

## Crossed Immunoelectrophoresis of Sera from Adult through Aged Mice (41301)

ALFRED J. CROWLE AND JANET L. MILLER

*Webb-Waring Lung Institute and Department of Microbiology and Immunology, University of Colorado School of Medicine, Denver, Colorado 80262*

---

**Abstract.** In male and female A/J mice ranging from young adults (3 months old = 0.11 mean life span) through senility (1.0 mean life span), 21 serum antigens were analyzed quantitatively and qualitatively by crossed immunoelectrophoresis (X-IEP). No age-related qualitative changes were seen. C-3 globulin, transferrin, and unknown antigen No. 32 remained quantitatively unchanged in this life range. Ceruloplasmin and  $\alpha_2$ -macroglobulin peaked at 6 months and thereafter also remained constant. Eleven antigens fell, 3 after 3 months, 5 after 6 months, and 3 after 12 months of age. Hemopexin and  $\alpha_1$ -antichymotrypsin tended to rise during aging. The immunoglobulins were quite variable, reaching "mature" levels at 6 months and later varying independently of each other. The 5 constant antigens will be useful directly, and as comparators for X-IEP analyses of all mouse serum antigens, to assess health and disease. The 11 regularly changing antigens will be useful as multiple indicators of aging. With the X-IEP characteristics now established of these 21 serum antigens in adult mice of different ages, mouse serum X-IEP offers uniquely multifactorial objective testing by which to study the biology and pathology of this popular species of laboratory animal.

---

Detailed qualitative and quantitative knowledge of the serum antigens throughout the life span of an animal species will be useful in several ways. Thus, it is a multifactorial baseline reference of "normality" for that species from which age-related changes can be identified and studied, different strains of the species compared, and physiologic or pathologic abnormalities used to examine antigen metabolism and function. This kind of knowledge can be obtained most efficiently with crossed immunoelectrophoresis (X-IEP), because X-IEP can simultaneously both identify and quantitate over 40 serum antigens and, most importantly, compare them all with one another within individual tests for multiple ratio analyses that definitively identify even minor changes among the antigens (1-4).

Life-span analyses of some human serum antigens have been done (5, 6), and X-IEP has been used to quantitate multiple human serum antigens in children (1) and young to old adults (5). But the heterogeneity of human populations and the impossibility of freely manipulating them makes life-span X-IEP analyses of sera from laboratory animals an especially attractive alternative for studying aging and physiology as re-

flected by serum antigens, particularly from the mouse with its many inbred and thoroughly characterized strains. X-IEP patterns of sera from different individuals of A/J mice of the same age and sex, for example, are so much alike as almost to have been taken from one mouse (4). Data from an aging colony of inbred mice therefore should be much more uniform than could be obtained from any human population, and age-related trends of serum antigen composition that much more evident.

We have mapped the most prominent A/J mouse serum antigens detected by X-IEP (4) and have charted their development in newborn through juvenile to adult mice (7). Here we present the adult life-span continuation of the latter study with X-IEP analyses of 21 serum antigens from A/J mice extending from young adults through terminal senility.

**Materials and Methods.** *Mice and sera.* A/J mice bred in our own animal quarters were maintained under uniform conditions, as previously described, to a mean life span (MLS) of 26.6 months (8). Sera from four healthy female mice of each age were individually analyzed, the good health (lack of infection or tumors) being confirmed for

each donor by autopsy immediately after bleeding. The animals were bled out from the jugular vein. Serum was collected from blood clotted for 1 hr at 37° and analyzed the same day as bleeding to avoid storage-induced changes in serum X-IEP patterns. Preliminary experiments had shown that antigens like  $\alpha_1$ Lp and C-3 change qualitatively and progressively if serum is stored at -20° (see Refs. (4, 9)). Storing serum at -70° and thawing a sample no more than once seemed to prevent these changes, but we still preferred using fresh serum.

*Antisera.* Rabbit antisera to normal human serum and to normal mouse serum were prepared as previously described (4).

*Crossed immunoelectrophoresis.* This technique, identification of the measured antigens, and our methods of planimetric quantitation have been described earlier (4, 7).

For every X-IEP pattern, the ratio of the area of each antigen loop to that of added carbamylated human transferrin marker was calculated (7, 10). But then to simplify comparisons between quantitations for all the 21 antigens of the nine different ages examined, the data have been plotted as ratios to the quantities of each antigen found in serum from mice 3 months old, because this is the age of mice most commonly accepted as "young adult" (11) and because it is the standard we used in a similar quantitative study of newborn and juvenile A/J mouse serum antigens (7).

The spread of  $\pm$  "standard error of the mean" shown on each graph is larger than such a value was for any single antigen or set of sera because of the way it was derived. Thus, it was the mean of standard errors of the mean for each antigen depicted in a graph over the entire period of study. For example in Fig. 2 it is the mean of all the standard errors of the means for all measurements of C-3 globulin, antigen No. 32, and transferrin. The spread thus amply brackets chance variations in the quantities of any single antigen at any given age, and values outside these areas are probably genuinely different from the "normal adult" (i.e., 3 months of age) value.

**Results.** Measurements and statistical evaluations are summarized in Table I.

These data have been converted to proportions of the values for each antigen to the same values in sera from 3-month-old mice, an age of mice most popularly used as "adults" in immunological experiments, in order to reveal changes in their serum concentrations with aging. These proportional values, graphed in Figs. 2-8, indicate that the antigens are divisible into groups that remain constant throughout life, drop with aging, rise with aging, or are highly variable. Figure 1 shows a typical X-IEP pattern for senile mice to both illustrate its overall "normality" and to identify antigens discussed in this paper.

*Constant antigens.* The antigens which remain constant are divisible into two subgroups: those which have reached ultimate levels at 3 months of age, and those which reach such levels at 6 months of age (Figs. 2 and 3, respectively). C-3 globulin, transferrin, and unidentified antigen No. 32 remained at 3-month levels throughout adulthood into old age (24 months), and C-3 and No. 32 into senility (27 months). There was some indication that transferrin rose slightly in this last period of life. This rise in extreme old age was seen for several other serum antigens, as will be noted below.

Once ceruloplasmin and  $\alpha_2$ -macroglobulin had reached "mature" levels, at 6 months (Fig. 3), they remained constant through old age. Even more than transferrin, however, ceruloplasmin increased significantly (more than four times the standard error of the mean) in senile mice.

These data identify C-3, No. 32, transferrin, ceruloplasmin, and  $\alpha_2$ -macroglobulin as essentially unchanging antigenic comparators by which to measure other antigens in mouse serum in X-IEP from 6 to 24 months in A/J mice. As a corollary, should a mouse exhibit moderate variations in any of these five antigens during this span of life, those variations could be considered abnormalities.

*Dropping antigens.* Most of the measured antigens dropped with aging, 11 of the 21. They could be divided into three subgroups: those which drop after 3 months (Fig. 4), those which drop after 6 (Fig. 5), and those which drop after 12 (Fig. 6). Note that they all increased between old age (24

TABLE I. RELATIVE CONCENTRATIONS OF 21 SERUM ANTIGENS IN A/J MICE AGED FROM 3 MONTHS TO SENILITY

Antigen	Concentration at age (months) <sup>a</sup>								
	3	6	9	12	15	18	21	24	27
IgG <sub>1</sub>	0.25 (0.04)	0.35 (0.02)	0.29 (0.01)	0.30 (0.02)	0.26 (0.02)	0.28 (0.03)	0.30 (0.07)	0.30 (0.02)	0.30 (0.04)
Ig2	0.13 (0.01)	0.26 (0.01)	0.22 (0.01)	0.23 (0.01)	0.19 (0.02)	0.20 (0.03)	0.21 (0.02)	0.18 (0.01)	0.21 (0.03)
IgM	0.17 (0.01)	0.22 (0.01)	0.22 (0.02)	0.25 (0.02)	0.25 (0.01)	0.24 (0.01)	0.21 (0.02)	0.30 (0.01)	0.27 (0.05)
C-3 globulin	0.41 (0.03)	0.44 (0.04)	0.39 (0.01)	0.39 (0.02)	0.38 (0.03)	0.37 (0.01)	0.40 (0.04)	0.41 (0.01)	0.40 (0.03)
Transferrin	0.60 (0.04)	0.63 (0.03)	0.57 (0.02)	0.59 (0.01)	0.53 (0.03)	0.57 (0.03)	0.61 (0.03)	0.58 (0.02)	0.66 (0.05)
Hemopexin	0.69 (0.03)	0.80 (0.06)	0.67 (0.02)	0.77 (0.05)	0.76 (0.05)	0.76 (0.06)	0.84 (0.11)	0.79 (0.03)	0.80 (0.07)
Antigen No. 32	0.31 (0.02)	0.33 (0.02)	0.32 (0.01)	0.34 (0.03)	0.33 (0.03)	0.30 (0.02)	0.31 (0.03)	0.31 (0.01)	0.32 (0.01)
Antigen No. 10	1.57 (0.13)	1.24 (0.06)	1.27 (0.05)	1.27 (0.06)	1.16 (0.05)	1.12 (0.07)	1.26 (0.06)	1.11 (0.04)	1.33 (0.04)
Ceruloplasmin	0.39 (0.01)	0.44 (0.01)	0.41 (0.02)	0.44 (0.02)	0.41 (0.03)	0.42 (0.02)	0.44 (0.04)	0.43 (0.02)	0.48 (0.03)
Gc globulin	0.47 (0.03)	0.48 (0.02)	0.43 (0.02)	0.44 (0.01)	0.42 (0.02)	0.42 (0.04)	0.39 (0.02)	0.41 (0.02)	0.48 (0.02)
$\alpha_2$ -HS glycoprotein	0.61 (0.02)	0.57 (0.02)	0.54 (0.03)	0.51 (0.02)	0.50 (0.02)	0.48 (0.04)	0.48 (0.01)	0.44 (0.01)	0.53 (0.03)
$\alpha_2$ -Macroglobulin	0.42 (0.01)	0.44 (0.01)	0.45 (0.02)	0.47 (0.02)	0.42 (0.03)	0.41 (0.02)	0.40 (0.03)	0.43 (0.01)	0.45 (0.03)
$\alpha_1$ -Antichymotrypsin	0.57 (0.03)	0.67 (0.02)	0.60 (0.01)	0.64 (0.03)	0.65 (0.03)	0.67 (0.03)	0.74 (0.07)	0.67 (0.02)	0.72 (0.06)
Inter- $\alpha$ inhibitor	0.50 (0.01)	0.50 (0.01)	0.47 (0.02)	0.51 (0.03)	0.43 (0.02)	0.45 (0.03)	0.42 (0.03)	0.42 (0.02)	0.47 (0.02)
Antigen No. 21	0.66 (0.04)	0.57 (0.01)	0.50 (0.01)	0.55 (0.05)	0.43 (0.02)	0.51 (0.02)	0.46 (0.05)	0.41 (0.01)	0.47 (0.02)
$\alpha_1$ -Lipoprotein	1.47 (0.02)	1.27 (0.03)	1.16 (0.07)	1.29 (0.07)	1.12 (0.07)	1.23 (0.04)	1.13 (0.04)	1.07 (0.02)	1.21 (0.04)
Albumin	1.80 (0.10)	1.78 (0.13)	1.60 (0.09)	1.63 (0.06)	1.30 (0.03)	1.40 (0.06)	1.29 (0.08)	1.19 (0.07)	1.54 (0.04)
$\alpha_1$ -Antitrypsin	1.22 (0.06)	1.16 (0.04)	1.05 (0.04)	1.06 (0.01)	0.96 (0.03)	1.04 (0.05)	0.93 (0.03)	0.85 (0.02)	1.04 (0.03)
Antigen No. 26	1.07 (0.05)	1.10 (0.04)	1.03 (0.06)	1.01 (0.03)	0.88 (0.02)	0.91 (0.03)	0.84 (0.02)	0.79 (0.03)	0.93 (0.05)

<sup>a</sup> Given as proportions of loop areas of precipitation to that of the added standard antigen, carbamylated human transferrin, means of four determinations each together with parenthesized standard errors of means. Note that concentrations of different antigens cannot be compared with each other, because precipitate loop areas in X-IEP depends on antibody quality and quantity as well as on quantity of antigen, and antibodies to different antigens precipitate them differently. For example, a ratio at 3 months of 0.50 for inter- $\alpha$  inhibitor cannot be interpreted as meaning that there is more of this antigen, for example, than IgG<sub>1</sub>, because IgG<sub>1</sub> has a lower rate of 0.25. Statistical analyses assumed normal distributions for the quantities of antigens in the separate age groups, based on previous information obtained with larger numbers of young adult mice (4).

months) and senility (27 months). The only recognized antigen dropping after 3 months was  $\alpha_1$ -lipoprotein; the other two, Nos. 10 and 21, have not yet been identified. Four of the five dropping after 6 months are identified: albumin,  $\alpha_1$ -antitrypsin,  $\alpha_2$ HS-glycoprotein, and Gc globulin. The fifth was unidentified antigen No. 22. Uniden-

tified antigens Nos. 26 and 33 together with inter- $\alpha$  inhibitor remained stable to 12 months of age, then dropped.

*Rising antigens.* Two antigens other than the immunoglobulins, which had their own peculiar responses, increased with aging:  $\alpha_1$ -antichymotrypsin and hemopexin. Both reached adult levels at about 6 months of

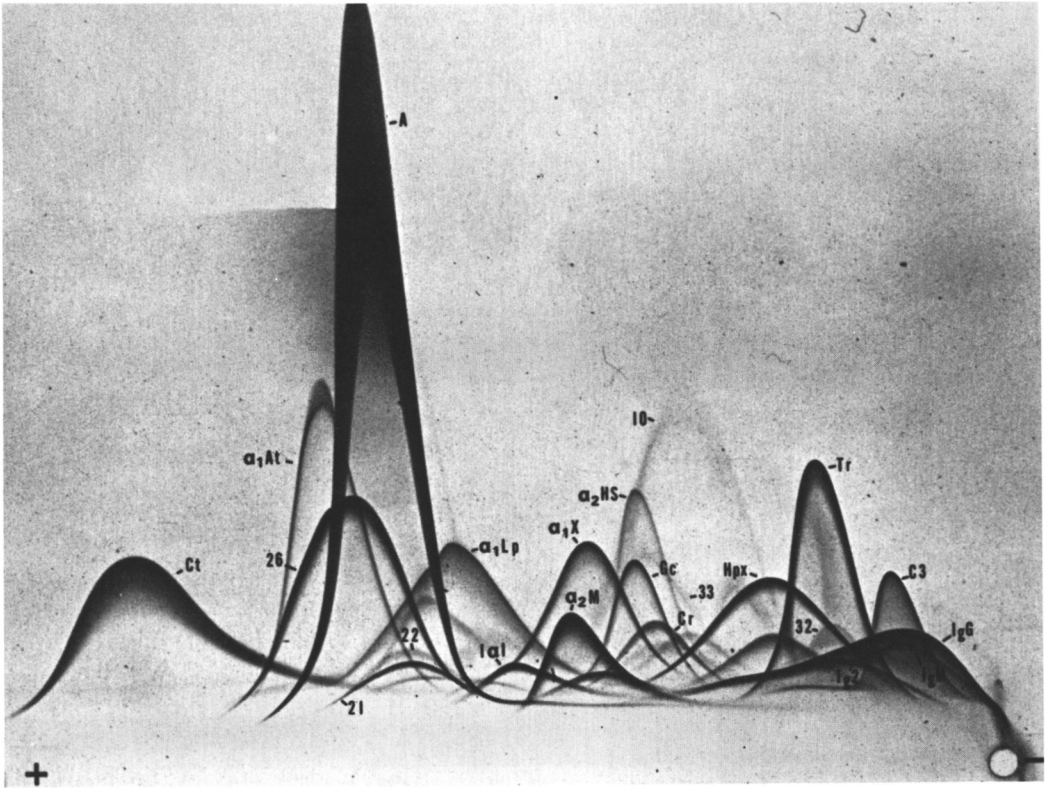


FIG. 1. X-IEP of serum from aged (24-month, 0.9 MLS) A/J female mouse identifying the antigens studied. The serum is generally quite similar to sera of younger A/J mice (cf. Ref. (7)). Identified are the added marker antigen carbamylated human transferrin (Ct),  $\alpha_1$ -antitrypsin ( $\alpha_1$ At), albumin (A),  $\alpha_1$ -lipoprotein ( $\alpha_1$ Lp), inter- $\alpha$  inhibitor ( $I\alpha I$ ), antichymotrypsin ( $\alpha_1$ X),  $\alpha_2$ -macroglobulin ( $\alpha_2$ M),  $\alpha_1$ HS-glycoprotein, transcalciferin (Gc globulin), ceruloplasmin (Cr), hemopexin (Hpx), transferrin (Tr), C-3 component of complement, the three major classes of immunoglobulin (Ig), and several unknown numbered antigens that were quantitated (10, 21, 22, 26, 32, 33). The several antigens that can be seen but have no numbers or names were not measured in these studies.

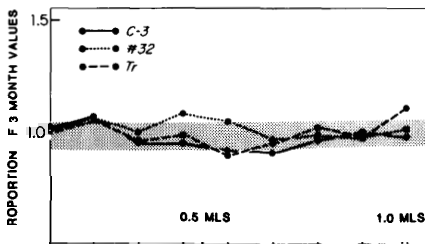


FIG. 2. Antigens whose quantities remained constant from 3 months of age. In this and succeeding graphs, mean quantities for each age of sampling are in proportion to corresponding quantities of the 3-month-old mice. Shaded area is 3-month-old value  $\pm$  SEM for all determinations recorded in each graph. See Materials and Methods for details, Table I for primary data. See Fig. 1 for names of antigens. MLS, Mean life span.

age (Fig. 7) and remained the same through 18 months of age. Then at 21 months they rose by approximately 20% and remained there through senility.

*The immunoglobulins.* Perhaps because

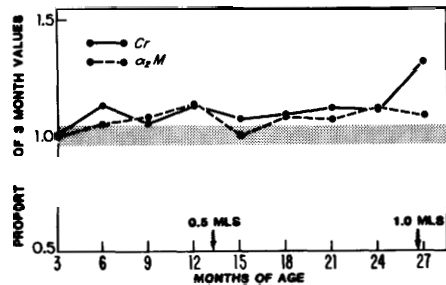


FIG. 3. Antigens whose quantities remained constant from 6 months of age.

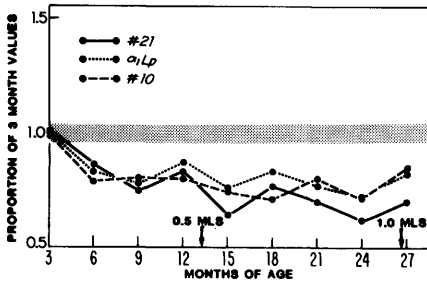


FIG. 4. Antigens whose quantities dropped after 3 months of age.

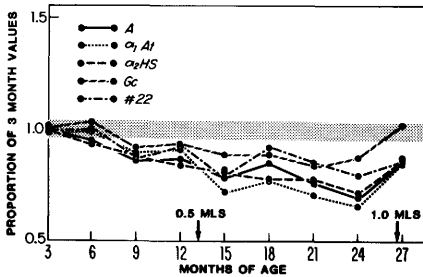


FIG. 5. Antigens whose quantities dropped after 6 months of age.

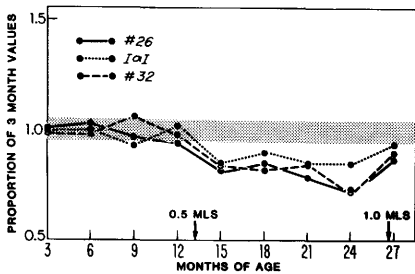


FIG. 6. Antigens whose quantities dropped after 12 months of age.

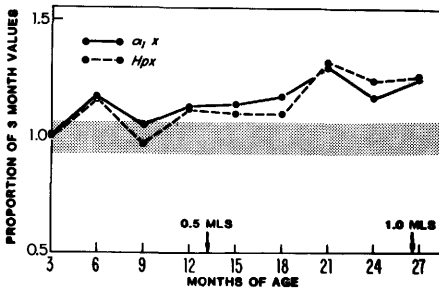


FIG. 7. Antigens whose quantities rose with age.

of their responsiveness to environmental antigens, such as from food, bedding, and microorganisms, the three classes of immunoglobulin measured were more variable than any of the other antigens. For all three, "mature" levels apparently were not reached until 6 months of age (Fig. 8). This was especially noticeable for the minor 7 S class (Ig2) which by 6 months had risen to double its 3-month value, then declined moderately and leveled off after 15 months of age. The other (and major) 7 S immunoglobulin (IgG<sub>1</sub>) also peaked at 6 months, though at a much lower proportion, then dropped back at 9 months to a level that remained constant and slightly higher than the 3-month level throughout life. IgM was higher at 6 months than at 3 months and tended to rise irregularly throughout life. If immunoglobulin levels are taken as indicators of immunologic reactivity, without considering what kind they might be (e.g., protective vs regulatory, vs autoimmune), A/J mice mature at about 6 months of age, then retain full reactivity throughout life into senility.

*Qualitative changes.* No age-related qualitative changes were seen among the antigens measured.

*Discussion.* Our use of similarly housed inbred mice, freshly obtained individual sera, a single high-resolution multianalytic technique, and one batch of antiserum to follow the relative concentrations of 21 different serum antigens throughout the adult life span of A/J mice has resulted in

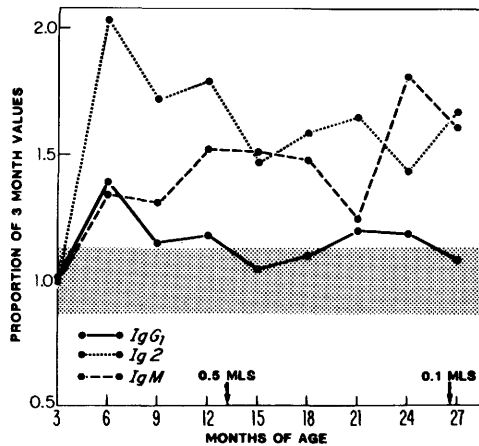


FIG. 8. Immunoglobulins.

enough uniformity of results for certain interesting patterns of age-related antigen concentration changes to become evident, and for the characterization of normal X-IEP patterns for A/J mice of different adult ages.

Particularly important for our analyses is the ability of X-IEP to show ratios of individual serum antigens to one another and to an added standard, with all the antigens being analyzed simultaneously in a single test. Changed ratios indicate truly changed relative concentrations (1, 3, 5, 6, 10), because the ratio of each antigen to any other for the 21 we measured, and to the carbamylated transferrin marker we added in each test, provides population data for each analysis and thus rigid internal control of its reliability. For all of these reasons, our data reliably show several age-related trends for the concentrations of the 21 antigens even though for each age sera from only four separate mice were analyzed.

Three months is widely accepted as the "normal adult" age for mice (11, 12). So we used X-IEP values for this age as unity to most conveniently compare all the other values graphed in the Figures of results. Our data show that "adulthood" as defined by attainment of maximum levels of serum antigens (1) is approximately correctly chosen at this age for A/J mice (at 0.11 MLS). Thus, such levels were reached by most of the antigens (17 of 21) at this age when for some they had not at earlier ages (7). This is both convenient and important to know, since subsequent changes in any of these 17 antigens in healthy mice can be attributed to aging, providing at once a much-needed (13) relatively simple objective multifactorial gauge of aging. However, the data also show that for Ig2 maturity as just defined is reached later (6 months; cf. (14) for similar conclusions), and for IgM, by this definition, it may not be reached until midlife or later (Fig. 8). Of course, these immunoglobulins must be increasing in response to antigenic stimuli (15), and since these stimuli continue and may vary considerably throughout life, the levels of immunoglobulins cannot be so regularly or closely controlled as the levels of other serum anti-

gens. This is clearly evident in their much greater variability (i.e., standard errors of means) in our measurements than among the other antigens. In view of this, the good constancy of IgG<sub>1</sub> from 9 months through senility is mildly surprising. Our data confirm that immunologic responses, if measured by antibody (immunoglobulin) production, do not decrease (14, 16) but rather may increase (17–19) with aging, although the data say nothing about inducibility of new humoral antibody responses or about the nature or actions of the measured immunoglobulins (cf. (20)). Since classes of immunoglobulins reach mature levels at different ages in different strains of mice, and different classes predominate (21), our data will apply only approximately to these other strains.

Levels of 11 antigens of the 18 nonimmunoglobulins declined progressively with aging (Figs. 4–6), which is significant because at the same time the levels of 5 others remained steady (Figs. 2 and 3), and of another 2 rose (Fig. 7). Levels of some of these antigens, which declined the most and most constantly (e.g., albumin), could be used in well-characterized inbred populations of animals, such as the mice used here, as objective measures of aging. A progressive drop in albumin with aging in people (1, 6, 22, 23) lends credibility to this proposition. The data for these antigens further suggest that age-related decline begins at different ages for different groups of antigens, some after 3 months (Fig. 4), some after 6 months (Fig. 5), and some after 12 months (Fig. 6) of age. Considering that many of these antigens are made in the liver (23–25), it is evident that within one organ different synthetic processes "age" at different rates. Thus, while diminished output of albumin could be considered to reflect aging of the liver, by output of transferrin, which never dropped (Fig. 2), the A/J mouse liver, and similarly the human liver in which the same contrast is seen (5, 6), could be judged "young" throughout life into terminal age. Learning why the levels of a few antigens remain constant throughout life while those of others begin to drop starting at different ages will be most in-

teresting and perhaps informative about the events of aging.

Not all these mouse serum antigen data may be applicable to humans, for some antigens appear to behave differently with aging in the two species. For example, while albumin and  $\alpha_2$ HS-glycoprotein drop in both, and  $\alpha_1$ Lp and  $\alpha_1$ At dropped in mice, in humans these have been reported either to remain the same or rise (5, 6). Also, in early human life,  $\alpha_2$ -macroglobulin begins high and then drops (5, 6), while in A/J mice it remains the same from early youth (7) into senility (Fig. 3).

Perhaps more remarkable than the declines with aging seen in the above-discussed 11 antigens is the life-span invariability of 5: C-3, transferrin, ceruloplasmin,  $\alpha_2$ -macroglobulin, and unidentified antigen No. 32. It will be interesting to learn why and how the aging body keeps their levels to such close tolerances. In man also, transferrin and ceruloplasmin have been found to retain constant levels in aging (5, 6). But C-3 seems to vary more than in mice, for example, being reported higher in people over 80 years old than in 20 to 60-year-old controls (19), and so do levels of  $\alpha_2$ -macroglobulin (5, 6). Previously we found significant quantitative or qualitative changes in C-3 globulin, ceruloplasmin, and  $\alpha_2$ -macroglobulin in juvenile A/J sera (7), so that of all the mouse serum antigens we have studied, transferrin alone is quantitatively and qualitatively constant from birth until death.

These five unvarying antigens more than being physiologically interesting for their constancy provide a superb set of independent internal references for using X-IEP and coefficients of variations of ratios between them and other serum antigens to detect changes in any of these antigens easily and significantly (2-4, 26) throughout adult life in the A/J mouse. This presents many new opportunities for incisive physiologic and pathogenetic research in mice.

Two of the nonimmunoglobulin serum antigens,  $\alpha_1$ -antichymotrypsin and hemopexin, showed some tendency to rise in aging mice (Fig. 7). Both of these tend to rise also in aging people (5, 19). We know

too little about the functions of these two antigens, one an antiprotease and the other a scavenger of heme, as related to aging to speculate usefully on this finding.

Fourteen of the eighteen nonimmunoglobulins were increased in sera taken in terminally aged but still-healthy mice; for example, compare 24- and 27-month values in Figs. 5 and 6. These mice while not obviously sick nevertheless were feeble and emaciated from old age and bled poorly compared with younger mice. Blood volume may drop in terminal agedness (27), and it is possible that this may have accounted for these increases. If so, the steadiness with which the constant antigens C-3,  $\alpha_2$ -macroglobulin, and No. 32 held underlines the apparent importance of their regulation to the body.

Note that our data were obtained for verifiably healthy aging mice. Mice that have tumors or infections show highly variable amounts of antigens including increased IgG and very low amounts of albumin. Similar results have been seen in human serum (unpublished observations) and could be responsible for some of the variation in serum studies of humans where the relative health of the aging individual cannot be determined by autopsy as was done with mice.

This project was supported by Grant 5 R01 AG00008 awarded by the National Institute on Aging.

1. Weeke B, Krasilnikoff PA. *Acta Med Scand* 192:149, 1972.
2. Davidsen O. *Acta Paediatr Scand* 63:833, 1974.
3. Kashimura S, Umetsu K, Suzuki T, Tohoku J. *Exp. Med.* 129:169, 1979.
4. Crowle AJ, Miller JL. *J Immunol Methods* 43:15, 1981.
5. Weeke B, Krasilnikoff PA. *Protides Biol Fluid* 18:173, 1970.
6. Lyngbye J, Krøll J. *J Clin Chem* 17:495, 1971.
7. Miller JL, Crowle AJ. *Proc Soc Exp Biol Med* 169:27, 1982.
8. Crowle AJ, Miller JL, Grove N. *Gerontology* 27: 241, 1981.
9. Cline LJ, Crowle AJ. *Clin Chem* 25:1749, 1979.
10. Clarke HGM, Freeman T. *Clin Sci* 35:403, 1968.
11. Hijmans W, Hollander CF. In: Makinodan T, Yunis E, eds. *Immunology and Aging*. New York, Plenum, p23, 1977.

12. Kay MMB. In: Waters H, ed. Handbook of Cancer Immunology. New York, Garland STPM Press, Vol 1:p63, 1978.
13. Hayflick L. Proc Soc Exp Biol Med 165:206, 1980.
14. Haaijman JJ, Hijmans W. Mech Ageing Develop 7:375, 1978.
15. Gitlin D, Gitlin JD. In: Putnam FW, ed. The Plasma Proteins: Structure, Function, and Genetic Control. New York, Academic Press, p264, 1975.
16. Makinodan T, Kay MMB. Advan Immunol 29:287, 1980.
17. Buckley CE III, Buckley EG, Dorsey FC. Fed Proc 33:2036, 1974.
18. Mackay IR, Whittingham SF, Mathews JD. In: Makinodan T, Yunis E, eds. Immunology and Aging. New York, Plenum, p35, 1977.
19. Riesen W, Keller H, Skvaril F, Morell A, Barandun S. Clin Exp Immunol 26:280, 1976.
20. Adler WH, Jones KH, Brock MA. In Behnke JA, Finch CC, Moment GB, eds. The Biology of Aging. New York, Plenum, p221, 1978.
21. Natsuume-Sakai S, Motonishi K, Migita S. Immunology 32:861, 1977.
22. Karel JL, Wilder VM, Beber M. J Gerontol 4:667, 1956.
23. Blombäck B, Hanson LA. Plasma Proteins. New York, Wiley, 1979.
24. Putnam FW. The Plasma Proteins. New York, Academic Press, 1975.
25. Alper CA, Raum D, Awdeh ZL, Petersen BH, Taylor PD, Starzl TE. Clin Immunol Immunopathol 16:84, 1980.
26. Quintero DS, Crowle AJ. Clin Chem 25:723, 1979.

---

Received June 22, 1981. P.S.E.B.M. 1982, Vol. 169.