patterns of development of epiphyses of knee and head of femur of mice during second year of life were described(1,2). These studies were carried out to determine sources of difference in occurrence of spontaneous degenerative joint disease in various inbred strains; and more particularly, whether development of osteoarthritis of the knee in various strains paralleled progression of epiphyseal closure as animals aged. No such relationship was observed. The present studies deal with ossification and closure of epiphyses during first 2 months of life rather than the second year. It was thus hoped to learn (1) whether there is a correspondence between rates of early and late epiphyseal development within individiual strains and (2) whether there is a relationship between early skeletal maturation, and later development of osteoarthritis.

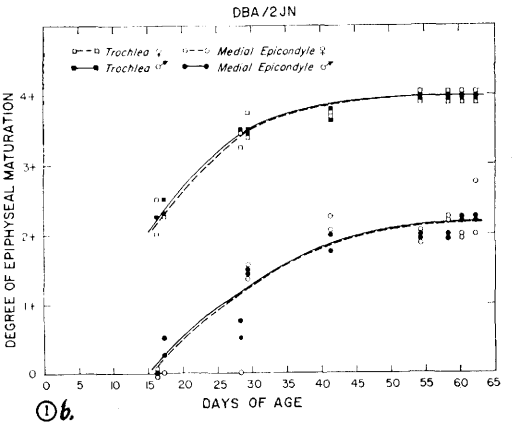
Materials and methods. Two experiments were carried out: *Exp. A.* Development of epiphyses at distal end of right humerus was traced histologically in 2 groups of mice during the first 2 months of life as follows: Two male and 2 female littermates were killed at daily intervals during the first month and, non-littermates following weaning at intervals of 2 days until day 62. In the *first* group were strains A/HeN, A/LN, C3H/HeN and BALB/cAnN, born in February, and housed in groups of 4 or 5. All animals received Purina Lab. Chow and water *ad lib.* In the *second* group were C3H/HeN and DBA/2JN mice, born July and August, housed initially following weaning, in groups of 12 to 15 in large cages containing 2 food hoppers and water bottles, rather than shoebox type cages as in the previous group. As space became available, they were separated into smaller groups as in the preceding. Tissues were fixed either in neutral formalin or Bouin's solution. Decalcification and staining procedures were as previously described. Serial sections were made in a coronal plane. *Exp. B.* Specific ages of mice were chosen for comparing ossification of medial epicondyle with osteoarthritis of the knee, since some strains varied largely in these 2 measurements. The experiment included strains having earlier and later maturation times of the medial epicondyle and knee, as well as differences in occurrence of the joint disease (A/LN, DBA/2JN, STR/1N and C57BL/6JN). An attempt was made to obtain 15 pairs (littermates) of males for each strain. In each pair, 1 animal was killed at 60 days of age for examination of elbow, while the other was killed at 16 months for evaluation of arthritis. Intercurrent diseases reduced the number of animals in several instances (Table I). Production difficulties made consistent use of littermates impossible in STR/1N and precluded use of certain other strains, such as C57L/HeN which might have been desirable because they have a high degree of epiphyseal closure of the knee(1). Knees were macerated with papain and evaluated for osteoarthritis according to criteria described previously. The following scheme was employed for grading degree of epiphyseal maturation: 0 = no ossification of epiphysis; 4+ = complete ossification and union of epiphysis. It was possible to estimate the extent of ossification and of closure to the closest ½ in units of 1 to 4+. "Degree of epiphyseal maturation" was computed by dividing the sum of the grade of ossification and of closure by 2. It was noted, as observed by others, that foci of degenerated cartilage were often present in the epiphyseal plate without immediate vascular penetration or bony union. These were disregarded, and only vascular resorption of the plate or communication of hematopoietic tissue between diaphyseal and epiphyseal spaces recorded.

Description of epiphyses of distal humerus: Configuration and sequence of epiphyseal ossification of the distal humerus of mice corre-

sponded closely to that described in the rat by Becks and coworkers(3). There was a main center of ossification in the trochlea that united with the diaphysis of the humerus immediately distal to the olecranon fossa, and a secondary center in the medial epicondyle. The plate of the latter was perpendicular to the former. The earliest changes in formation of the secondary center were a vesicular enlargement of lacunae of cartilage cells and penetration of blood vessels from the perichondrium into them. Epiphyseal plates were intact for a short time, focal disruption by penetrating blood vessels taking place before the epiphysis had completely expanded or ossified. The present method of grading degree of epiphyseal maturation has taken into account both degrees of ossification and of closure of the plate. The epiphyses of the distal end of the humerus of mice have been studied previously by Johnson(4) and Dawson(5). Secondary centers in the capitellum and trochlea were found by the former at 5 days, 11 hours of age; the medial condyle center at 9 days; and one in the lateral condyle at 19 days. Dawson observed the distal epiphysis to close at 21 days.

Results. Exp. A. In first and second groups, there was little variation between animals in rate of development of the distal epiphysis of the humerus within individual strains. Development was completed at 28 days of age in the A/LN mice; 32 days in BALB/cAnN; and at 36 days in A/HeN. Development of the medial epicondyle epiphysis was uniform in most strains (A/LN, C3H/HeN, BALB/cAnN; also DBA/2JN and STR/1N in Exp. B), but less so in 2 (A/HeN and, in experiment B, in C57BL/6JN. There was considerable variation between strains in degree of maturation achieved by the medial epicondyle by day 62. In strain A/LN, there was virtually complete ossification with fusion in all animals 52 or more days of age (Fig. 1A) while in others, as DBA/2JN (Fig. 1B), no fusion had taken place. The findings in the C3H/HeN animals were comparable regardless of whether animals were born in February or July. In neither trochlear nor medial epicondyle was an unequivocal difference in rate of maturation found between





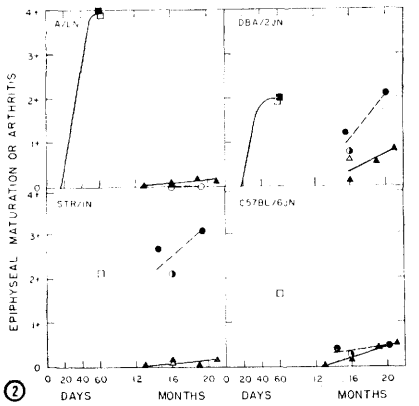


FIG. 1. Rate of epiphyseal maturation at distal end of humerus in 2 inbred strains of mouse. *a.* A/LN; *b.* DBA/2JN.

FIG. 2. Comparison of development of epiphyses of medial epicondyle, knee and osteoarthritis in 4 strains of mice. ■ avg epiphyseal maturation of medial epicondyle (Exp. A); □ avg epiphyseal maturation of medial epicondyle (Exp. B); △ avg epiphyseal maturation of knee (concurrent exp., Ref. 9); ▲ avg epiphyseal maturation of knee (previous exp., Ref. 1); ○ (half-solid) avg osteoarthritis of knee (Exp. B); ● avg osteoarthritis of knee (previous exp., Ref. 1).

TABLE I. Maturation of Epiphysis of Medial Epicondyle and Osteoarthritis of Knees in 4 Strains of Mice (Males).

Strain	Epiphyseal maturation* No. animals	Avg degree (0-4+)	Osteoarthritis of knees† No. animals	Avg severity (0-4+)
A/LN	15	4.0 ± .2	14	0
DBA/2JN	15	2.0 ±0	15	.8 ±.18
STR/1N	14	2.1 ± .03	15	2.1 ±.31
C57BL/6JN	15	1.4 ± .33	11	.2 ±.26

* 60 days old. † 16 mo old.

the sexes; the process was more rapid in males, if anything.

Exp. B. Development of the epiphyses of the A/LN and DBA/2JN at 60 days was identical with those noted in Exp. A, and degree of osteoarthritis of the knees of the 4 strains quite comparable to that observed in the same strains several years earlier(2).

Our findings are summarized in Table I. The 2 strains having a low degree of osteoarthritis, A/LN (0) and C57BL/6JN (0.2), which are not significantly different with respect to this measurement ($t = .6$, $p \sim .5$), had, however, highly significantly different degrees of epiphyseal maturation (A/LN was 4.0; C57BL/6JN was only 1.4; $t = 7.67$, $p < .01$). The STR/1N and DBA/2JN mice had nearly identical degrees of epiphyseal maturation (2.1 and 2.0 respectively) but differences in severity of osteoarthritis (2.1 and 0.8) were highly significant ($t = 3.58$, $p < .01$) although there was wide variation within a strain.

Discussion. Our studies demonstrate that early closing epiphysis of the medial epicondyle of the humerus develops at different rates in various strains of mice. These rates are quite constant in these strains over many generations. They were not affected, within limits of experiments, by season in which animals were born or numbers housed together. Although infantile diarrhea occurred in the C3H/HeN colony in Exp. A, first group, the mice studied here were selected to be unaffected by it and had bone changes comparable to those in Exp. A, second group, where they had normal weight curves.

No distinct sex differences in rate of epiphyseal maturation of the elbow were observed. This is in contrast to findings in late closing epiphyses of hip and knee(1,2) in which ossification is more advanced in males. The findings are at some variance with the observations of Silberberg and Silberberg(6), that during early development the female mouse skeleton is physiologically older than the male, although the male thereafter may overtake and exceed the female. Comparison of humeral maturation with that of the knee of the same strain indicates that there is no consistent relationship between rate at which epiphyses mature early in life in mice and ultimate progression of those that remain open till late age (Fig. 2).

Silberberg and Silberberg observed that the rate at which epiphyseal cartilage undergoes the sequence of proliferation, regression and bony union, *i.e.*, skeletal time curve, varies among different strains of mice. C57BL mice, showing slow skeletal growth, had more spontaneous degenerative joint disease and responded to high fat diet with accelerated skeletal aging and more osteoarthritis than did DBA mice that had a rapid skeletal growth under ordinary conditions(7). Furthermore, young mice were more susceptible to accelerated epiphyseal and articular cartilage aging, induced by high-fat intake for 5 months, than were older ones(8). Because previous studies(1,2) failed to disclose a close relationship between late epiphyseal aging and articular degeneration in mice, we inquired whether the differences in prevalence of spontaneous osteoarthritis in different strains of mice correspond to early changes in skeletal development rather than late ones. We demonstrated that maturation of early closing medial epicondyle epiphysis does not bear any relationship to ultimate development of osteoarthritis in the same strain. Thus, sources of differences in which spontaneous osteoarthritis occur with varying frequency in different strains of mice must be sought outside early or late rates of skeletal "aging" of these strains.

Summary and conclusions. Rate of ossification and closure of epiphyses of the medial epicondyle of the humerus was studied in 7 inbred strains of mice. The rate of develop-

ment varied significantly in different strains but bore no relationship to occurrence of degenerative joint disease, or to ultimate epiphyseal development of knee or head of femur previously found to occur late in second year of life.

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Heterogeneity of Insulin-Antibody Complexes in Rabbits and Guinea Pigs. (25570)

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Presence of insulin-binding antibodies in human subjects treated with commercial mixtures of beef, pork insulin(1) and in guinea pigs and rabbits immunized with beef or pork insulin(2) has been established with use of insulin-I^{131} and paper chromatoelectrophoresis of insulin antiserum mixtures. Kinetic studies of rates of formation and dissociation of insulin-antibody complexes and analysis of equilibrium mixtures have indicated the heterogeneity of complexes formed in human antiserums(3-6). Heterogeneity in this case appears to be due to presence of 2 (or more) distinct orders of antibody combining-sites rather than to differences in stoichiometric proportions of insulin and antibody in the complexes(5,6). Since human antiserums contain antibodies to both beef and pork insulins, heterogeneity of insulin-antibody complexes might be due to antibodies directed against different antigenic determinants in beef and pork insulin or to separate antibodies directed against multiple distinct antigenic sites in each insulin derived from a single species(6). In our study guinea pigs and rabbits have been immunized with *either* beef *or* pork insulin. Insulin-binding antibodies in antiserums of these animals react with insulin to form heterogeneous antigen-antibody complexes.

Methods. Analytic and technical methods follow procedures described by Berson and Yalow(1,5,6). In "equilibrium state" studies, mixtures of antiserum and beef or pork insulin (containing trace amounts of same species' insulin-I^{131}) were incubated at 37° until equilibrium was reached (usually about 4 hours) and then analyzed by paper chromatoelectrophoresis for insulin bound to antibody(B) and unbound ("free") insulin (F). Considerations based on the law of mass action indicate that when antiserum concentration is kept constant while insulin concentration is varied, the ratio B/F is a linear function of concentration of bound insulin (B) only *if* insulin is univalent *and* all antibody combining-sites are of a single order(5,6). A curvilinear relationship is expected if insulin is multivalent and/or if there is more than a single order of antibody combining-sites. In 5 guinea pig and 3 rabbit antiserums studied, plots of B/F *vs.* B were curvilinear (Fig. 1), a finding which is consistent with either of the last 2 alternatives.

Results. In "transient state" studies, only dissociation experiments are analyzed. Following attainment of equilibrium between

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