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Loss of microsomal β-glucuronidase and structural change of its lysosomal form in cultured mouse hepatocytes

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In some organs, lysosomal β-glucuronidase is also localized in the endoplasmic reticulum (microsomes) and bound to the anchor protein egasyn. In freshly prepared hepatocytes, approximately 40% of total activity was present in the microsomal form. During culture, this form disappeared within 6 days and concomitantly, a new form appeared in the lysosomes. This suggested a correlation between the absence of β-glucuronidase in microsomes and a lysosomal enzyme of particular structure. The correlation was confirmed in spleen which normally lacks the microsomal enzyme and was found to contain a lysosomal form electrophoretically identical with that of 6-day-cultured hepatocytes. From this observation and from the fact that the microsomal enzyme, fully active at acid pH, was less than 5% active at neutral pH we conclude that the accumulation of β-glucuronidase in the endoplasmic reticulum may be important not for catalysis but for the control of directing and packing the enzyme into the lysosomes.

Mapping of malaria resistance genes in the mouse

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A long search for resistance genes to malaria has resulted in a likely location of such a gene in the distal part of chromosome 1 of the mouse. We have been using both conventional back-crosses and BXD RI strains for the tests. So far we have not been able to confirm, in the BXD strains an earlier finding of a resistance gene on chromosome 9. When BXD-14 which is resistant was crossed to the sensitive BXD-5 strain, resistance was found to be dominant. There also seems to be complementation, as F_1 animals between C57BL/6 and DBA/2 are far more resistant than the parental strains.

Detection of mouse mutants with altered enzyme activities

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Specific activities of several erythrocyte enzymes are determined in order to detect gene mutations (*Mutation Res.* **97** (1982), 177–178). Male (101 × C3H)F_1 mice were treated with ethylnitrosourea (ENU), and mated to untreated T-stock females. Activities of 10 enzymes (LDH, TIP, MDH, PGI, PGK, PGAM, GAPDH, G-6-PD, PK, GR) were measured in the blood of the offspring (derived from spermatogonia) in an automatic analyser. To date, no mutant in 2713 controls, 5 mutants in 800 offspring of a 160 mg/kg ENU group ($P < 0{\cdot}001$), and 2 mutants in 505 offspring of a 250 mg/kg ENU group ($P = 0{\cdot}024$) were found. In the same ENU 250 mg/kg experiment, specific locus mutants and dominant cataract mutants were scored (Ehling *et al.*, *Mutation Res.* **92** (1982), 181–192). The mutation frequency (mutants per gamete) of the studied enzymes is by a factor 1·5 lower than that of the specific loci, but by a factor 3·7 higher than that of the cataracts. Combining these tests in one experiment, more than 40 loci can be scored per animal. Of the 7 enzyme-independent mutants, 6 caused reduced activity, i.e. 1 LDH, 3 MDH, 1 PGI and 1 G-6-PD, and 1 caused increased PK activity. The G-6-PD mutation is *X*-linked and the first detected in experimental animals.

The structural and genetical basis of the multiple forms of human lysosomal α-D-mannosidase

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The gene for human lysosomal (acidic) α-D-mannosidase (EC 3 . 2 . 1 . 24) has been assigned to a single locus on chromosome 19. However, the enzyme exists in at least two major forms, A and B, which have similar properties but which can be separated by differences in charge and size. The proportions of these two forms vary from one tissue to another. In mouse two other loci have been found to affect the proportions of the corresponding forms of α-D-mannosidase but as yet a similar locus has not been found in man. To understand the structure and genetical basis of the multiple forms of α-D-mannosidase the sub-unit comparison of human liver α-D-mannosidase and the biosynthesis of fibroblast α-D-mannosidase have been investigated.

Genetic variation in the production of TCDD-induced porphyria

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One aspect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity is the induction of porphyria in mice. In crosses between C57BL/6J and DBA/2J the degree of TCDD-induced porphyria segregates with the Ah^b (aryl hydrocarbon hydroxylase induction) allele. C57BL/6J (Ah^bAh^b, AHH responsive) are susceptible and DBA/2J (Ah^dAh^d, AHH non-responsive) are resistant. Imferon (12·5 mg Fe/mouse, s.c.) seven days before TCDD (75 μg/kg, p.o.) maximizes the porphyric response. Differences in the degree of porphyria were found between *Ah* non-responsive strains. DBA/2 showed a negligable porphyria which AKR had high hepatic porphyrin levels. *Ah* responsiveness is not therefore the sole requirement for the induction of porphyria and liver damage by TCDD. A series of crosses between DBA/2J and AKR showed that the inheritance of susceptibility to TCDD is complex.

Alterations in the non-ribosomal protein pattern from human skin fibroblasts and meningiomal tumour cells with normal karyotype and monosomy 22

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Primary cell cultures from human meningiomas and skin fibroblasts from the same patients were incubated in the presence of S 35-methionine at 37 °C and 42 °C, respectively. The ribosome-associated protein fraction (mostly initiation factors of protein synthesis) was analysed by 2D-PAGE. The comparison was carried out in order to find out whether the lack of chromosome 22 leads to any visible change in the overall synthesis of ribosome-bound proteins under normal (37 °C) and hyperthermal (42 °C) growth conditions. Our findings show that a set of high mol. wt. proteins which are mostly acidic in nature and which are absent in cells grown at 37 °C are synthesized at 42 °C. The tumour cell-derived protein patterns differ from normal fibroblasts inasmuch as protein 'K' is lacking in cells grown at elevated temperatures. However, so far no difference in the protein pattern was found between tumour cells with a normal karyotype and those with monosomy 22. The present findings favour the idea that chromosome 22 is not directly involved in the cellular response to heat shock conditions.

Variation in the hepatotoxic effects of carbon disulphide in the rat

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Phenobarbitone pretreatment increases the hepatotoxicity of carbon disulphide in rats. Earlier work in this aspect of CS_2 toxicity had used an outbred Lac : P rat, and variation in this stock had made further studies difficult. Considerable genetic variation in the responses of rats from eight inbred strains was observed, with a good correspondence between measures of histopathological damage to the liver and biochemical indices. A series of crosses between AGUS, the least affected strain, and PVG, a strain showing extensive liver damage, indicated that the variation could not be explained by a simple genetic model.

More about tasting in mice

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The gene *Qui* determines sensitivity to the bitter taste of quinine. The gene *Soa* determines sensitivity to the bitter taste of sucrose octa-acetate and also strychnine. Sucrose is a disaccharide. I have now extended this genetical analysis to include the acetates of monosaccharides (glucose and glucitol) and of a trisaccharide (raffinose). The results of surveys in inbred strains and RI strains indicate that (1) sensitivity to glucose penta-acetate is determined by *Soa*; (2) sensitivities to glucitol hexa-acetate and raffinose undeca-acetate are each determined by different genes (provisional symbols *Gha* and *Rua*); (3) *Rua* may be linked to *Qui*. I suggest that before a molecule can reach the taste receptors it must first pass through a molecular sieve containing pores of different shapes and sizes. The genetical variation of different classes of pores could influence the ease of passage of molecules through these pores and hence the sensitivity of mice to the different classes of bitter molecules.

Two genes controlling hormone regulation of mouse histidine decarboxylase (HDC)

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A gene determining oestrogen-inducibility of histidine decarboxylase, *Hdc-e*, is linked within the gene complex on chromosome 2, has a co-dominant mode of inheritance, does not have pleiotropic effects and is probably *cis*-acting. *Hdc-e* is particularly unusual since alternative alleles are widely distributed amongst inbred strains and differ between C57BL/6 and C57BL/10. A fully recessive allele of a second locus *hti* (HDC thyroxine inducibility) alters the extent, but not the kinetics, of induction of the enzyme by thyroxine.

Genetics of carbohydrate metabolism in animal cells

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Chinese hamster ovary cells, CHO-K1, cannot grow in medium in which glucose is replaced by ribose. A ribose$^+$ variant has been isolated and shown to be unable to glycolyse glucose. This variant lacks glucose phosphate isomerase (GPI) and phosphoglycerate kinase (PGK) activities. To determine whether or not the ability to grow on ribose is related to the inability to glycolyse glucose we fused the ribose$^+$ GPI$^-$ PGK$^-$ variant with wild-type cells. Analysis of the hybrids revealed that the ribose$^+$ character was dominant and not dependent on the absence of GPI and PGK activities. No GPI$^+$ recombinant was isolated following the fusion of this GPI$^-$ PGK$^-$ variant with a GPI$^-$ variant obtained from Dr Jacques Pouyssegur. It is likely therefore that the lack of GPI activity is due to a mutation in the structural gene. Analysis of glucose utilization shows that these glycolysis mutants are absolutely dependent on respiration to furnish their energy. This work is supported by MRC Programme Grant G977/997.

The genetics of fructose biphosphatase (EC 3.1.3.11) in the mouse

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Evidence from starch gel electrophoresis suggests that there are at least two structural genes in the mouse for this important glucogenic enzyme. One gene

product appears to be confined to the liver and kidney and is monomorphic in the strains tested. The other, found in most tissues, is polymorphic, and the evidence suggests three alleles at a structural locus (*Fbp-1*).

Production of 'biochemical' mutants in the mouse and the analysis of a new haemoglobinopathy

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Electrophoretic techniques have been used to screen for germinal mutations induced by ethylnitrosourea in the mouse, since by this approach mutations arising at loci coding for well-defined proteins can be detected and investigation of the nature of the mutational lesion is possible. In a recent experiment eight electrophoretically detectable mutations have been found, three of which have been at the *Hbb* locus, coding for the β-globin chain of haemoglobin. One of these leads of polycythaemia, and is probably the result of a single amino acid substitution due to a single base alteration in the DNA. This mutant is the first example of a β-globin haemoglobinopathy in the mouse, and may be analogous to polycythaemias in man which are associated with altered β-globin structure.

Detection of enzyme mutants in mice by isoelectric focusing

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The aim of our research is to detect gene mutations in mice leading to electrophoretical alterations of enzymes. In order to score in the same experiment for specific locus mutations, (101 × C3H)F_1 males were injected with 600 mg/kg procarbazine hydrochloride (PHC1), 160 or 250 mg/kg ethylnitrosourea (ENU) and mated to untreated T-stock females. Liver proteins from the F_1 offspring were separated by isoelectric focusing on polyacrylamide gels (Electrophoresis **3**, 1982, 142–145). Banding patterns of 6 enzyme system (ES, LDH, SDH, GDC, PGM, DIA) were checked by using the agar contact replica technique and specific activity stainings. No mutant was found in 5278 offspring of the control group. The frequency of enzyme mutants was increased after paternal treatment of spermatogonia, namely 1 mutant/5630 offspring (600 mg/kg PHC1), 1/1892 (160 mg/kg ENU) and 4 (2 died)/4136 (250 mg/kg ENU). In the 250 mg/kg ENU group, the frequency of the 4 mutants (2 confirmed, 2 presumed) is significantly different from that of the control ($P = 0{\cdot}037$). An LDH mutant has been further characterized (*Biochem. Genet.* **19**, 1982, 301–309). It presents an interesting model to study the regulation of haemopoeisis by disturbed haematologic basic values.

Resistance to 2,6-diaminopurine in a Friend leukaemia cell clone

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Resistance to the adenine analogue, 2,6-diaminopurine (DAP) has been examined in clone 707 of the Friend leukaemia cell line. The frequency of cells resistant to 100 μg/ml DAP was found to be high ($2{\cdot}25 \times 10^{-5}$), with a mutation rate of $1{\cdot}8 \times 10^{-6}$ cell^{-1}. Fluctuation test analysis has revealed that the mutations occurred spontaneously and were not induced by the selective agent. DAP-resistant subclones were found to have less than 10 % of the wild-type level of incorporation of radioactivity labelled adenine into nucleic acids. It is suggested that the high forward mutation rate to DAP resistance may be due to only one functional adenine phosphoribosyl transferase allele being present in cells of clone 707. It is possible that considerable areas of the clone 707 genome may be inactivated, as a similarly high mutation rate have been observed at the thymidine kinase locus in this clone (McKenna, P. G. & Hickey, I., *Mutation Res.* **80**, 187–199). This research was supported by a grant from the Ulster Cancer Foundation.

Genetic differences in the responses of zona pellucida and cumulus oophorus to hyaluronidase and pronase

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A reproductive advantage may occur in female C57 mice that compensates for male deficiencies resulting from sub-normal androgen levels. Dissolution rates of zona pellucida and cumulus oophorus levels have been measured in C57BL/6By, Balb/cBy and the CXB Recombinant-Inbred lines. Clutches of ova isolated from superovulated females were treated with hyaluronidase followed by pronase. Breakdown of both peri-ovum layers was considerably faster in the C57 females, indicating a greater efficiency in reaction to spermatozoal enzymes and therefore a more rapid penetration of the ovum. C57BL/10ScSn sublines were similar to C57BL/6By. Both parental phenotypes were found amongst the CXB lines, in addition to lines with intermediate phenotypes. No correlation existed between reaction times for the R-I lines. A difference between reciprocal F1 mice in their response to hyaluronidase suggests that separable mechanisms underlie the responses to the two enzymes.