

Genes and genotypes affecting embryonic fluid relations in the mouse

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SUMMARY

The contribution of maternal and foetal genotype to the regulation of extra-embryonic (exocoelomic and amniotic) fluid and embryonic fluid in the mouse is discussed. Three of the four inbred strains studied (A, BALB/c and CBA) had similar patterns of fluid accumulation. The fourth (C57BL) differed significantly. In the reciprocal F_1 crosses between CBA and C57BL a maternal effect was present. In the F_2 no increase in variance of extra-embryonic fluid was noted.

The effects of two mutant genes were studied. *Blebs* (*my*) had no effect on the total fluid in the conceptus, but led to a rearrangement of fluid within the embryo. *Brachyphalangy* (Xt^{bph}) caused an overall increase in fluid within the conceptus. Distribution of the excess depended upon the phenotype (exencephalic versus non-exencephalic) of the embryo.

1. INTRODUCTION

The developing mammalian embryo contains a number of cavities and manufactures several membranous enclosures. Each of these is filled with fluid and follows a specific course of development, some remaining relatively uninflated, others enlarging rapidly. This implies the presence of specific mechanisms to create and maintain these bodies of fluid, i.e. regulation must be in force. This paper discusses the sac fluid weights of mouse embryos derived from matings arranged to give information on the possible contribution of maternal and foetal genotype to the control of fluid regulation. These matings were within or between inbred strains of mice or between mice carrying mutant genes for which a *prima facie* case for fluid derangement exists.

2. MATERIALS AND METHODS

One male was kept with each five nulliparous females, who were inspected daily for the presence of vaginal plugs. The day of the plug was considered day 0 of gestation. Pregnant females were killed with ether and their uteri dissected out. The uteri were either processed immediately or labelled, wrapped in Saran-wrap and placed in a deep freeze at -20°C until required. Individual conceptuses were dissected free, blotted and weighed. Each conceptus was then placed on a square of filter paper and the embryonic membranes ruptured. After allowing 15 sec for

drainage the embryo and placenta were separated by severing the umbilical cord, using an eye-cautery apparatus which eliminated blood loss. Embryo and placenta + membranes were then weighed, placed in an oven at 105 °C for 24 h and reweighed on cooling. Embryos carrying mutant genes were classified under a dissecting microscope during drainage.

At least two litters of each nominal age were used wherever possible (Table 1). In some cases, notably where CBA mothers were involved, breeding performance was poor and this ideal was not fulfilled. Litters containing less than five or more than ten embryos were discarded to eliminate variation due to extremes of litter size.

Table 1. *Numbers of embryos used*

| Inbred strains | Age (days) | | | | | | | | |
|-----------------------|------------|----|----|----|----|----|----|----|----|
| | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| A | 14 | — | 9 | — | 17 | 5 | 15 | 13 | 7 |
| BALB/c | 15 | 17 | 15 | 26 | 17 | 18 | 20 | 21 | 19 |
| CBA | — | — | 8 | 7 | 12 | 13 | 10 | — | 7 |
| C57BL | 14 | 14 | 13 | 17 | 14 | 16 | 13 | 19 | 13 |
| Crosses | | | | | | | | | |
| CBA × C57BL (CB) | — | — | 9 | 8 | 10 | 13 | 10 | — | 7 |
| C57BL × CBA (BC) | — | 6 | — | 6 | 7 | 5 | 7 | 5 | — |
| CB × CB | 14 | 15 | 21 | 19 | 19 | 19 | 18 | 17 | 14 |
| Mutant stocks | | | | | | | | | |
| Xt^{bph}/Xt^{bph} * | — | — | 5 | 6 | 4 | 1 | 3 | 1 | — |
| Xt^{bph}/Xt^{bph} | — | — | 4 | 2 | 7 | 1 | 5 | 1 | — |
| $Xt^{bph}/+$ | — | — | 5 | 8 | 10 | 10 | 14 | 15 | — |
| + / + | — | — | 9 | 8 | 4 | 5 | 6 | 5 | — |
| + / <i>my</i> | — | 9 | 8 | 16 | 7 | 11 | 6 | 6 | — |
| <i>my/my</i> | — | 8 | 7 | 7 | 8 | 10 | 4 | 8 | — |

* Indicates exencephalic.

(i) Terminology

Previous studies of the foetal sac fluids in the mouse (e.g. McCafferty, 1955) have referred to amniotic fluid when it is clear that what has been measured is the entire mass of fluid lying within the yolk-sac-membrane/Reichert's-membrane complex, i.e. the amniotic fluid plus exocoelomic fluid (Fig. 1). McCafferty mentions the perforation of the amnion 'at the point of least vascularity'; the amnion is avascular but the yolk sac is highly vascularized. In this study the term *sac fluid* is used to represent exocoelomic fluid plus amniotic fluid. In the rat the volume of exocoel fluid waxes and wanes in parallel with the volume of the amniotic fluid (Barker, in Adolph 1967), so sac fluid weight may be taken to represent amniotic fluid weight.

3. RESULTS

One of the fundamental differences between inbred strains of mice is in their rate of development. Because of this, and because of the error implicit in the timing of matings by vaginal plug (litters of embryos of the same nominal age may vary by

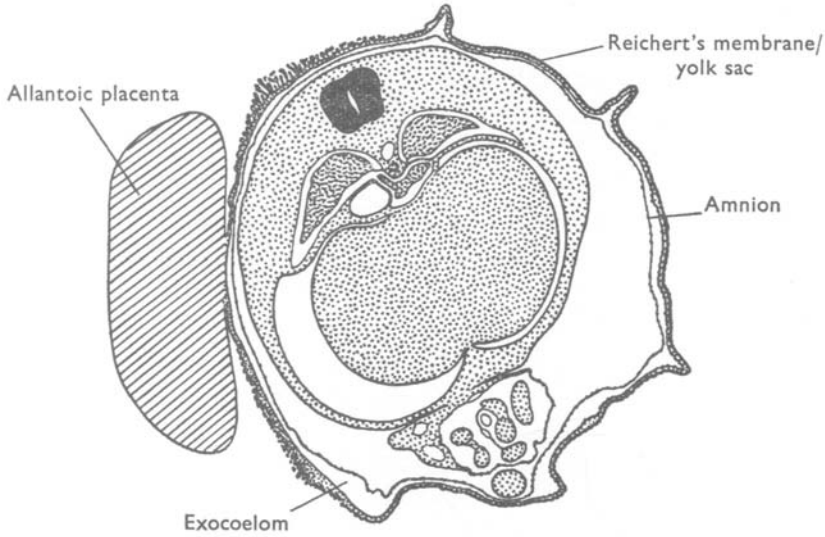


Fig. 1. Semi-diagrammatic cross-section of mouse embryo and its membranes. 13 days.

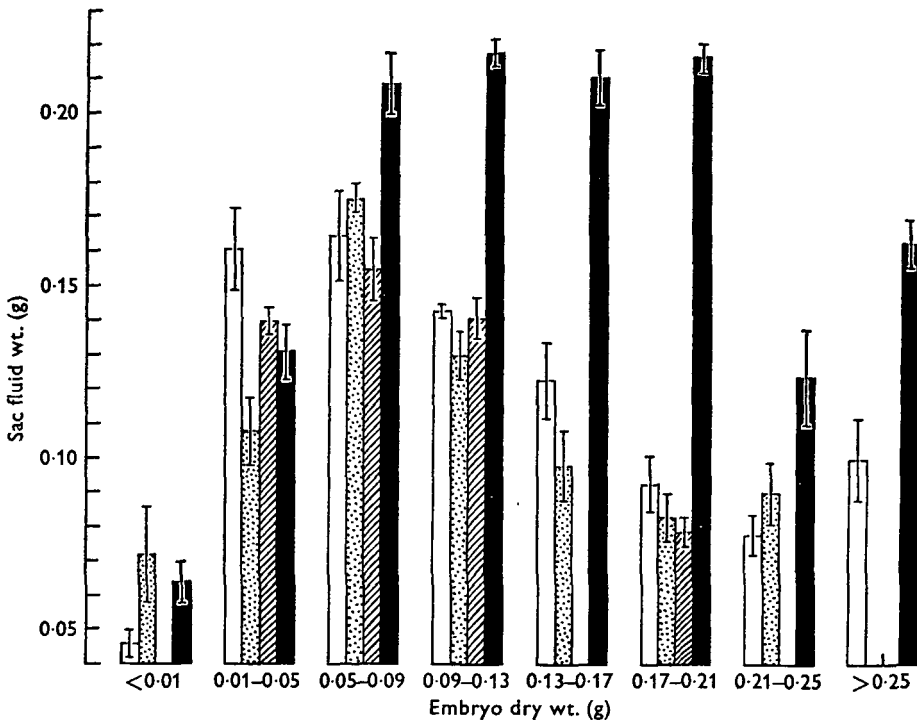


Fig. 2. The relationship between sac fluid weight and embryo dry weight for four inbred strains of mice. Sac fluid weight is expressed as mean \pm its standard error: each point is based upon at least five observations. \square , A; stippled , BALB/c; hatched , CBA; \blacksquare , C57BL.

up to 12 h in conceptual age according to the time of mating), the data from the inbred strains and crosses between them are expressed with reference to embryo dry weight. In the case of mutant genes, where comparisons are within strain and often within litter, the data have been presented simply with respect to day of gestation.

It is clear (Fig. 2) that large differences can exist in the amount of sac fluid surrounding embryos of different strains, even if they are of the same weight. Three of the strains used – A/Gr, BALB/c/Gr and CBA/Gr – show similar patterns of sac fluid weight. C57BL/Gr differs significantly, showing a large increase in embryos weighing 0.05 to > 0.25 g (approximately 15–18 days of gestation). In the reciprocal F_1 's between CBA and C57BL (Fig. 3) BC (C57BL ♂ × CBA ♀) has significantly more sac fluid than CB (CBA ♂ × C57BL ♀) at one of the two points where a comparison can be made. The F_2 studied (CB × CB) is similar to CB throughout.

The $X^{t^{bph}}$ homozygote is a polydactylous monster with multiple abnormalities, whilst the heterozygote can be classified from day 13 onwards on footplate morphology (Johnson, 1969). $X^{t^{bph}}$ was chosen for investigation because of the excess of sac fluids easily seen when dissecting out litters of embryos. Also a proportion of $X^{t^{bph}}/X^{t^{bph}}$ embryos are exencephalic, with persistently open neural tubes.

The $X^{t^{bph}}/X^{t^{bph}}$ embryo already has an excess of sac fluid on day 13 of gestation (Fig. 4). The value for non-exencephalic homozygotes parallels that of normal litter-mates, but is higher throughout. The exencephalic $X^{t^{bph}}/X^{t^{bph}}$ figure rises

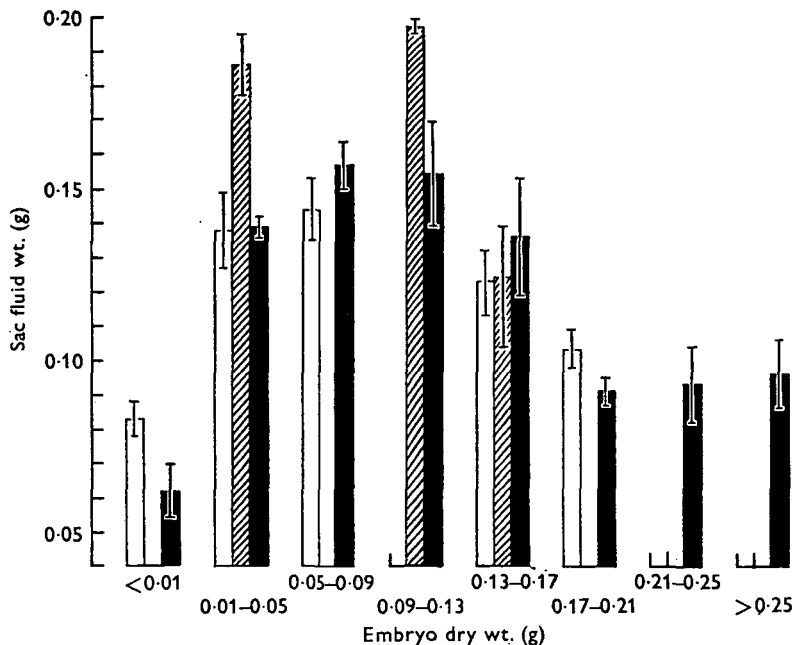


Fig. 3. The relationship between sac fluid weight and embryo dry weight in reciprocal F_1 crosses between CBA and C57BL (CBA ♂ × C57BL ♀ = CB; C57BL ♂ × CBA ♀ = BC) and the CB × CB F_2 . □, CB; ▨, BC; ■, CB × CB.

even higher (from day 14), peaks at 16 days then drops to the non-exencephalic level. This sharp drop coincides with the failure of exencephalics to gain further dry weight, i.e. they are moribund.

The distribution of excess fluid varies with phenotype (Table 2). In exencephalics the embryo wet weight is stable at $1.2 \times$ the normal value from 13 to 16 days, while the sac fluid weight rises to $3.76 \times$ the normal. In non-exencephalics the embryo wet weight increases to $1.34 \times$ the normal value at 15 days then falls again, while the sac fluid weight increases to twice the normal value late in gestation.

Table 2. Fluid distribution in Xt^{bph}/Xt^{bph} exencephalic (*) and non-exencephalic embryos (data expressed as A/N values)

| | | Age in days | | | | | |
|----------------------|-------------------|-------------|------|------|------|------|------|
| | | 13 | 14 | 15 | 16 | 17 | 18 |
| Xt^{bph}/Xt^{bph*} | Embryo wet weight | 1.19 | 1.22 | 1.22 | 1.23 | — | — |
| | Sac fluid weight | 1.51 | 1.87 | 1.69 | 3.76 | — | — |
| Xt^{bph}/Xt^{bph} | Embryo wet weight | 1.22 | 1.23 | 1.34 | 1.28 | 1.22 | 1.03 |
| | Sac fluid weight | 1.21 | 1.33 | 1.18 | 1.99 | 1.79 | 2.01 |

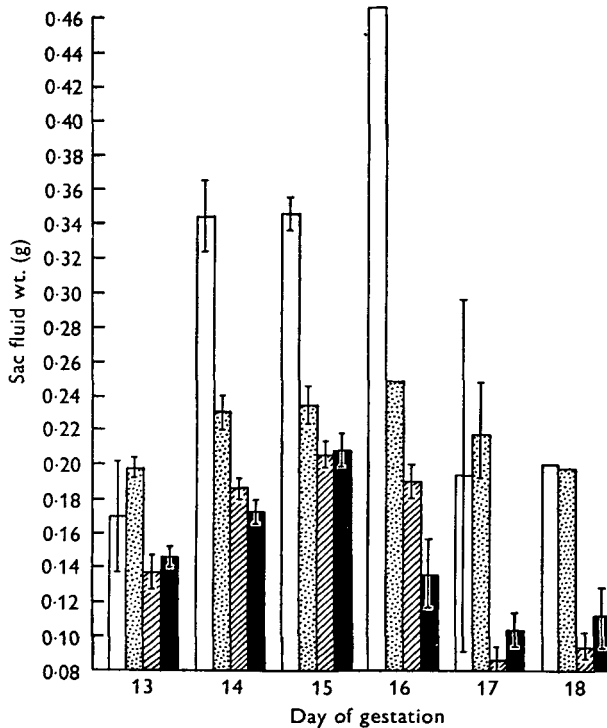


Fig. 4. The relationship between sac fluid weight and day of gestation in the Xt^{bph} stock. Where no s.e. is shown the value is based on a single observation. □, Xt^{bph}/Xt^{bph} exencephalic; ▨, Xt^{bph}/Xt^{bph} ; ▩, $Xt^{bph}/+$; ■, $+/+$.

Accumulations of subepidermal fluid, 'blebs', have been recorded in both exencephalic and non-exencephalic embryos of this genotype, and the heterozygote may have a central face bleb (Johnson, 1969), although its gross fluid relationships are essentially normal.

Much larger and more persistent fluid filled blebs, often accompanied by subcutaneous oedema, are a regular feature of mice homozygous for blebs (*my*, Carter, 1959). In this stock, however, there is no sign of excess fluid in or around mutant embryos (Fig. 5, Table 3).

Table 3. *Embryonic fluid in my/my and normal (+/my) litter-mates*

(Embryonic fluid is calculated as wet weight of embryo minus dry weight of embryo and is expressed as g/g embryo dry weight.)

| Age (days) | Embryonic fluid | | A/N |
|---------------|-----------------|-------------|------|
| | <i>my/my</i> | <i>+/my</i> | |
| 12 | 9.99 | 11.49 | 0.87 |
| 13 | 8.71 | 8.74 | 0.99 |
| 14 | 8.00 | 8.08 | 0.99 |
| 15 | 8.18 | 8.16 | 1.00 |
| 16 | 6.18 | 6.16 | 1.00 |
| 17 | 5.60 | 5.53 | 1.01 |
| 18 | 5.57 | 5.26 | 1.06 |

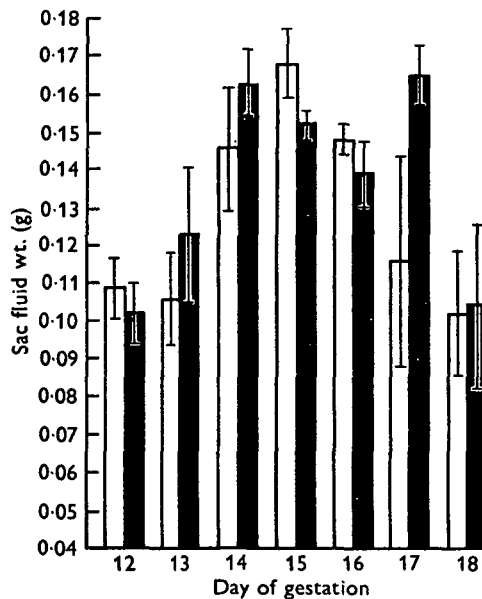


Fig. 5. The relationship between sac fluid weight and day of gestation in the *my* stock. □, *my/my*; ■, *+/my*.

4. DISCUSSION

Previous investigators have used a variety of methods to estimate the sac fluids of the mouse. McCafferty (1955) employed direct measurement with graduated pipettes. Gulienetti, Kalter & Davis (1962) rightly criticize direct measurement for such small volumes and suggest a sophisticated radio-isotope procedure as an alternative. Fraser, Chew & Verruso (1967) used a method almost identical to that of this paper, whilst Harris (1964) used both volumetric and weighing methods. The results presented here are in good agreement with those of Gulienetti, Fraser and Harris, but considerably higher than those of McCafferty. This suggests that weighing and radio-isotope methods are of the same order of accuracy, and that McCafferty's values are too low.

There is a marked difference in sac fluid weight between C57BL and the other three inbred strains studied, which tend to resemble each other closely throughout, once differences in developmental rate have been eliminated.

The pattern of fluid regulation in the F_1 embryo between CBA and C57BL differs from both parent strains, and maternal genotype seems to exert a considerable modifying effect in this pattern. It is interesting that the effect is patroclinal rather than matroclinal. In both $Ph/+$ and $X^{t^{ph}}/+$ a central face bleb is sometimes present and in both cases the frequency of the face bleb is higher in embryos from a $+/+$ mother (Johnson, 1969).

In the F_2 one might expect that the segregation of large numbers of genes would lead to an increase in the variance of sac fluid weight, if not in its mean. This was not seen (Fig. 3), although there is an increase in the variance of embryonic wet weight (Table 4) showing that segregation is present and detectable by the methods used. It therefore seems that in this situation the genotype of the embryo is of no great importance in determining the mass of the fluids which surround it.

Table 4. Mean embryo wet weight \pm standard error of litters of embryos from C57BL, CBA and $CB \times CB$ (litter size 6-10)

| Stock | Age in days | | |
|----------------|-------------------|-------------------|-------------------|
| | 14 | 16 | 18 |
| C57BL | 0.228 \pm 0.009 | 0.477 \pm 0.012 | 1.043 \pm 0.037 |
| CBA | 0.193 \pm 0.006 | 0.483 \pm 0.010 | — |
| $CB \times CB$ | 0.273 \pm 0.005 | 0.601 \pm 0.024 | 1.136 \pm 0.063 |

The embryonic genotype is important, however, in the case of embryos homozygous for $X^{t^{ph}}$, which have an excess of sac fluid from day 13. The fluid imbalance is worse in exencephalics; this may be due to fuller expression of the genotype, the most severely affected individuals having a persistently open neural tube and poorer fluid regulation. The failure of the neural tube to close leads to an altered distribution of fluids within the conceptus. With the neural tube closed some of the excess fluid which finds its way into the embryo (or fails to find its way out) is retained as subcutaneous oedema. With the neural tube open it escapes and accumulates in the amnion and/or exocoelom.

There is a possible parallel here with anencephaly in man, which is also strongly correlated with polyhydramnios. The phenomenon of one member of a pair of monozygous twins being anencephalic and the other not has been reported on many occasions (Litt & Strauss, 1935; Stevenson *et al.* 1966) but there is only one report of both members of a pair of what were probably monozygous twins being affected (Josephson & Waller, 1933). This points to the importance of local intra-uterine environment, maternal/foetal relationships and/or embryonic nutrition in anencephaly. The same factors may decide whether or not an Xt^{bph}/Xt^{bph} embryo is to become exencephalic.

Grabowski (1964) has shown that exposure of the 3-day-old chick embryo to moderate hypoxia produces a syndrome resembling that seen in *my*, i.e. blebs, haematomata and consequent tissue and organ damage, and was able to demonstrate a generalized oedema involving both circulating and extra-cellular fluids. He goes on to review similar cases of the 'oedema syndrome' in mouse, chick and axolotl, including *my* amongst his examples. In fact, *my* is not representative of the 'oedema syndrome'. Plagens (1933) suggested that the excess fluid within the blebs of *my/my* embryos originated within the blood stream, found that 13½-day-old blebby embryos had only half as much plasma per million erythrocytes as normal litter-mates, and suggested that the subcutaneous oedema and consequent blebs were due to a redistribution of fluid within the embryo. Although Plagens' assumption that capillary leakage was due to the abnormal situation of certain capillaries has to be discarded (Carter, 1959), his general interpretation is in agreement with the results of this study.

In some cases it is possible to distinguish between excess fluid (Xt^{bph}) and misplaced fluid (*my*). Patch (Grüneberg & Truslove, 1960) is another example of fluid excess, while the excess of cerebrospinal fluid seen in the dural sac of the neck region of 13-day-old *tk/tk* embryos is fluid misplaced (Grüneberg, 1955). More often we see blebs which seem to cause damage by their mere presence, sometimes with the intervention of haematomata. Such blebs may appear flanking the unclosed or closing neural tube (9 days, *Ph*; Grüneberg & Truslove, 1960), at the base of a defective tail (11 days, *tc*; Theiler, 1959; *Sd*; Gluecksohn-Schoenheimer, 1945) or may be more widely distributed (12 days, *my*; 13 days, Xt^{bph}). They are also frequently seen as a result of the action of a variety of teratogens (Grabowski, 1964).

Whether these blebs are due to a total excess of fluid or merely a local one, the causal factors which underlie them still remain to be elucidated.

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