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Protein polymorphisms in strains of golden hamsters

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Introduction

Compared to other experimental animal species the golden or Syrian hamster (*Mesocricetus auratus*) has only a recent laboratory history. But during this short period it has become the model of choice in many fields of biomedical research (rev. v. *Hoosier & McPherson* 1987) represented by a variety of strains which are now available (*Festing* 1979, *Altman & Katz* 1979).

From the genetical point of view it is of interest that all laboratory strains were derived from very few wild caught animals. The first gene pool was contributed by Aharoni in the early thirties consisting of three or four littermates which served as founders for all strains until 1970 (*Altman & Katz*, 1979, *Heinecke* 1990).

This pool was supplemented twice: 1970 by Murphy and 1978 by Duncan adding another dozen of wild hamsters (rev. *Heinecke* 1990).

However, according to *Streilein* (1987) most of the strains available at present are originally derived from the first capture of Aharoni.

Considering this narrow genetic "bottleneck" and the short time of controlled breeding the degree of genetic variability of the golden hamster could be expected to be rather low. But the great number of coat colour variants, marked strain differences concerning sensitivity against tumours, bacterial and viral infections, and hereditary diseases (*Altman & Katz* 1979) are indications of a substantial genetic variability.

However, despite its frequent use in research a comprehensive genetic characterization of the laboratory strains of this species is still missing. But this would be of urgent necessity for providing characters which could serve as a basis for genetic monitoring procedures.

The present study will contribute to such a characterization giving a preliminary overview on phenotypic electrophoretical variation comparing different strains of hamsters.

In a second step the variants have to be tested for their genetic background and in case of inheritance for their value in genetic control systems.

Materials and methods

Animals

Males and females of 16 inbred strains and one random-bred stock were included in this study:

Twelwe inbred lines derived from the outbred stock Han:AURA of the Central Institute for Laboratory Animal Breeding (Hannover, FRG). It came to the institute in 1974 originating from the stock Hö:SYK of the Höchst company (Frankfurt/Main, FRG). Inbreeding was performed at Hannover since 1981 by fullsib mating which was interrupted in a few cases by parent × progeny mating (*Sickel* 1991). In 1988 the following agouti coloured Han strains were available (the number of inbreeding generations is given in parantheses):

AUA1/Han (F27)
AUA2/Han (F23)
AUA3/Han (F26)

AUB1/Han (F28)
AUB3/Han (F24)

AUD1/Han (F30)
AUD2/Han (F28)
AUD3/Han (F30)

AUE2/Han (F28)

AUG1/Han (F30)
AUG2/Han (F28)
AUG3/Han (F29)

In addition to the Han lines the following inbred strains were included:
– APRICOT /Bga (coat colour: apricot): this strain was originally maintained at the Clinical Research Centre of the Medical Research Council in Great Britain. With F50 it came to the Bundesgesundheitsamt (Berlin, FRG) from the Institute of Virology of the university of Gießen (FRG) (ZVA Informationen, no. 14, 1981). Animals of F68 were obtained from the Bundesgesundheitsamt for this study.
– BIO 1.5 (coat colour: acromelanic white): animals of this strain were obtained with F62 from the Central Laboratory Animal Facility of the university of Essen (FRG). In Essen inbreeding was applied since 1978. The founder animals came from the BIO-Research Institute of Cambridge (Mass., USA) (*Militzer* 1987, *Militzer et al.* 1990). Just before shipping the animals from USA to Germany there was a break in the fullsib mating followed by a short period of random mating within the strain (*Van Dongen* 1978). However, all generations bred at Essen were produced by brother × sister mating (*Militzer* 1992).
– LSH/SsLak (coat colour: agouti): originating from Charles River (USA) with F75. In 1965 Charles River Lakeview received the first breeders from Billingham and Silvers, university of Pennsylvania, USA, with F31 (CR Bulletin, no. 1, 1982).
– MHA/SsLak (coat colour: acromelanic white): originating from Charles River (USA) with F67. The Charles River colony was founded in 1965 using also animals from Billingham and Silvers having reached F23 (CR Bulletin, no. 1, 1982).
In addition to the inbred strains one random-bred stock was used:
– BIO R (coat colour: agouti): obtained from the Institute for Experimental Pathology of the Medical High School of Hannover (FRG) via Dr. Dasenbrock (Fraunhofer Institut für Aerosolforschung, Hannover, FRG). The information available about the breeding history of these animals was short. However, the founders originated from the BIO-Research Institute (USA). Breeding was continued at Hannover by random mating (*Dasenbrock* 1992).
All animals included were maintained under conventional conditions at the Institute for Laboratory Animal Science of the university of Aachen (FRG). They were housed in groups of two or three on soft wood bedding in macrolon cages, type III, with free access to food (Altromin, no. 1314) and drinking water (ozone treated and acidified to pH 4.5). Cellulose sheets were given as nesting material. The light/dark cycle was 12 : 12 hrs, the mean room temperature 20 ± 2 °C, the relative humidity 50–70 %.
From each strain samples of different age were tested including 4 consecutive generations: 6–8 weeks, 4–6 months, and retired breeders having reached one year of age and more. The number of animals per test group varied between 10 (APRICOT, BIO 1.5, BIO R, LSH, MHA) and 50 (AU/Han strains).

Samples and electrophoresis

The following tissue were used for electrophoresis: plasma, hemolysate, liver, kidney, pancreas, skeletal muscle, duodenum, and urine. Blood was collected from the anesthesized (ether) animals by cardiac puncture using EDTA stabilized syringes. Plasma was separated by centrifugation for 10 min. at 5,000 g. The red cells were lysed by adding an equal volume of distilled water and subsequent centrifugation for 10 min. at 10,000 g.
The organs were homogenized in 2 ml of a 0.1 % Triton ×-100 solution using an ultraturrax homogenizer (IKA–Werk, Staufen, FRG). The homogenates were centrifuged for 1 h at 20,000 g. Urine was centrifuged like plasma and used without further treatment. The supernatant of each sample was divided in several parts:
One was used immediately, the others were frozen at – 80 °C for different intervals to study the effect of storage: one week, a fortnight, 4 weeks, 8 weeks and more.

Table 1. Proteins studied electrophoretically in golden hamsters.

Proteins	Abbr.	EC no.	Tissues
Aconitase	ACON	4.2.1.3	K
Alcohol dehydrogenase	ADH	1.1.1.1	L
Erythrocyte proteins	HBB, EP		E
Esterases	ES	3.1.1.1	E, K, L, M, PA, PL, T
Glutamate oxalacetate transaminase	GOT	2.6.1.1	L
Glucose-6-phosphate dehydrogenase	GPD	1.1.1.49	K, E
Glucose phosphate isomerase	GPI	5.3.1.9	E
Isocitrate dehydrogenase	IDH	1.1.1.42 L	
Lactate dehydrogenase	LDH	1.1.1.2	M, E
Leucine arylaminopeptidase	LAP	3.4.1.1	D
Malic enzyme	MOD	1.1.1.40	L
Malate dehydrogenase	MDH	1.1.1.27	L
Muscle proteins	MP		M
Peptidases	PEP	3.4.11	L, T
6Phosphogluconate dehydrogenase	PGD	1.1.1.44	K
Phosphoglucomutase	PGM	2.7.5.1	E
Plasma proteins	TRF, ALB		PL
Urine proteins	MUP		U
Xanthine dehydrogenase	XDH	1.2.3.2	L

E: erythrocytes, D: duodenum, K: kidney, L: liver, M: muscle, PA: pancreas, PL: plasma, U: urine, T: testis.

Nineteen groups of enzymes resp. unspecific proteins were included (Table 1). As most of them were present as two or more isoforms (differentiated according to their electrophoretical migration behaviour), at least 40 isoenzymes/-proteins were stained. Separation was carried out applying horizontal electrophoresis systems in starch gel (STAGE) and for the esterases additionally in polyacrylamide gel (PAGE) at a cooling temperature of 4°C. The starch gels (300×200×2 mm) consisted of 13 % hydrolyzed starch (Sigma Chemie, Deisenhofen, FRG). About 10 µ of the samples were inserted using filter papers (no. 1450, Schleicher & Schüll, Dassel, FRG) having a size of 2×5 mm. PAGE was run in a multiphor II chamber (LKB, Bromma, Sweden) using ultrathin gels (0.2 mm) with a pore gradient between 4–22.5 % T. 5 µl of the tenfold diluted (w:v, bidistilled water) samples were applied.

Staining was carried out using 1 % amido black for the unspecific proteins (EP, HBB, MP, TRF, ALB, MUP) while the substrate / coenzyme / dye reaction was utilized for demonstration of the specific enzyme phenotypes.

The buffer and staining conditions are given in principle by *Shaw & Prasad* (1970).

Results

The following proteins from Table 1 revealed electrophoretical variants: EP, ES isozymes from plasma (2×), pancreas (3×), and liver (1×), LAP, and PEP from liver.

The phenotypes could be shown to be reproducible concerning their banding pattern (using fresh samples) and were independent from sex and the covered period of age. In the following a short description is given for each of the polymorphisms:

EP (erythrocyte protein) (Figure 1) is an unspecific protein of the red cells migrating in STAGE at pH 9.0 anodally to the hemoglobin fraction in the albumin region. Three variants could be observed in the present animals:

One type consisted of a strong band with slow mobility (Figure 1c) sometimes in combination with a faster migrating zone of minor activity (not shown).

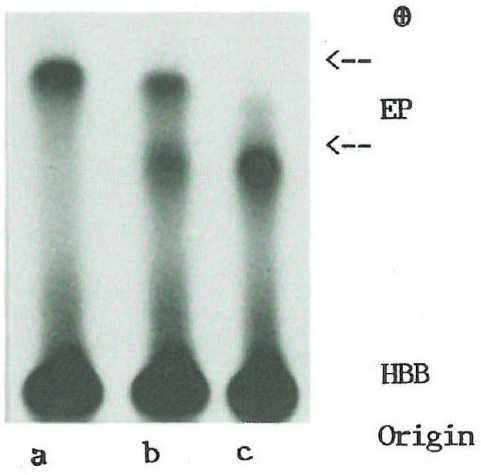

Figure 1. Phenotypes of the erythrocyte protein (EP). a. Fast type; b. Double banded type; c. Slow type.

The second type expressed a single fast band (Figure 1a). The third phenotype was characterized by two strong bands of similar activity (Figure 1b). A marked effect of storage could be observed for the slow band: single freezing of the samples lead to an increase of its mobility compared to that of the original fast type. Thus, the application of fresh samples is absolutely recommended.
First results of family studies indicate a monogenetic codominant inheritance of the protein variants. However, despite the degree of inbreeding of the animals no clear strain dependency of the phenotypes could be observed.
Six of the esterase isozymes (ES) were found to be polymorphic. Their distribution was strictly organ specific and activity was expressed only in a particular tissue respectively.
In liver the most anodal ES showed variation at pH 8.6 characterized by the presence (Figures 2a, b) or absence of a prominent band (Figures 2c, d). The optimum staining was achieved using 1-naphtyl acetate as substrate combined with Fast Red TT salt as

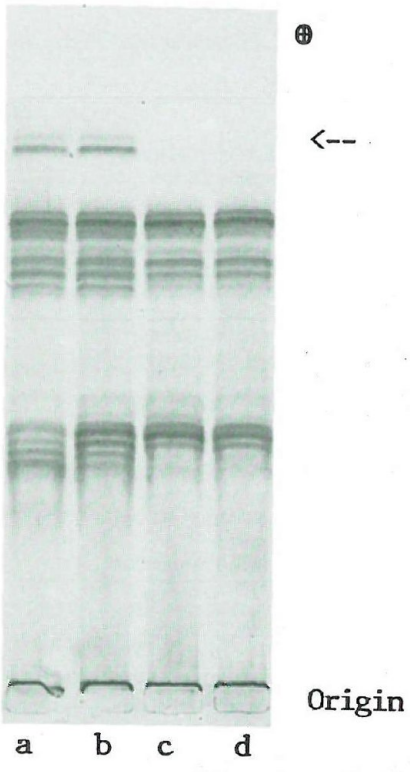

Figure 2. Phenotypes of the polymorphic liver esterase. a., b. Active band; c., d. No activity.

dye. The phenotype distribution of this ES was clearly strain dependent. The active type was present in the strains BIO 1.5, BIO R, and MHA while the non-active type was found in all other strains.
In plasma two variable ES-zones could be stained using 1-naphtyl butyrate as substrate (Figure 3). Both ES either showed an active band (Figures 3a, b) or not (Figures 3c, d). Similar to the liver ES there was a clear strain association of phenotypes. The active types could be confirmed for the following strains: APRICOT, LSH, and the AU lines A1, A2, A3, B3, D1, D2, D3, E2. No activity was proved for BIO 1.5, BIO R, MHA, and the AU lines B1, G1, G2, G3.
Electrophoresis of pancreas revealed three ES showing variants (Figures 4, 5), numbered from I to III, beginning with the most

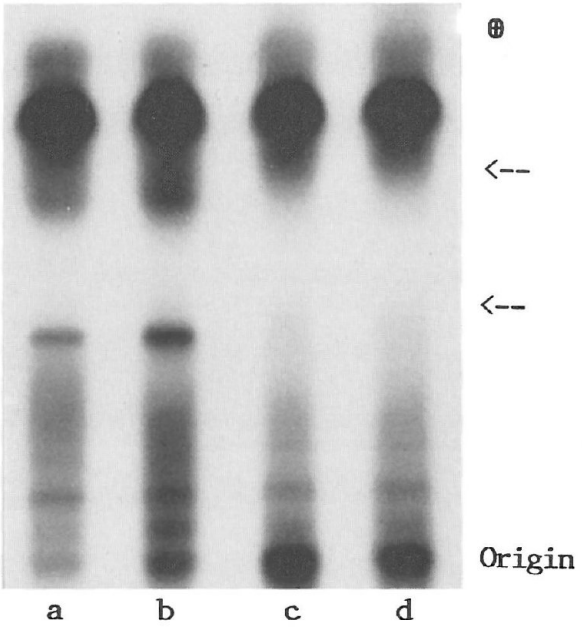

Figure 3. Variants of plasma esterase. a., b. Active bands. c., d. No activity.

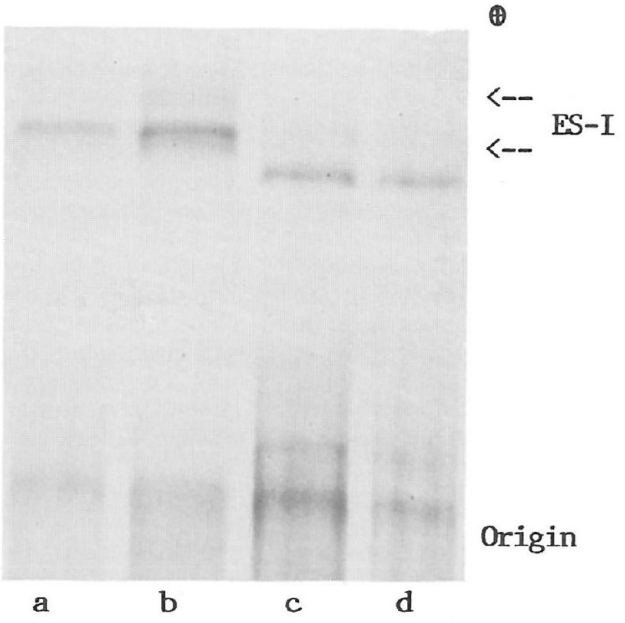

Figure 4. Pancreas esterase (ES-I). a., b. Fast type. c., d. Slow type.

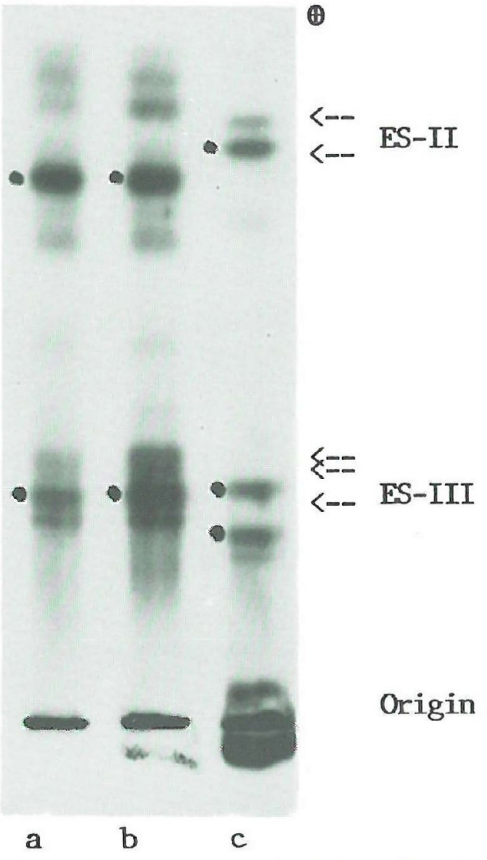

Figure 5. Pancreas esterases (ES-II, III). a., b. ES-II slow complex, ES-III one major band. c. ES-II fast complex, ES-III double banded pattern.

anodal one (Figure 4). Optimum separation of ES-I was achieved applying PAGE (pH 8.6) while ES-II and III were finally typed in STAGE (pH 8.6). Two phenotypes could be observed for ES-I, consisting of a fast (Figures 4a, b) or slow complex of bands (Figures 4c, d) respectively. ES-II also had a multibanded pattern with one prominent zone, migrating either slow (Figures 5a, b) or fast (Figure 5c).

Two phenotypes could be differentiated for ES-III having either one major band (Figures 5a, b) or a double band with similar activity (Figure 5c).

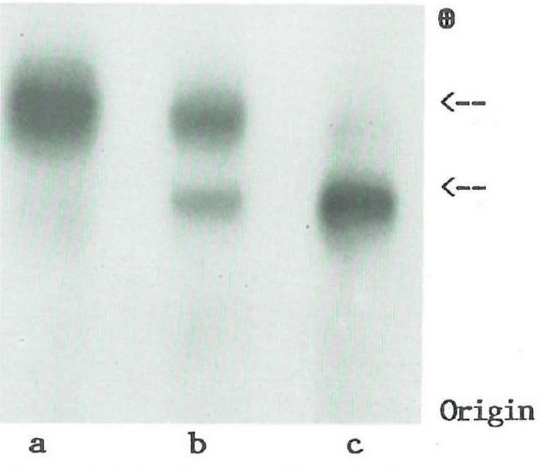

Figure 6. LAP phenotypes from duodenum. a. Fast type. b. Double banded type. c. Slow type.

A strong effect of storage was obvious for ES-I and II. After freezing the samples once for two days at – 80 °C the activity markedly decreased and typing was not longer possible. However, storage for up to three days at + 4 °C did not lead to a significant loss of activity.

The variants of pancreas ES were expressed in all strains.

Close linkage could be observed between the phenotypes of plasma ES as well as between those of pancreas (ES-I, II, and III). No linkage relationship was established for the liver ES.

Three LAP phenotypes could be determined from duodenum (Figure 6) characterized by a fast migrating band (Figure 6a), a band with slow mobility (Figure 6c), and a double banded pattern (Figure 6b) respectively. The variants were present in all strains investigated here.

A dipeptidase from liver was found to be polymorphic (Figure 7) using leucylalanine as substrate. First backcross results indicate a codominant inheritance of the phenotypes which could be differentiated according to their migration rate. The following strain distribution of the variants has been recor-

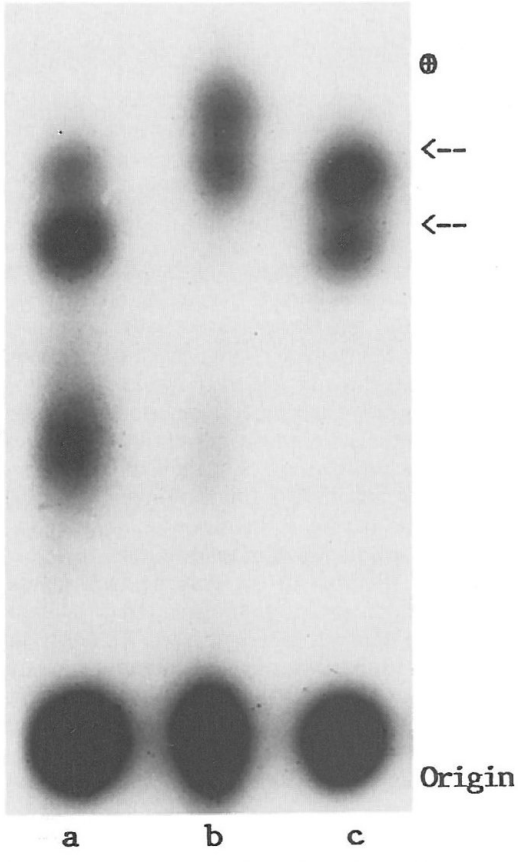

Figure 7. Polymorphic dipeptidase from liver. a. Slow type. b. Fast type. c. Heterozygous type.

ded: Slow type (Figure 7a): BIO 1.5, BIO R, LSH, and the AU-lines A1, B2, D1, D2, D3, E2, G3. Fast type (Figure 7b): APRICOT, AUA2, AUB1. Segregating strains, expressing e.g. the heterozygous phenotype (Figure 7c): AUA3, AUB3.
The MHA strain was not included in PEP typing.

Discussion

The present study revealed a considerable degree of electrophoretical variation in the golden hamster. It is comparable to that calculated from the results of *Csaikl* (1984). He typed animals from an outbred stock finding different proteins to be polymorphic than in the present material. This variability on the protein level is quite similar to other laboratory animal species (*Rice & O'Brien* 1980, *Bender et al.* 1984, *Kluge et al.* 1994). Referring to the close genetic relationship of the founder animals of the laboratory strains (*Heinecke* 1990) this could not be expected in any case but agrees with the variation found for other characters (*Altman & Katz* 1979). However, in contrast to mouse and rat the genetic background of most of the hamster variants still has to be elucidated. As shown by the results only four of the nine polymorphisms are strictly strain dependent (liver ES, plasma ES |2×|, liver PEP) giving a first strong hint on a genetic origin. This is supported by the preliminary results of interstrain crosses which agree with a monogenetic inheritance of these variants (*Kluge*, unpublished results).
The intrastrain variation of the other proteins indicates at first sight a non-genetic background of the phenotypes because the individual lines are completely inbred except for BIO R. In case of verification this would be species specific for the Syrian hamster because such a number of non inherited electrophoretic variants is unknown from other laboratory animals. Excluding methodical reasons first of all posttranslational metabolic influences (e.g. differences in phosphorylation or oxidation) may count for the maintenance of phenotypical polymorphisms of isoenzymes within a species or a population (*Cassman & Vetterlein* 1974, *Wynn* 1978).
Jagiello et al. (1986) published remarkable results investigating the influences of hibernation on spermiogenesis in Syrian hamsters. They pointed out that a hibernation of about ten weeks leads e.g. to a prolonged premeiotic DNA-synthesis, abnormal bivalent pairing, an increased non-disjunction rate, and a reduced crossing over rate especially for the smaller autosomes.
Thus, we can draw the conversial conclu-

sion that the omission of hibernation under usual laboratory conditions also may affect characters such as the structure and confirmation of active enzyme molecules leading perhaps to electrophoretical variants despite similar background alleles.

In this connection the tissue specific expression of the esterase isozymes should be mentioned, not knowing whether this is of special significance for the animals. But it is in contrast to e.g. mouse and rat showing a tissue overlapping distribution of most of the esterases (*Peters* 1982, *Bender et al.* 1984). On the other hand the findings agree with first results of the Chinese hamster (*Kluge* 1992) indicating perhaps another specificity of the hamster family.

Though first of all non-genetic reasons seem likely to be responsible for the polymorphic status of the enzymes the possibility of genetic influences should not be generally excluded. This assumption is supported by the first results of family studies and crossbreds which agree with a Mendelian inheritance of the variants at least for EP and LAP comparable to the strain dependent polymorphic proteins.

Supposing genetic effects on the enzyme variation the question arises how to maintain the variability within strains which are inbred for more than 25 generations. One essential prerequisite would be a substantial heterogeneity of the original founder animals resp. of the feral hamster population. To preserve different alleles at a given locus during a long period of inbreeding a selective advantage or disadvantage of single variants can be imagined comparable to other species (*Johnson* 1972, *Ayala et al.* 1974). Concerning the golden hamster, an advantage of heterozygotes could serve as a surviving strategy of the ferals resulting from the very limited size of the whole population. According to *Billingham & Silvers* (1963) the geographical distribution of this species is restricted to the narrow vicinity of Aleppo in north-west Syria. Thus it can be assumed to exist only in some thousand individuals especially considering its behaviour as an outsider. An increased mutation rate could be another explanation for a persisting genetic heterogeneity but at present there is no hint of differences between the golden hamster and other species concerning this character. However, the animal of choice in mutation studies is the Chinese hamster which is known to have a quite normal mutation rate (*Buselmaier* 1990).

Summarizing the results the establishment of a satisfactory genetic monitoring system to control the strains for genetic identity and authenticity based on heritable biochemical polymorphisms seems to be difficult at present because of the small number of protein variants having a distinct strain association and a simple mode of inheritance.

Thus, the risk of an unrecognized genetic contamination of a hamster strain is much greater than in mice and rats. Probably the application of molecular methods as e.g. satellite DNA tests will provide a suitable method for genetic monitoring of hamsters as it could be shown for rabbits (van Lith & van Zutphen 1994).

Summary

At present only a few data is available about biochemical polymorphisms in the golden hamster. But the great number of strains of this species used in biomedical research requires the establishment of specific marker traits which can be used for genetic control purposes.

This study gives an overview on the degree of variability on the protein level among a series of 17 strains of hamsters.

The results show 9 of about 40 stained isoproteins to express electrophoretic variants (22.5 %).

Unexpectedly most of the phenotypes occur not only between but also within the strains despite more than 25 generations of inbreeding.

The reason for this level of heterogeneity is not yet really obvious. Several possible points of influence are discussed, e.g. an increased mutation rate or physiological specificities. At present, non-genetic reasons seem to be the most likely explanation for most of the polymorphisms.

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