PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 27–35 (1982)

Crossed Immunoelectrophoresis of Neonatal and Juvenile Mouse Serum (41302)

JANET L. MILLER AND ALFRED J. CROWLE

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 mouse sera taken at intervals from birth through early adulthood, 16 antigens were analyzed quantitatively and qualitatively by crossed immunoelectrophoresis. Only 3 (α_1-antichymotrypsin, α_2-macroglobulin, C-3 globulin) began and remained at normal adult levels. Another 3 (α_1-lipoprotein, ceruloplasmin, transferrin) began slightly low, rose above normal levels at 2–3 weeks, then returned to normal. Eight (albumin, No. 26, α_1-antitrypsin, inter-α-inhibitor, No. 21, Gc globulin, hemopexin, and IgG) were very low or undetectable at birth, then rose in various nonparallel ways to, or sometimes, above normal. Two (α_2-HS glycoprotein, No. 10) began above normal and then rose higher before falling back to normal as the mice entered adolescence. The natures and functions of the numbered antigens are unknown. Serum concentrations of 4 antigens (α_1-lipoprotein, α_1-antitrypsin, Nos. 21 and 10) were significantly different in 6-week-old males and females, and this difference persisted into adulthood for α_1-antitrypsin. Certain antigens also exhibited "juvenile" forms: in newborn sera α_1-antitrypsin and α_1-lipoprotein reversed their normal immunoelectrophoretic positions, most C-3 globulin in neonatal serum was of the C-3b anodic form, and Gc globulin was an α_1 instead of the normal adult α_2 globulin. These various data for juvenile mouse sera are discussed and compared with analogous but sometimes quite contrasting data for juvenile human sera.

Knowledge of the quantities and characteristics of serum antigens in early life enhances our understanding of body functions, and our abilities to detect actual or impending misfunctions. This knowledge has been obtained for humans from analyses of cord blood and neonate sera (reviewed in (1); see also (2–5)). It has been shown that during very early life different serum antigens fluctuate independently of each other in quantities and sometimes in qualities, often quite markedly. But little is known about serum antigens in the early lives of laboratory animals, except for the immunoglobulins. The studies of the immunoglobulins have shown that while there are several close similarities between mice, for example, and children in acquiring these serum antigens before and after birth, there are also wide differences (1). Studying multiple serum antigens in juvenile laboratory animals therefore would seem to be worth while. We had an opportunity to do this while examining changes of immunologic responses in mice throughout life. Thus, we used crossed immunoelectrophoresis (X-IEP) to analyze samples of A/J male and female mouse serum taken neonatally and at regular intervals into young adulthood to obtain data on the quantities and immunoelectrophoretic qualities of 16 different serum antigens. We report these data here and discuss them in comparison with similar data from analyses of children sera.

Materials and methods. *Mice.* A/Jax mice bred in our own animal quarters were weaned at 6 weeks and separated by sex. Mothers and nursing mice were fed water and Breeder Blox (Wayne, Chicago, Ill.), weaned animals Rodent Laboratory Chow (Purina, St. Louis, Mo.). All animals for a particular age group were litter mates. Two males and two females were used for each group. Animals were bled out from the jugular vein. They ranged from newborn (less than 24 hr old) to adult (12 weeks of age). Serum was collected from clotted blood and analyzed immediately to avoid storage-induced changes in serum X-IEP patterns.

Antisera. Rabbit antisera to normal human serum and to normal mouse serum were prepared as previously described (6).

Crossed immunoelectrophoresis. Our technique has been described previously (6). The following modification was made

0037-9727/82/010027-09$01.00/0

to improve relative quantitation in our analyses of the mouse serum antigens.

An antiserum to human serum was mixed with the antiserum to mouse serum so that carbamylated human serum transferrin added to the mouse serum before electrophoresis could be detected as a quantitative constant against which each mouse antigen could be compared. This substituted for using mouse transferrin, of which we had none when these analyses were done. A constant portion of human carbamylated transferrin thus was added to each sample of mouse serum to be analyzed to make a final concentration of 1.75 mg/ml.

Identification of mouse serum proteins. The proteins studied in these analyses were identified as described previously (6). Briefly, this consisted of identification by a minimum of two independent criteria. For many of the antigens, this was selective reaction with specific antibodies. Additional criteria included electrophoretic mobility, morphologic correlation, molecular seiving electrophoresis, selective reaction with appropriate substrates and enzymes, and changed appearance in X-IEP following biological (e.g., anaphylactic reaction, antigenic boosting) and physical (e.g., freeze–thawing) manipulations. To the number originally mapped (6) has now been added α_2-HS glycoprotein, identified by its selective absorption from that serum by hydroxyapatite (Bio-Rad; 10 mg added to 50 μl of mouse serum in an adaptation of the technique of Ashton and Höhling (7)). This caused a unique diminution of the α_2-HS loop in the X-IEP pattern.

Planimetry and quantitation. Mouse serum antigens detected by X-IEP were quantitated by planimetric measurement of their loops of precipitate in X-IEP patterns as previously described (6). The area of each loop on every slide was compared with the area of the reference loop formed by the carbamylated human serum transferrin on the same slide. For each age, the four sera obtained by bleeding out two females and two males (see above) were analyzed separately. For every X-IEP pattern, the ratio of the area of each antigen loop to that of the added carbamylated transferrin was calculated. Data reported in the tables therefore are the mean ratio values for each set of four figures. These data and their statistics are given in the tables. Because trends are hard to follow from the tabular data, figures also have been prepared which plot ratios of these values at different ages to corresponding values in the adult (12-week) mice.

Results and discussion. Since in every test of each sample of serum all 16 antigens were quantitated simultaneously with one batch of antiserum, changes that we detected among them relative to each other and taken from succeeding ages of mice are real and significant (cf. (6, 8)). But the values we are reporting are relative to the carbamylated transferrin marker used in each analysis, not absolute. Therefore, while comparing changes among the different antigens is legitimate, comparing their quantities, i.e., loop areas, can be misleading, because each antigen is precipitated differently by its antibodies. Of course, comparing relative quantities of one antigen in sera from mice of different ages should be valid.

Our data are more uniform than those hitherto reported for children, (note the small standard errors of means, Table I), because our subjects were inbred littermates and were kept in one environment, and their sera were analyzed fresh with one antiserum by one unchanging technique. Our data are for A/J mice and may be only approximately true for other mouse strains. As will become evident, they vary from some to much from analogous data reported for children.

Age-related changes in serum antigen concentrations. Table I shows all the numerical data from our analyses of 16 antigens in sera from A/J mice ranging from newborn through 12-week-old adults. Age-related nonparallel fluctuations of any antigens in any samples of sera from mice of succeeding ages reveal genuine variations in age-related changes, for if there were no differences in their collective values in succeeding ages, the ratios of all to each other would have to remain constant. Remarkably, this was true for only two sets

TABLE I. RELATIVE CONCENTRATIONS OF 16 SERUM ANTIGENS IN A/J MICE AGED FROM NEWBORN TO ADULT

Antigen	Concentration at age[a]								
	<24 hr	1 week	2 weeks	3 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
IgG	0.06	0.23	0.29	0.28	0.20	0.21	0.22	0.26	0.32
	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)
C-3 globulin	0.38	0.44	0.44	0.46	0.39	0.42	0.40	0.39	0.46
	(0.02)	(0.03)	(0.03)	(0.02)	(0.02)	(0.03)	(0.02)	(0.003)	(0.02)
Transferrin	0.55	0.63	0.75	0.75	0.61	0.59	0.59	0.53	0.63
	(0.04)	(0.04)	(0.02)	(0.06)	(0.06)	(0.05)	(0.03)	(0.004)	(0.03)
Hemopexin	ND[b]	0.36	0.59	0.74	0.73	0.75	0.88	0.76	0.82
	(0.00)	(0.01)	(0.04)	(0.02)	(0.03)	(0.04)	(0.17)	(0.04)	(0.05)
Antigen No. 10	1.02	2.25	2.35	2.19	2.05	1.44	1.55	1.21	1.50
	(0.12)	(0.06)	(0.09)	(0.08)	(0.07)	(0.12)	(0.09)	(0.04)	(0.11)
Ceruloplasmin	0.29	0.45	0.50	0.57	0.49	0.45	0.46	0.44	0.45
	(0.01)	(0.05)	(0.03)	(0.07)	(0.02)	(0.05)	(0.02)	(0.01)	(0.01)
Gc globulin	ND	0.34	0.38	0.41	0.41	0.45	0.47	0.45	0.47
	(0.00)	(0.03)	(0.01)	(0.02)	(0.02)	(0.05)	(0.02)	(0.02)	(0.01)
α_2-HS glycoprotein	0.72	1.17	0.85	0.64	0.61	0.59	0.52	0.54	0.63
	(0.06)	(0.08)	(0.05)	(0.03)	(0.03)	(0.05)	(0.02)	(0.01)	(0.04)
α_2-Macroglobulin	0.36	0.45	0.46	0.46	0.44	0.46	0.48	0.44	0.49
	(0.01)	(0.04)	(0.03)	(0.02)	(0.03)	(0.05)	(0.04)	(0.01)	(0.02)
α_1-Antichymotrypsin	0.59	0.64	0.72	0.70	0.63	0.66	0.64	0.63	0.70
	(0.03)	(0.06)	(0.02)	(0.02)	(0.02)	(0.05)	(0.04)	(0.01)	(0.03)
Inter-α-inhibitor	ND	ND	0.42	0.54	0.56	0.66	0.63	0.53	0.57
	(0.00)	(0.00)	(0.04)	(0.03)	(0.02)	(0.09)	(0.05)	(0.02)	(0.01)
Antigen No. 21	ND	ND	0.37	0.72	1.02	1.15	1.30	0.86	0.84
	(0.00)	(0.00)	(0.02)	(0.06)	(0.03)	(0.16)	(0.08)	(0.07)	(0.13)
α_1-Lipoprotein	1.11	1.50	1.73	1.60	1.52	1.51	1.50	1.28	1.43
	(0.08)	(0.05)	(0.09)	(0.09)	(0.05)	(0.11)	(0.07)	(0.04)	(0.07)
Albumin	0.71	0.92	1.03	1.13	1.18	1.09	1.25	1.09	1.41
	(0.04)	(0.07)	(0.11)	(0.04)	(0.03)	(0.04)	(0.05)	(0.04)	(0.10)
α_1-Antitrypsin	0.79	0.82	0.97	1.12	1.31	1.33	1.53	1.22	1.33
	(0.01)	(0.03)	(0.06)	(0.01)	(0.03)	(0.16)	(0.17)	(0.10)	(0.15)
Antigen No. 26	0.29	0.48	0.61	0.80	0.79	0.68	0.73	0.68	0.80
	(0.02)	(0.05)	(0.03)	(0.01)	(0.05)	(0.05)	(0.01)	(0.01)	(0.02)

[a] 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.
[b] Not detectable.

of three antigens (Figs. 1 and 2). Evidently, most mouse serum antigens change concentrations independently of each other during the first 12 weeks of life.

The accompanying graphs most clearly show these age-related changes. According to them, the antigens fall into four groups: established (Fig. 1), transitional (Fig. 2), incremental (Figs. 3 and 4), and decremental (Fig. 5).

There were 3 antigens in the established category (Fig. 1): C-3 globulin, α_2-macroglobulin, and α_1-antitrypsin. These began slightly below adult concentration in the newborn, but within 1 week they had reached adult concentration and did not vary significantly thereafter. However, as noted below, neonatal C-3 globulin is qualitatively different from adult. Therefore, only α_2-macroglobulin and α_1-antichymotrypsin of the 16 antigens studied were both quantitatively and qualitatively normal from the time of birth.

In neonatal and juvenile humans, α_2-macroglobulin levels are significantly higher than in adults (1, 5–9), so with respect to the juvenile concentration of this antigen, mice are quite different from humans. Like mice, juvenile rats have unelevated, adult concentrations of α_2-macro-

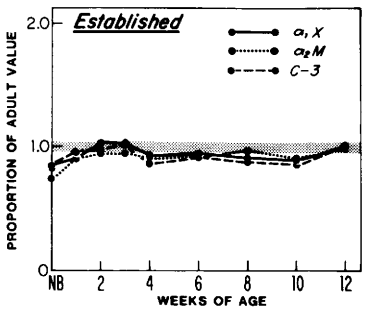

FIG. 1. Levels of A/J mouse serum antigens, relative to adult levels (12 week = 1.0), whose quantities do not change significantly from shortly after birth (NB, newborn) through early adulthood. Each data point is the mean of values from four different samples of serum (see text). Shaded bar indicates the mean of the 12-week values ± the standard error of means for all data graphed. See Table I for primary data, Fig. 7 for antigen nomenclature used in Figs. 1 through 5.

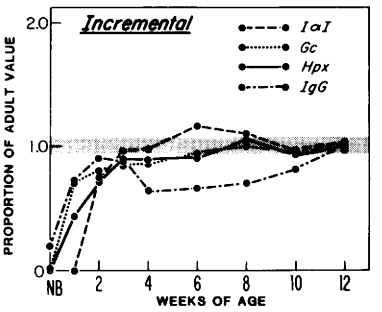

FIG. 3. Relative levels of four of eight A/J mouse serum antigens which are low or undetectable in newborn mice, then rise with aging. See Fig. 4 for the other four.

globulin (10). C-3 (11) and α_1-antichymotrypsin (4) begin below normal in neonatal children like they do in mice.

The transitional antigens transferrin, ceruloplasmin, and α_1-lipoprotein are plotted in Fig. 2. They began below normal, rose to moderately above normal at 2 and 3 weeks, then dropped back to adult levels. For transferrin this corresponds closely to the findings of Geiger and Hoffman (3) but not to those of Abrams and Freeman (2) for transferrin levels in children. The latter workers found transferrin higher in children from birth to 6 months than in adults, but it is possible that the standard that they were using to quantitate serum antigens by X-IEP may have been unsuitable for certain antigens, since it was a pool of freeze-dried serum.

Correspondence between youthful changes in mouse and human α_1-lipoproteins (1, 2) seems to be close, as it does also for ceruloplasmin (3, 5, 27); see also (12). This antigen rises above adult levels in early childhood and then drops back toward normal with the approach of adolescence.

Figures 3 and 4 include half of the measured serum antigens, the incremental antigens that begin far below normal at birth (IgG, albumin, α_1-antitrypsin, No. 26), or are unmeasurable (hemopexin, inter-α-inhibitor, No. 21), then rise with aging by different kinetics. Note that while Fig. 3 shows an "absence" of Gc at birth, a small amount of an anodic form of this glycoprotein nevertheless was present (cf. Fig. 7).

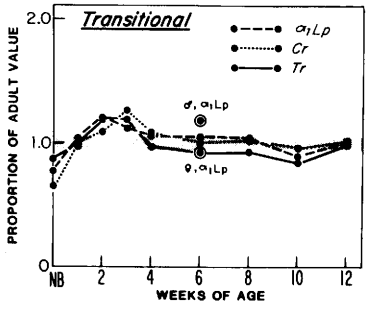

FIG. 2. Relative levels of A/J mouse serum antigens whose quantities rise in juvenile mice, then drop back to adult level. Divergence in values for α_1 Lp in 6-week males and females also indicated by circled data points.

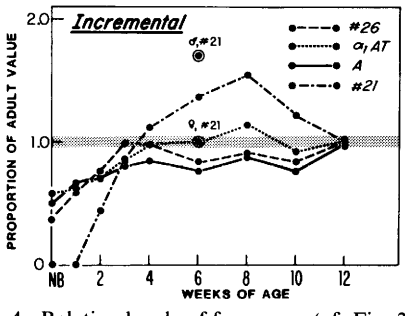

FIG. 4. Relative levels of four more (cf. Fig. 3) A/J mouse serum antigens which are low or undetectable at birth, then rise with aging. Divergence in values for antigen No. 21 in 6-week males and females also indicated by circled data points.

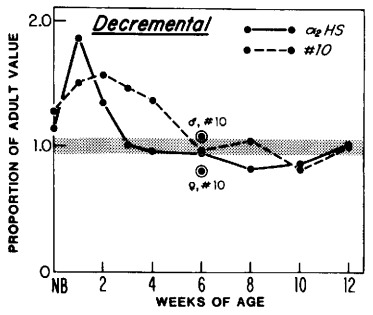

FIG. 5. Relative levels of A/J mouse serum antigens which are higher than adult at birth, rise more in juvenile sera, then fall just before or during mouse adolescence. Divergence in values for antigen No. 10 in 6-week males and females also indicated by circled data points.

Gc and hemopexin rose rapidly in the first 2 weeks after birth, then more slowly to adult levels by the eighth week of age, changing similarly, if one allows for differences in rates of maturation, to what is seen in children (1).

The other 2 antigens in this group (Fig. 3) began similarly but diverged strikingly between 4 and 8 weeks of age. The inter-α-inhibitor rose to normal at 4 weeks, then above normal at 6 weeks, then back to normal. This antigen also begins low in newborn children (4), though considerably higher in proportion to adult levels than we saw in the mice until they were 2 weeks old. Unlike the newborn baby, in whom IgG is found at adult levels (1, 13–15), in mice IgG was only 0.2 of normal. But this finding agrees with earlier data for juvenile mouse serum IgG (16), and with IgG being very low in serum from the near-term mouse fetus (17). Also, unlike in children in which it falls steadily after birth, in mice IgG rose for 3 weeks. After that it fell, then rose once again very slowly. These data can be explained from what is known about factors determining IgG levels in neonatal mouse serum (1, 16, 17). The neonatal level is low because of both limited synthesis and transplacental accumulation, which is lower in mice than humans (16). After birth it rises rapidly because of increasing synthesis combined with absorption through the gastrointestinal tract from mother's milk. Then, after the third week it drops as the gastrointestinal tract closes to the milk IgG (16), and the young mice must sustain levels entirely by synthesis.

In some respects, our X-IEP analyses of mouse serum suggest that neonatal mice are less mature than newborn humans. For example, albumin in the neonatal mouse is at 0.5 of the adult level and does not come into normal range until 3 weeks of age (Fig. 4). Children are born with adult levels (1, 3, 12). Similarly, α_1-antitrypsin levels were below normal in mice for the first 3 weeks, while according to several reports children are born with levels that are higher than adults (1, 4, 13, 17). However, on this the literature disagrees: sometimes α_1-antitrypsin levels have been reported equal to (18) or lower than (9, 19, 20) in adults. According to Kueppers and Offord (21), children are born with adult levels of α_1-antitrypsin that rise for a few days, then return to adult levels by 30.

Changes in the levels of mouse antigen No. 21 were interesting. Negligible in neonatal and 1-week mice, its level climbed rapidly to well above normal by 8 weeks, then fell gradually back to normal by 12 weeks. We do not know the identity or function(s) of this antigen.

Two of the sixteen antigens that we studied began in newborn mice at adult levels, then increased markedly before dropping back to adult concentrations (Fig. 5). One of these decremental antigens was α_2-HS glycoprotein, which peaked sharply at 1 week, to be nearly double the adult level, then dropped to adult levels by 3 weeks. This is quite different from what happens in children. In the human fetus there is a negative correlation between α_2-HS concentration and gestational age, and at birth the level of α_2-HS is below adult levels (4) and continues the same or may fall a little more in the next few days and weeks of life (3). While certain functions of α_2-HS are known, like opsonization (22, 23) and regulation of calcification (7), these do not provide any obvious explanation for this disparity between neonatal mice and neonatal humans for this antigen.

Levels of decremental antigen No. 10, a β_1-globulin, rose and fell more slowly than those of α_2-HS (Fig. 5), remaining well

above adult levels through the fourth week of life. The functions of this antigen are unknown.

Sexual differences in serum concentrations at certain ages. Through 4 weeks of age, no sexual differences were evident. But by the next age of sampling, 6 weeks, there were such differences for α_1-lipoprotein, antigen Nos. 10 and 21, and α_1-antitrypsin, with values for males being higher than for females. These are shown in Figs. 2, 4, 5, and 6. Only the difference for α_1-antitrypsin, however, persisted (see Fig. 6). Similar differences have been seen between male and female children for the antitrypsin. Thus, at adolescence the human sexes segregate into "normal" levels for males and low for females (5). Other data that we have from adult mice older than 12 weeks (not shown here) indicate that it is the female levels which remain "normal," with young male levels being high and later dropping to the female levels shown in Fig. 6. At adolescence, male children also have higher prealbumin than female, but lower β-lipoprotein. We did not quantitate these antigens. The sex difference we noted for α_1-lipoprotein in mice (Fig. 2) apparently has not been reported in children, perhaps because it would be too small to see except in an inbred and uniform population like we studied.

Qualitative changes in the serum antigens of developing mice. As illustrated in Fig. 7, certain antigens in neonatal mouse serum are different from their adult mouse analogues. For example, the positions of α_1-antitrypsin and α_1-lipoprotein are electrophoretically reversed. While this may occur in neonatal child serum, judging from figures published by Abrams and Freeman (2) and by Studd *et al.* (24), it appears not to have been commented on. A major portion of C-3 globulin in newborn mice appears to be in C-3b, or anodic, form instead of the normal C-3 form. In human neonates the C-3 is nearly all of the normal cathodic form (4). All the Gc globulin has α_1 mobility, holding a position anodic to α_2-macroglobulin and α_1-antichymotrypsin in the newborn mouse. It is present in changing proportions of both α_1 and α_2 forms at 3 and 4 weeks (Fig. 8), and thereafter assumes its adult α_2 mobility cathodic to α_2-macroglobulin and α_1-antichymotrypsin. This kind of difference also has been seen for Gc globulin between adult and neonatal humans (2), but its significance is not yet known. It could represent a change in molecular structure, such as accounts for differences between human neonatal and adult transferrins (1), or a change in its functioning, such as in binding 25-dihydroxyvitamin D (25).

There is an "immature" form of transferrin in neonatal children (1), and in sera from mouse fetuses correlating inversely with gestational age (26). But we did not find any in newborn mouse serum, the transferrin in which appears to be entirely of adult type.

We have compared the respective antigenic compositions of sera from newborn mice and humans throughout this paper because mice are frequently used for experiments by interpolation on human medical problems. Quite obviously, and extending earlier more general evidence (28), there are numerous interesting differences between these sera. How much these are determined by contrasts in mother-to-fetus transfer of antigens as opposed to disparities in antigen synthesis by the conceptus is a question inviting some potentially fascinating experimentation.

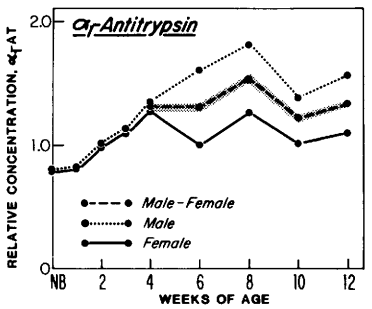

FIG. 6. Plots by sex of the mean relative concentrations of α_1-antitrypsin in A/J newborn, juvenile, and young adult mice showing a divergence between the sexes after 4 weeks of age. Shaded bar beginning at 4 weeks indicates standard errors of means as projected from 4 weeks and bounding means for the values of both sexes combined to compare with mean values from males or females alone.

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

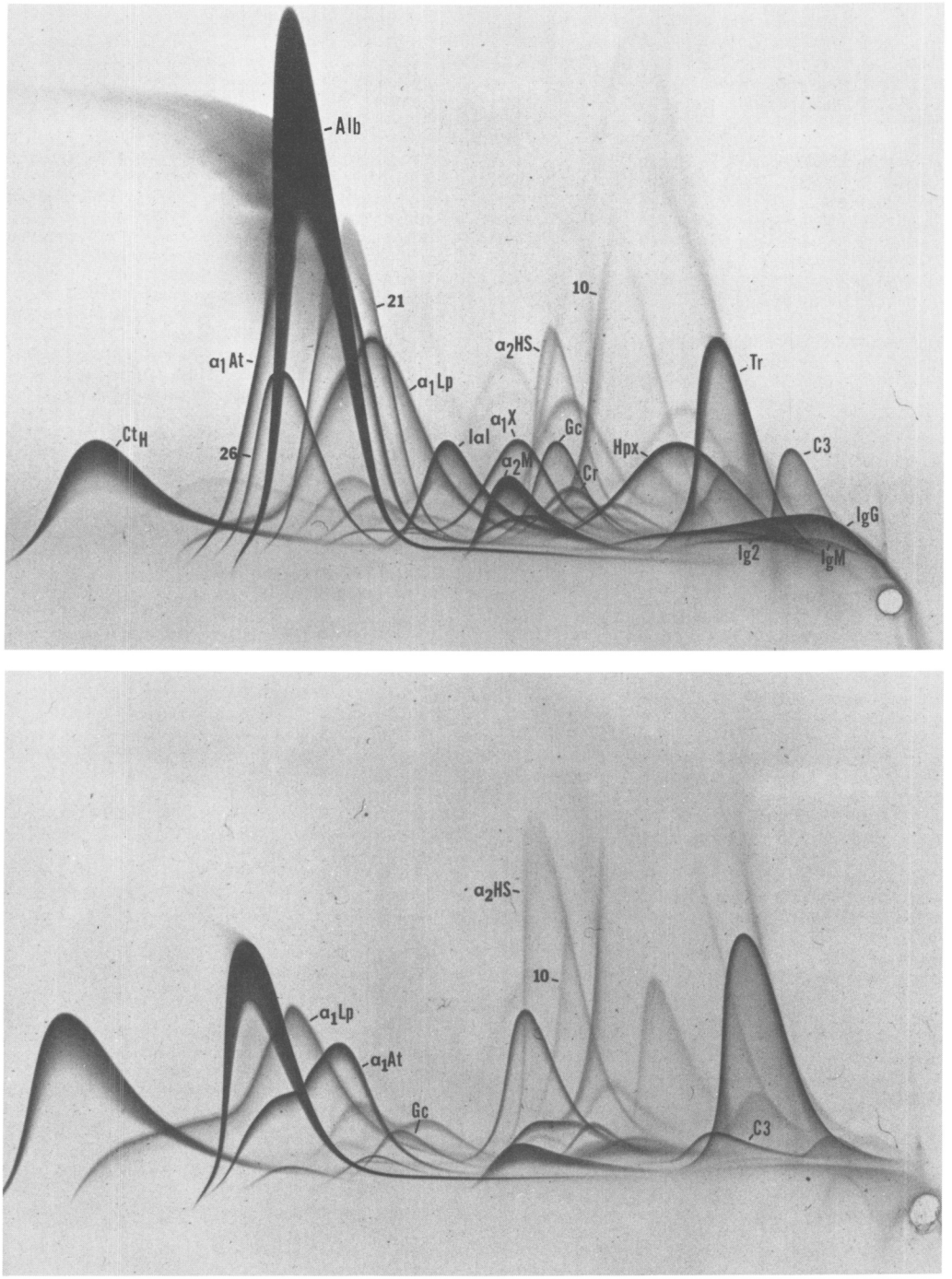

FIG. 7. X-IEP patterns for adult (upper) and newborn (lower) A/J mouse sera contrasted and illustrating both quantitative and qualitative differences as discussed in text. Symbol key: Ct_H, carbamylated human transferrin (added antigenic marker); A, albumin; α_1At, α_1-antitrypsin; α_1Lp, α_1-lipoprotein; IαI, inter-α-inhibitor; α_2M, α_2-macroglobulin; α_1X, anti-chymotrypsin; α_2HS, α_2-HS glycoprotein; Gc, Gc globulin (transcalciferin); Cr, ceruloplasmin; Hpx, hemopexin; Tr, transferrin; C3, C-3 globulin; Ig2, IgG, and IgM are immunoglobulins. The identities of numbered antigens are unknown. The very large, open loop (unlabeled) of antigen cathodic to the α_2HS in the newborn serum is hemoglobin from lysed blood cells invariably contaminating our bleedings from newborn mice. For first and second electrophoresis, the cathode was to the right and at the bottom, respectively. Six antigens are identified in the lower pattern because of notable qualitative (α_1Lp, α_1At, Gc, C3) and quantitative (α_2HS, 10) differences of the neonatal serum antigens from the adult.

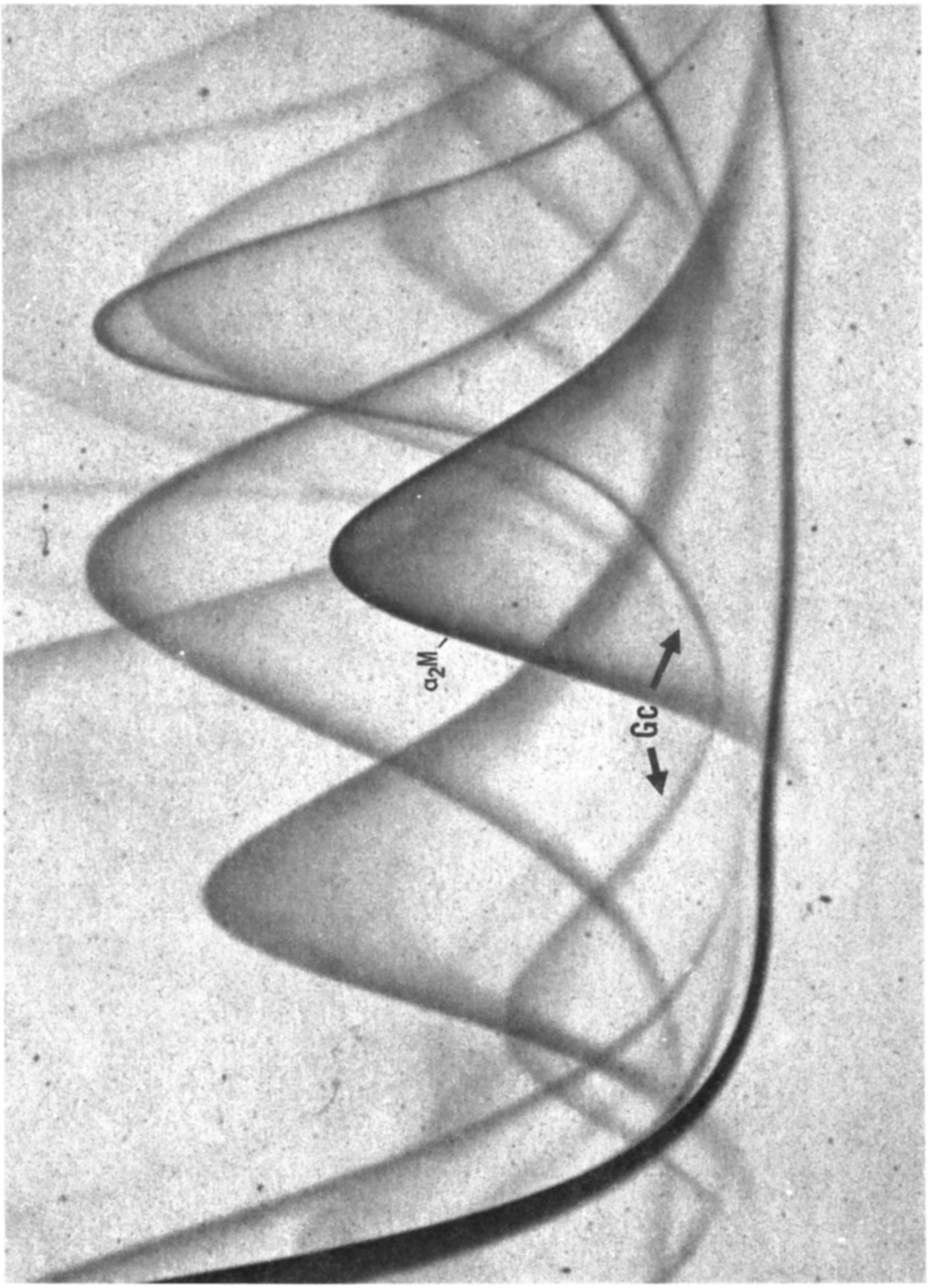

FIG. 8. Detail in X-IEP pattern of serum from a 3-week-old mouse showing dimorphism of Gc globulin, the adult form on the right (cathodic) and juvenile form on the left of α_2-macroglobulin.

1. Gitlin D, Gitlin JD. In: Putnam FW, ed. The Plasma Proteins New York, Academic Press, Vol 2:p264, 1975.
2. Abrams B, Freeman T. Clin Sci **37**:575, 1969.
3. Geiger H, Hoffmann P. Z Kinderheilk **109**:22, 1970.
4. Davidsen O. Acta Paediatr Scand **63**:833, 1974.
5. Podolsak B, Peter G, Öller J. Eur J Pediat **132**:21, 1979.
6. Crowle AJ, Miller J. J Immunol Methods, **43**: 15, 1981.
7. Ashton BA, Höhling H-J. Calcif Tissue Res **22**: 27, 1976.
8. Quintero SD, Crowle AJ. Clin Chem **25**:723, 1979.
9. Gobel U, Petrich C, vonVoss H, Schmelzer L. Eur J Pediatr **129**:117, 1978.
10. Schade R, Burger W. Experientia **36**:125, 1980.
11. Johnson RB, Altenburger KM, Atkinson AW, Curry RH. Pediatrics **64**:781, 1979.
12. Hitzig VH. Helv Paed Acta **16**:46, 1961.
13. Mendenhall HW. Amer J Obstet Gynecol **106**:581, 1970.
14. Hitzig WH. In: Grabar F, Burtin P, eds. Analyse Immuno-electrophoretique. Paris, Masson et Cie, p121, 1960.
15. Cederqvist LL, Ewool LC, Litwin SD. Amer J Obstet Gynecol **131**:520, 1978.
16. Schlamowitz M. Immunol Commun **5**:481, 1976.
17. Gitlin D, Morphis LG. Nature (London) **223**:195, 1969.
18. Singer AD, Thibeault DW, Hobel CJ, Heiner DC. J Pediatr **88**:87, 1976.
19. Lee SK, Thibeault DW, Heiner DC. Pediatr Res **12**:775, 1978.
20. Bardos P, Luthier B, Avenet JM, deRusse J, Muh JP, Soutoul JH, Weill JD. Clin Chim Acta **66**:353, 1976.
21. Kueppers F, Offord KP. J Lab Clin Med **94**:475, 1979.
22. Lewis JG, Andre CM. Immunology **39**:317, 1980.
23. van Oss CJ, Bronson PM, Border JR. J Trauma **15**:451, 1975.
24. Studd JWW, Shaw RW, Bailey DE. Amer J Obstet Gynecol **114**:582, 1972.
25. Steichen JJ, Tsang RC, Gratton TL, Hamstra A, Deluca HF. N Engl J Med **302**:315, 1980.
26. Renfree MB, Hensleigh HC, McLaren A. J Embryol Exp Morphol **33**:435, 1975.
27. Krasilnikoff PA, Weeke B. Prot Biol Fluids **18**: 169, 1970.
28. Schultze HE, Heremans JF. Molecular Biology of Human Proteins, with Special Reference to Plasma Proteins. New York Elsevier, p557, 1966.

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