# Gene Dosage Effect of Serum Concentration of a Complement Component, MuBl.* (31249)

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The protein composition of individuals is directly determined by genes. Details of the mechanism by which this control is exercised in mammalian cells are not understood and many questions that have been answered in bacterial systems cannot yet be resolved in mammals. One question that can, however, be answered concerns the relation between protein concentration and number of allelic genes available in cells. This problem has been studied in many inborn metabolic errors (1) of man, but the nature of the measurement of the proteins has resulted in difficulties of interpretation(2). Since most inborn errors of metabolism are due to abnormalities of enzymes, they have been studied by measurement of catalytic activity. Interpretation of the resulting data is complicated by the fact that genetic changes can lead to loss of enzyme activity without loss of molecules. Low enzyme activity may thus be attributable either to a reduction in the number of molecules or to a decrease in the catalytic capacity of an unaltered number of molecules. In other words, it needs to be established whether abnormalities are caused by catalytically incompetent molecules *(allotypy)* or by complete deletion of synthesis *(eniotypy)* (3). To resolve this difficulty, a method is required which measures molecular concentration rather than activity; immunological assays of molecules can provide such estimates. The discovery of the antigen MuBl present in mice(4) allows study of a system in which molecular concentration can be determined by unispecific antibody.

MuBl is a component of the complement system(5) and probably identical with C'5 (6). An inherited complement deficiency in mice (Hc of Rosenberg *et al*)(7, see also 8,5, 9) is closely linked with deficiency in MuBl (5). Failure to produce MuBl therefore constitutes an inborn error of metabolism as defined by Garrod(1) and may be studied in this context.

The synthesis of MuBl is under the control of a single gene and strains of mice are available which lack MuBl completely(5,10). One can thus obtain animals which have none, one, or two copies of the gene and we shall examine the concentration of MuBl in the circulation of such mice.

Sex plays an interesting role in the concentration of some human enzymes and a further reason for the study of relative concentration of MuBl arises from the fact that the serum concentration of MuBl is sex-dependent(5).

*Methods.* Inbred MuBl-positive mice (C57 BL/6J, C57L/J) and hybrids between them and MuBl-negative A/J mice (C57BL/6J ♀ × A/J ♂; C57L/J ♀ × A/J ♂) were obtained from Jackson Memorial Laboratory, Bar Harbor, Maine. Animals of various ages were bled individually from the tail within 2 days of arrival. Blood samples were taken into tubes which were immersed in an ice water bath and were kept at +2°C for 60 minutes; the serum was then separated at +2°C. Serum samples so obtained were kept frozen at −10°C until used. Rabbit antibody to MuBl was raised by immunizing rabbits with immune precipitates, separated from mixtures of murine MuBl antiserum with MuBl-positive normal mouse serum. The precipitates were carefully washed, incorporated into complete Freund's adjuvant and in-

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TABLE I. Comparison of Concentration of MuBl in Mice Homozygous and Heterozygous with Respect to the MuBl Gene.

| Sex | Age (wk) | Relative concentration of MuBl in Homozygotes C57BL/6J Arithmetic mean | Confidence limits ±‡ | Heterozygotes $B6AF_1/J$† Arithmetic mean | Confidence limits ±‡ | Ratio Homozygotes/Heterozygotes For animals of identical age | Avg for all ages | P* |
|---|---|---|---|---|---|---|---|---|
| ♂ | 3 | .60 | .10 | .34 | .04 | 1.79 | 2.10 | .95>P>.9 |
| | 7 | 1.20 | .30 | .48 | .03 | 2.51 | | |
| | 9 | 1.58 | 1.12 | .64 | .12 | 2.47 | | |
| | 10 | 1.05 | .31 | .64 | .18 | 1.64 | | |
| ♀ | 3 | .63 | .12 | .31 | .06 | 2.03 | 2.06 | 1.0>P>.99 |
| | 6 | .82 | .13 | .34 | .08 | 2.42 | | |
| | 8–8½ | .85 | .13 | .35 | .15 | 2.47 | | |
| | 9 | .79 | .08 | .49 | .13 | 1.62 | | |
| | 10 | .68 | .06 | .38 | .03 | 1.76 | | |
| | | Homozygotes C57L/J | | Heterozygotes $LAF_1/J$† | | | | |
| ♂ | 3 | .52 | .16 | .28 | .04 | 1.89 | 2.02 | .95>P>.9 |
| | 7 | 1.43 | .10 | .46 | .06 | 3.13 | | |
| | 8 | .94 | .12 | .61 | .10 | 1.55 | | |
| | 9 | 1.25 | .46 | .68 | .38 | 1.85 | | |
| | 10 | .98 | .28 | .57 | .17 | 1.70 | | |
| ♀ | 3 | .58 | .21 | .27 | .06 | 2.13 | 2.07 | P>.99 |
| | 6 | .89 | .14 | .37 | .02 | 2.38 | | |
| | 7 | .83 | .10 | .37 | .04 | 2.26 | | |
| | 8 | .75 | .18 | .36 | .02 | 2.06 | | |
| | 9 | .71 | .05 | .47 | .11 | 1.52 | | |

* P is the probability of the assumption that the ratios for various age groups do not differ significantly from the average ratio.

† $B6AF_1$ is the abbreviation for a hybrid: C57BL/6J ♀ × A/J ♂. $LAF_1$ is the abbreviation for a hybrid: C57L/J ♀ × A/HeJ ♂.

‡ 95%

jected subcutaneously into rabbits. The resulting immune serum was absorbed by addition of MuBl-negative mouse serum; the absorbed antiserum was highly specific and was directed against MuBl only(5).

The relative concentrations of MuBl were measured by a modified single diffusion method(11) as previously described(5). Tubes were partly filled with a mixture of absorbed rabbit antiserum to MuBl and 0.6% agar. The agar was allowed to set, and the sera to be assayed were layered above it. The tubes were then sealed with plasticine and stored in a vertical position at 20.5° ± 0.2°C. After incubation for 7 days, the bands were photographed at the high magnification of the Cordis Immunodiffusion Camera. The distance between the interphase and the leading edge of the precipitation band was measured with a precision of ± 0.1 mm. The measurements so obtained were evaluated by comparison with a calibration curve, in which the distance between interphase and leading edge of the precipitation band was plotted against the logarithm of various concentrations of a standard serum.

Concentrations of MuBl were determined in groups of 4-8 male and female animals of various ages and genotypes.

*Results.* The MuBl concentration of groups of *homozygotes and heterozygotes* were compared. The animals in each group were of the same age, sex and strain. The MuBl concentration of homozygous animals was invariably greater than that of heterozygous animals of the same age, strain and sex and the ratios ranged from 1.52 to 3.13. Since the ratios for each age group did not significantly differ from the average ratios (P values ranging between 1 and 0.95), the average ratios for each strain-sex group, irrespective of age, were determined. For all groups examined this ratio was remarkably close to 2.0 (Table I).

The concentration of MuBl in animals of the same age, strain and sex showed considerable inhomogeneity but a general increase

with age was observed. There was invariably a significantly higher amount of MuBl in animals of the oldest age group (24-33 weeks) than in animals of the youngest age group (3 weeks). The level of MuBl showed considerable fluctuation with age; analysis of variance showed that most of these differences were not significant.

Groups of *male and female* animals, identical in age, strain and genotype, were compared with respect to MuBl concentration (Fig. 1). There was no difference in concentration of MuBl of 3-5 weeks old animals; in older animals, the concentration of MuBl in males and females was significantly different. This can be seen in Fig. 1 as the probability, (P), of the assumption that the MuBl concentration in males and females of a given age does not differ significantly. The ratios of MuBl concentration between males and females ranged from 1.21 to 2.0. The average ratio for C57BL/6J mice of age ranging from 6-33 weeks was 1.55; the average ratio for C57L/J mice of 6-24 weeks of age was 1.54. This pooling of data, without regard to age, was justified since the ratios of each age group (above 6 weeks) were not significantly different from the average ratios ($P>0.95$ for C57BL/6J, and $P>0.99$ for C57L/J). The ratios of MuBl concentration between male and female heterogygous mice were strikingly similar to the above values; the average ratio for $B6AF_1$ mice was 1.48, the ratio for $LAF_1$ was 1.47.

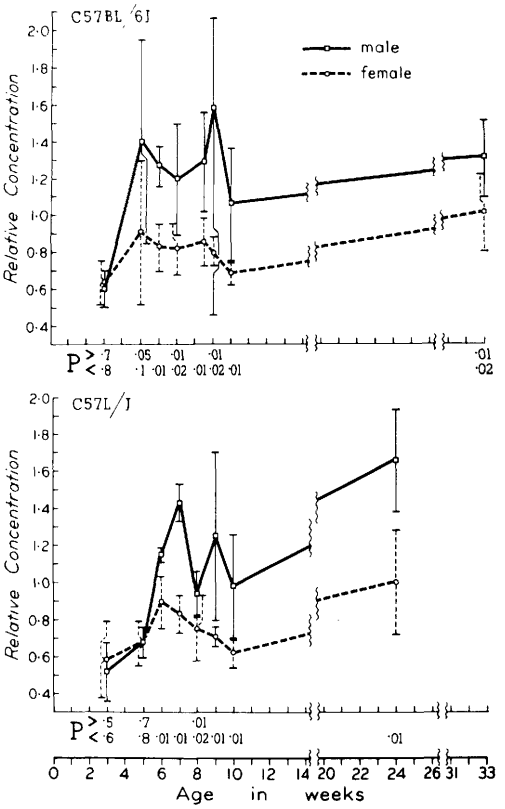


FIG. 1. Relative concentration of MuBl in mice of strains C57BL/6J (upper graph) and C57L/J (lower graph). —□— MuBl concentration in males. —○— MuBl concentration in females. Fine vertical lines show 95% confidence limit of MuBl concentration for a given age group. P values given at bottom of each graph indicate probability of the assumption that the differences in MuBl concentration between males and females of a given group are *not* significant.

*Discussion.* Our measurements of the serum concentration of MuBl reveal a fairly exact gene-dosage relationship. Animals with 2 copies of the gene contain twice as many MuBl molecules as animals with one copy of the gene. Gene-dosage effects of this kind are usually explained by the hypothesis that each gene copy in the cell controls the synthesis of the same quantity of protein and that 2 gene copies will, therefore, result in twice as much protein as one gene. This hypothesis accounts for our findings but does not rule out an alternative possibility.

It is conceivable that the mechanism which has been invoked to explain dosage compensation operates also in regulation of the gene product of autosomal genes. Dosage compensation has been explained(12,13,14) on the basis of the hypothesis that in each somatic cell of the female, only one of the two X chromosomes is functional; the possibility cannot be excluded that, in general, only one of 2 chromosomes functions, even among the autosomal chromosomes.

This seems to be the case in the production of allotypically marked immunoglobulins by heterozygous cells. Both allotypic specificities can be found in the serum, but each cell produces gamma globulin with only one of these 2 allelic allotypic markers(15).

If only one gene per cell were involved in the synthesis of MuBl, the number of cells producing MuBl would be twice as great in

homozygotes as in heterozygotes. At present we cannot distinguish between this possibility and the alternative in which each cell of the homozygote would produce twice as many molecules as each cell of the heterozygote.

Having considered the genetic control of MuBl concentration we turn next to other aspects of control.

The mutant gene of MuBl-negative individuals does not specify the synthesis of molecules. Therefore, one would expect that any feedback process would affect the heterozygote less than the homozygote. Since, however, a ratio of two has been observed in the relative concentration of MuBl in homozygotes and heterozygotes, it is clear that the critical factor in regulation is not the total amount of molecules present and that feedback repression does not play an important role in the limitation of MuBl concentration. It follows that the regulation must take place at the genetic level.

The over-riding control of the synthesis of MuBl is exercised at the genetic level. However, this is not the only control, except in very young animals. In adult animals, another factor enters into the control of the synthesis, and concentration of MuBl is greater in the male than in the female(5 *cf.* 16); the average ratio of concentration in the different sexes is the same in homozygotes and heterozygotes. It follows, therefore, that some factor operates in a different manner in male and female animals. We can safely conclude that this factor is not sex-linked. The reasons for this view are as follows: (1) No relation has been found in back-cross experiments between the presence or absence of MuBl and the sex of parents or sex of offspring(5,10), and (2) The concentration of MuBl is always lower in females than in male animals. Sex-linked control of the concentration of MuBl would lead one either to expect that females would have a larger circulating concentration of MuBl than males or that the concentration in both sexes would be identical. It would appear that a controlling mechanism must be involved which is not *directly* gene-dependent and which must be rate-determining at least in animals of one of the two sexes. This would explain why the concentration in homozygote females is always lower than in homozygote males. The output would be controlled by a factor which promotes or inhibits the output by a gene to the same extent whether this gene is in a homozygous or in a heterozygous cell.

*Summary.* The inherited absence of an effective hemolytic complement system is linked with a deficiency in an antigen, MuBl, and constitutes an inborn error of metabolism. MuBl is completely lacking in animals of certain inbred strains of mice. The regulation of the concentration of MuBl as a function of age, of sex and of the number of gene copies per cell, was studied. The concentration of MuBl was identical in 3-week-old males and females. In older animals, it was approximately 1.5 times as high in the male as in the female. The concentration of the antigen was twice as great in homozygous as in heterozygous animals.

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