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A lethal mutation (*cab*) affecting heart function in the mouse

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SUMMARY

A new autosomal recessive gene (*cab*, 'cardiac abnormality'), which affects heart differentiation appeared spontaneously in our mouse colony. The affected homozygotes die approximately half a day prior to birth or at term, with the myocardium exhibiting vacuolation and large areas of mesenchyme-like cells. A significant finding in mutant foetuses is the lack of histochemically detectable glycogen.

INTRODUCTION

Mutations can be useful tools in the analysis of developmental processes (Gluecksohn-Waelsch, 1963). We were, therefore, very intrigued by the spontaneous occurrence of a new lethal mutation in our colony of mice, which already carried the muscular dysgenesis (*mdg*) mutation. Mating between a phenotypically normal brother and two of his sisters yielded an unusually high number of dead newborns, which were quite distinct from the easily recognizable *mdg/mdg* newborns (Pai, 1965) with which we normally worked. In size and morphology the dead newborns were similar to their living littermates. However, the affected animals were cyanotic, their lungs were not inflated, and occasionally small hematomas were present on their extremities. Some backcross matings between phenotypically normal progeny and their parents and some $F_1 \times F_1$ matings resulted in the same proportions of dead newborns with the same phenotype as that described.

We suspected that a second mutation, different from *mdg*, was responsible for the new lethal phenotype and further work was done to isolate and characterize the new mutation.

MATERIALS AND METHODS

The *mdg* stock in which the new mutation appeared has been kept in a closed breeding colony here. The new gene was crossed into stock not carrying the *mdg* mutation, some with a C3H/HEJ background and some with the same initial background as the *mdg* animals.

From the appropriate crosses, pregnant females were isolated and checked twice

daily for delivery of newborns, whose phenotypes were recorded. For the analysis of 18½–19 day foetuses, timed matings based on the vaginal plug method were utilized.

Whole foetuses (slit in strategic areas to allow fixative penetration) or isolated organs were placed in 10% formalin. Five micron histological sections were prepared by standard methods and stained with hematoxylin and eosin. Foetal sections were also stained with Best's carmine for the localization of glycogen and control sections were treated with amylase.

Table 1. *Newborns from heterozygote × heterozygote matings*

	Total	Normal	Affected	Affected, %
Observed	2010	1526	484	24
Expected (3:1)	—	1507·5	502·5	25

Heterozygous carriers of the new mutation were identified on the basis of their giving mutant dead newborns when mated to known carriers. The newborn mortality rate from matings between known wild-type animals in the colony was less than 2%.

RESULTS AND DISCUSSIONS

The segregation data obtained from numerous breeding experiments provided evidence that the new unknown abnormality was due to an autosomal, recessive mutation. Twenty-four percent of the progeny from matings between phenotypically normal, positively tested new heterozygotes (determined by progeny testing) were affected, males and females equally (Table 1). Further evidence for a recessive mutation comes from the fact that 95 of a total of 150 tested normal survivors from heterozygote × heterozygote matings were themselves carriers of the gene. This finding that heterozygotes constitute approximately 67% of the surviving population is expected for alleles segregating in the cross indicated.

The new mutation was provisionally designated *cab* (cardiac abnormality) in view of one of its major phenotypic effects (see below). Data obtained from matings between $+/mdg$ and $+/cab$ animals, and genetic tests of their progeny indicated that the *mdg* and *cab* genes assorted independently. Further, the *cab* locus showed no evidence of linkage to the albino, brown, or agouti loci, the only other known markers available in our stocks.

The major question of interest concerned the functional and morphological effects of the mutation and the actual cause of death of the *cab/cab* homozygotes. Non-specific degenerative changes, such as pyknotic nuclei and membrane dissolution observed in various organs of affected newborns indicated that generalized post-mortem alterations were occurring. Therefore, 18½–19 day foetuses from heterozygote × heterozygote matings were examined. The distinguishing characteristic of homozygous *cab/cab* foetuses was greatly reduced spontaneous or induced movements. Reflexes which may have been present initially disappeared a few minutes after delivery. Upon dissection of mutant foetuses, their hearts contracted only a few times or failed to pulsate at all, unlike the hearts of control foetuses.

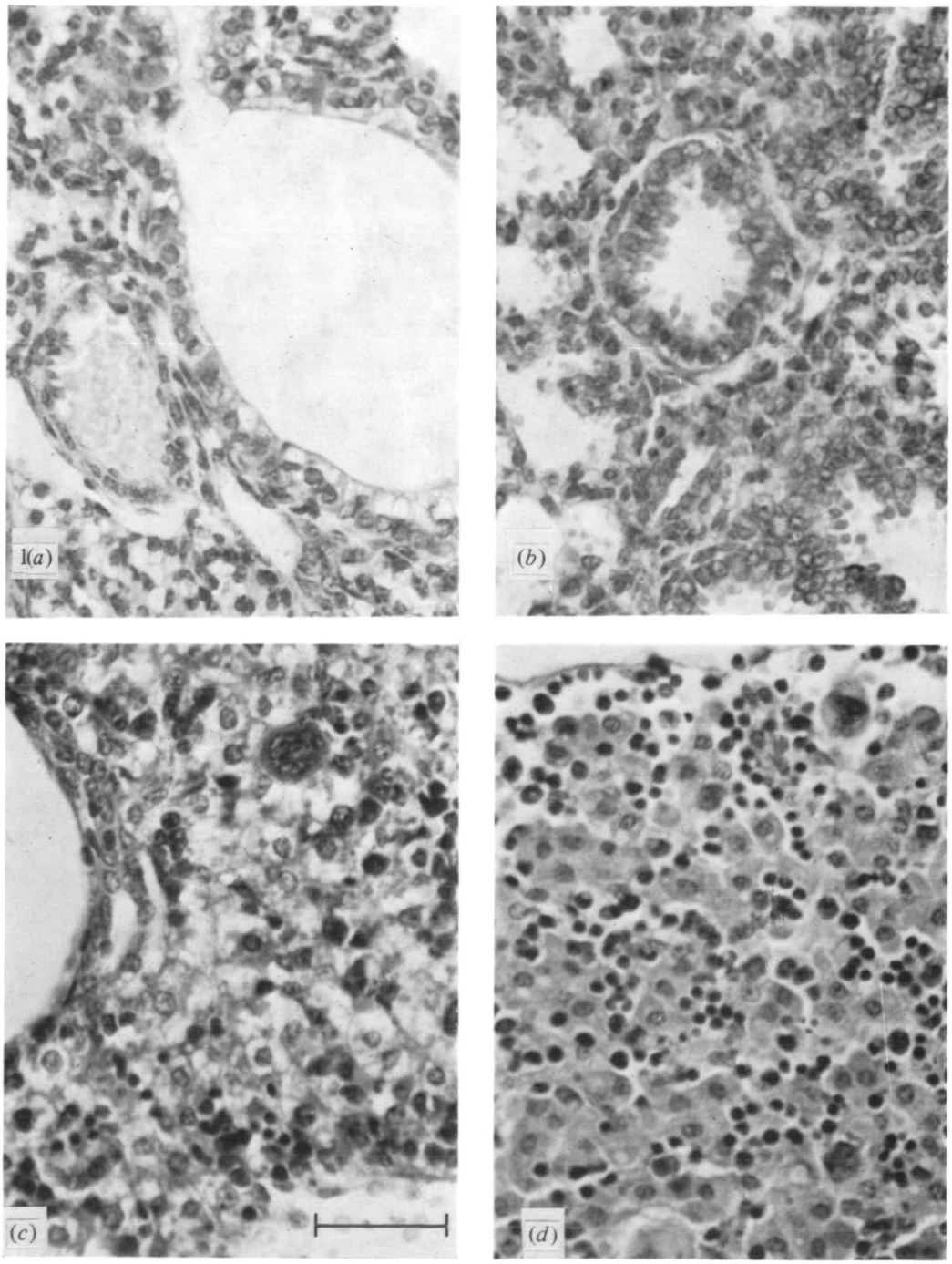

Fig. 1. Sections through the lung and liver of 19-day foetuses. H & E stain. (*a*) control lung; (*b*) mutant (*cab*/*cab*) lung; (*c*) control liver; (*d*) mutant liver. (The bar indicates 50 u.) In slides stained with Best's carmine the spaces in the control bronchiole and control liver are positive for glycogen; mutant organs are negative.

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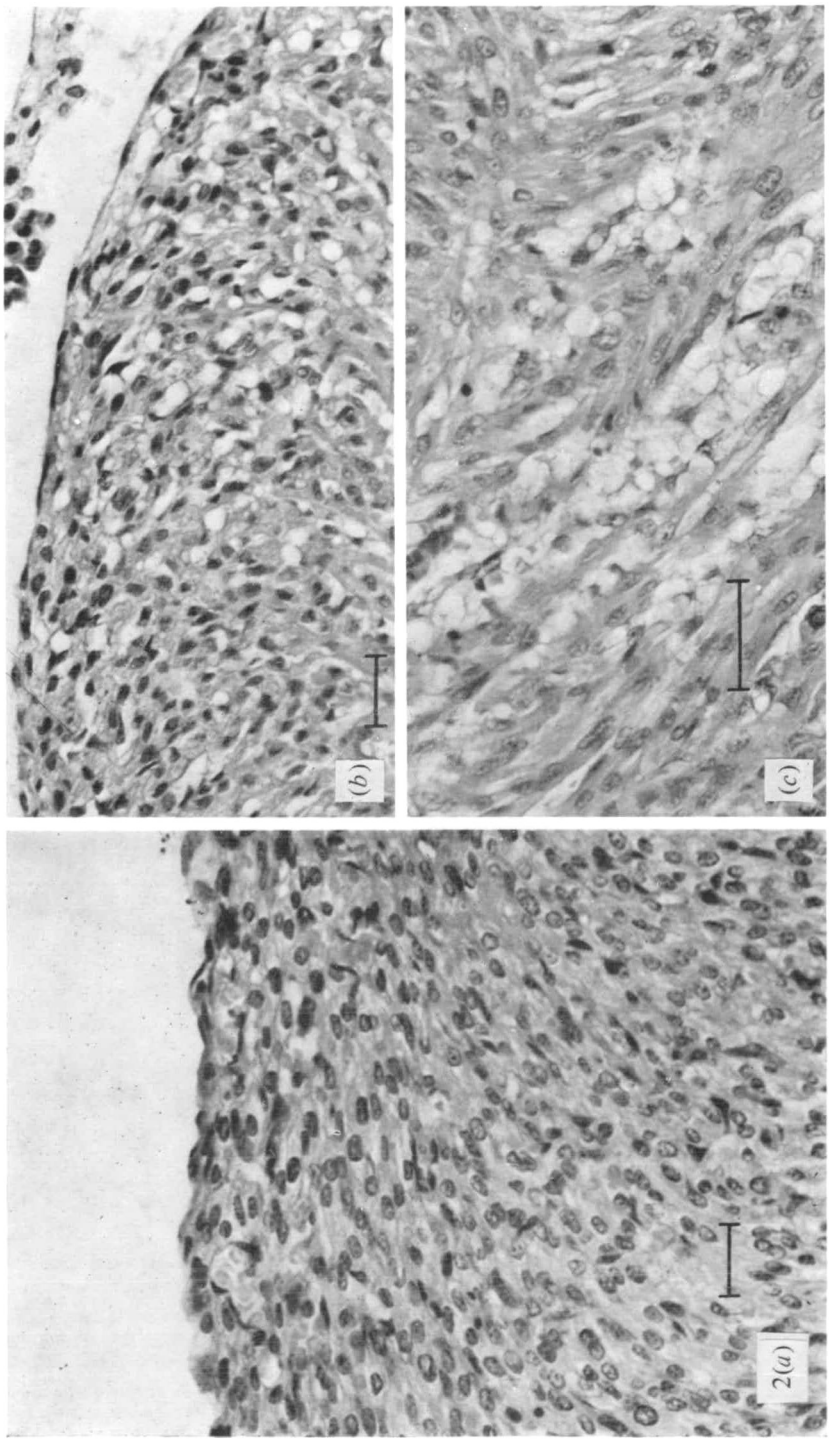

Fig. 2. 19-day foetal heart, H & E stain. (*a*) Control myocardium, gives positive reaction for glycogen; (*b*) and (*c*) ventricular myocardium of mutant foetus showing vacuolated cells; no glycogen is detectable in the *cab/cab* heart.

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The mutant foetal heart often had atrial and/or ventricular chambers engorged with blood. The great vessels, however, appeared normal.

Examination of histological sections revealed that most organs of the mutant foetus were comparable to controls. However, the duct epithelium of the mutant lung had no characteristic secretory cells (Figs. 1*a*, *b*). The *cab/cab* foetal liver also differed significantly from the control organ (Figs. 1*c*, *d*), lacking normally vacuolated regions in these formalin-fixed sections.

However, one of the most significant pathological changes observed was in the myocardium of *cab/cab* foetuses. There were numerous vacuolated cells present near the ventricular cavities and throughout the atrial and ventricular walls. (Fig. 2). In addition, the mutant myocardium contained prominent foci of mesenchyme-like cells. At present, the significance of either of these kinds of cells is unknown, but experiments in progress will permit their further characterization. The affected vacuolated cells did not have the features of rhabdomyomas observed in newborn mammalian hearts (Scotti, 1977), nor did they appear to be maturing cardiac cells undergoing fibrolysis as described for hereditary myocardial degeneration in the hamster (Bajusz, 1969). However, some of the features of the abnormal cells in *cab/cab* hearts are similar to cardiac cytological alterations reported in cases of infantile cardiomyopathy by Ferrans, McAllister & Haese (1976).

In normal foetal sections stained with Best's carmine the liver, lung, heart and skeletal muscle exhibited strong, positive reactions, with the amylase treated sections appearing negative. In striking contrast, there was no histochemically detectable glycogen in any of the *cab/cab* foetal organs. Thus, this new lethal mutation may cause a generalized defect in carbohydrate metabolism, with severe consequences for myocardial cell structure and function. Biochemical, histochemical and developmental studies in process should permit a better understanding of the lethality caused by the *cab* mutation.

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