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growing culture, chloroplasts are diluted out. All bleaching antibiotics act against plastids as ordinary germicides. The greater the concentration of the antibiotic, the faster and more effective is the lethality to plastids.

In addition to the substances listed in the Table, several other known inhibitors of DNA synthesis or mutagens have been shown by others to be highly effective bleaching agents. These include nitrosoguanidine[1], the nitrofurans[5,6], the mitomycins[2], UV-light[7] and some others. Many substances known to inhibit protein synthesis are not effective bleaching agents although they interfer with chloroplasts development. These include 5-fluorouracil, hadacidin, chloramphenicol, puromycin, the tetracyclin antibiotics etc. Among these inhibitors, chloramphenicol action on *Euglena* chloroplasts is the best known[8]. Perhaps streptomycin, a very potent bleaching antibiotic, which is believed to be an inhibitor of protein synthesis in bacteria[9] may act in chloroplasts by a different way.

The results obtained in this study suggest that only inhibitors of DNA synthesis are bleaching agents in *Euglena*. Since some known mutagens are also effective against chloroplasts, these substances may cause lethal mutation in plastids in the classical sense. It may be necessary to classify inhibitors of DNA synthesis, therefore, as agents which cause a 'killing' of chloroplasts. The pathological plastids produced by an antibiotic are gradually diluted out within the multiplying cells.

These results show that *Euglena gracilis* can be used as a model organism for selection of DNA inhibitors or possibly even for the study of anticancer drugs inhibiting DNA synthesis. On the other hand, the susceptibility of plastids to antibacterial antibiotics offers a new tool for helping to answer the question we have presently asked: 'Were chloroplasts microorganisms?'.

*Zusammenfassung.* Ermittlung einer allgemeinen Regel über die Relation der DNA-Synthesehemmer und die Elimination der Chloroplasten bei *Euglena gracilis*.

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*Department of Microbiology, Komensky University, Bratislava (Czechoslovakia), 12 October 1970.*

## Serum Albumin and Transferrin Variants in Italian Water Buffalo (*Bos bubalis* L.)

This report presents the results of a study into the identification and analysis of serum albumin and transferrin variants inheritance observed in Italian water buffalo. Electrophoresis was carried out at room temperature for approximately 2 h with a discontinuous buffer system (gel buffer: 0.19 *M tris* + 0.16 *M* cacodylic acid, pH 7.6–7.7, diluted, prior to preparation of the gel, 1:16 for albumins and 1:8 for transferrins; buffer for electrodes compartments: 0.3 *M* boric acid titrated to pH 8.7 with 0.1 *M* sodium hydroxide) and a voltage gradient between the electrodes of 350 V. Current drawn was approximately 2.5 mA/cm width of the gel. The starch (Connaught Lab. Toronto, batch 242/1) was used at 14% concentration. Confirmation that presumed transferrin bound iron was obtained by autoradiography. Each sample received about $^1/_5$ volume of $Fe^{59}$ (as ferric citrate; Amersham, Buckinghamshire, England) with specific activity of 3–30 mC/mg Fe.

The letters adopted (see figure illustrations) for these summary notations are the same as those used to define cattle albumin and transferrin phenotypes having approximately corresponding electrophoretic mobility. 3 albumin phenotypes (AlbA, AlbAB, AlbB) were observed in both sexes. Phenotypes AlbA and AlbB (Figure 1) were characterized by a single band with AlbA migrating faster than AlbB. The phenotype AlbAB (Figure 1) had 2 bands, the slower of which corresponded to AlbB and the faster to AlbA. Further, the bands in AlbA and AlbB sera occurred in approximately twice the concentration of those in the AlbAB sera. This pattern suggested that the 3 observed phenotypes were determined by 2 codominant alleles ($Alb^A$ and $Alb^B$) with AlbA and AlbB being the homozygous types and AlbAB the heterozygous.

Phenotypes TfD and TfE showed 4 bands each and the phenotypes TfDE 6 (Figure 2). Autoradiography with $Fe^{59}$ confirmed these bands as transferrins. Again, these patterns suggested that the polymorphism of transferrins was also regulated by 2 codominant alleles ($Tf^D$ and $Tf^E$) with the 4 band patterns referring to the homozygous types and the 6 band pattern to the heterozygous.

Autosomal inheritance is implied at both loci, because heterozygous animals were observed in both sexes. The distribution of albumin and transferrin progeny phenotypes (Tables I and II, respectively) is consistent with the above hypothesis of two codominant alleles at each locus. In fact, only progeny phenotypes expected under the hypothesis have been observed in each mating class.

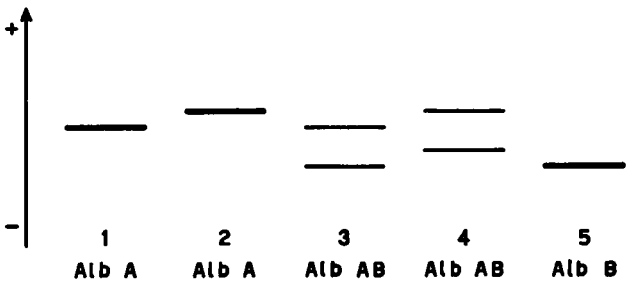


Fig. 1. Albumin phenotype comparisons between cattle (2 and 4) and water buffalo (1, 3 and 5).

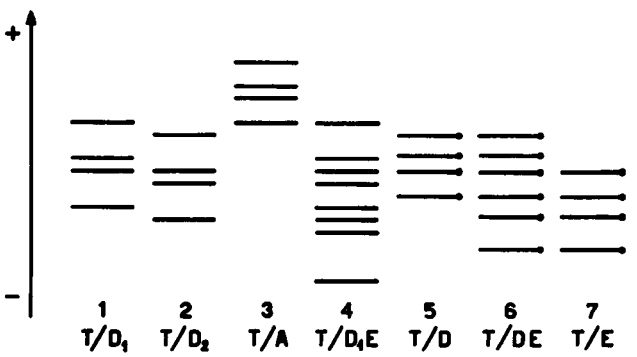


Fig. 2. Transferrin phenotype comparisons between cattle (1–4) and water buffalo (5–7). Dot marks on bands indicate those which bind iron as determined by autoradiograms.


Fig. 3. Effect of neuraminidase treatment of normal and abnormal TfD phenotypes. 1: TfD normal not treated; 2, 3 and 4: treated consequently for 24, 48 and 72 h; 5: TfD abnormal not treated; 6, 7 and 8: treated consequently for 24, 48 and 72 h; 9: TfE not treated; 10, 11 and 12: treated consequently for 24, 48 and 72 h.

Simultaneously, the same results as described above have been obtained by KHANNA and BRAEND[1] in their studies of albumin polymorphism of Indian water buffalo, and by MAKAVEEV[2], whose studies included both the albumin and transferrin polymorphism of Bulgarian water buffalo. The results of this study, together with those of the above-mentioned authors, provide substantial support for the hypothesis that European water buffalo originate from India[3]. However, great caution is necessary in suggesting relationships between breeds on the basis of few loci. Future studies on a more grounded basis concerning the various aspects and approaches to this argument are planned, particularly in the comparison of Indian and European water buffalo, utilizing the various reagents which also have been developed by the present researchers against erythrocyte and serum antigens of this species.

At both loci, heterozygotes have appeared to be equivalent to a mixture of the 2 homozygous phenotypes: artificial mixtures of equal quantities of homozygous sera have been electrophoretically indistinguishable from the true heterozygotes. No interaction product, therefore, has been notably associated with the albumin or transferrin genes. The albumin and transferrin patterns of a given animal have been found to be constant: 11 serum samples, collected from 4 animals over a period of almost 2 years were tested and found to give consistent results. No variation of albumin of transferrin patterns have been observed on storage.

Gene frequencies estimated on 350 unrelated animals occurred as follows: $Alb^A = 0.327$; $Alb^B = 0.673$; $Tf^D = 0.368$; $Tf^E = 0.632$. The observed distribution of transferrin and albumin types and those expected under genetic equilibrium proved to be in close agreement (at Alb locus $\chi^2 = 0.306$, 1 d.f., $P_{0.05} = 3.841$; at Tf locus, $\chi^2 = 0.179$; 1 d.f., $P_{0.05} = 3.841$).

In certain cattle breeds, the slow migrating $Alb^B$ allele and the blood factor Z′ both occur at particularly high frequency[4]. The same feature had not been observed in water buffalo, where Z′ had not been detected either by hemolytic or absorption techniques in any of the more than 100 individual red cell samples tested, despite the high frequency of the slow migrating albumin allele in the population.

This research also showed a few animals with an abnormal TfD phenotype. This abnormality consisted in that bands A and C (Figure 3) stained more intensely. To

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Table I. Distribution of albumin progeny phenotypes in Italian water buffalo

| Mating types | No. of matings | Offspring phenotype | | |
|---|---|---|---|---|
| | | AlbA | AlbAB | AlbB |
| AlbB × AlbA | 11 | 0 | 11 | 0 |
| AlbB × AlbAB | 15 | 0 | 7 | 8 |
| AlbB × AlbB | 43 | 0 | 0 | 43 |
| AlbA × AlbA | 1 | 1 | 0 | 0 |
| AlbA × AlbAB | 3 | 2 | 1 | 0 |
| AlbAB × AlbAB | 2 | 0 | 0 | 2 |

Table II. Distribution of transferrin progeny phenotypes in Italian water buffalo

| Mating types | No. of matings | Offspring phenotype | | |
|---|---|---|---|---|
| | | TfD | TfDE | TfE |
| TfD × TfD | 1 | 1 | 0 | 0 |
| TfD × TfDE | 11 | 7 | 4 | 0 |
| TfDE × TfDE | 38 | 13 | 17 | 8 |
| TfDE × TfE | 36 | 0 | 20 | 16 |
| TfE × TfD | 8 | 0 | 8 | 0 |
| TfE × TfE | 1 | 0 | 0 | 1 |

investigate further this abnormality, serum samples of animals with normal and abnormal TfD phenotype were treated as described by SPOONER[5], with the enzyme neuraminidase (BDH, 500 units/ml), known to remove sialic acid from macromolecules[6]. Upon digestion for 24 h, abnormal phenotypes proved electrophoretically indistinguishable from treated normal phenotypes. These results remain very much the same as those obtained by SPOONER[5] and can be explained with a similar hypothesis, namely that in abnormal TfD phenotypes, the indicated bands B and D (Figure 3) which contain less sialic acid, are slowed down, thus coinciding partially with bands A and C that consequently stained more intensely. The possibility that the abnormal phenotype might be gene-controlled is being investigated and the data so far available are encouraging. The fact that treatment prolonged for more than 24 h tended to cause progressive disappearance of the fast moving bands at the advantage of the slower migrating ones (Figure 3), is in agreement with the above hypothesis[7].

*Riassunto.* È descritto il polimorfismo delle albumine e delle transferrine nel bufalo allevato in Italia. Le tre varianti (AlbA, AlbAB, AlbB) dell'albumina sono controllate dai geni codominanti $Alb^A$ ed $Alb^B$ e le tre varianti (TfD, TfDE, TfE) delle transferrine dai due alleli dominanti $Tf^D$ e $Tf^E$.

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## Chromosome Polymorphism in the Malayan House Shrew, *Suncus murinus* (Insectivora, Soricidae)

Insectivora cytogenetics has recently been reviewed by BORGAONKAR[1] and GROPP[2]. The chromosome number, fundamental number (number of major chromosome arms) and the sex chromosomes of 7 families (including Tupaiidae) comprising 61 species were tabularized and the available information summarized by BORGAONKAR[1], while GROPP[2] discussed in greater detail 2 families, viz. Talpidae and Erinaceidae.

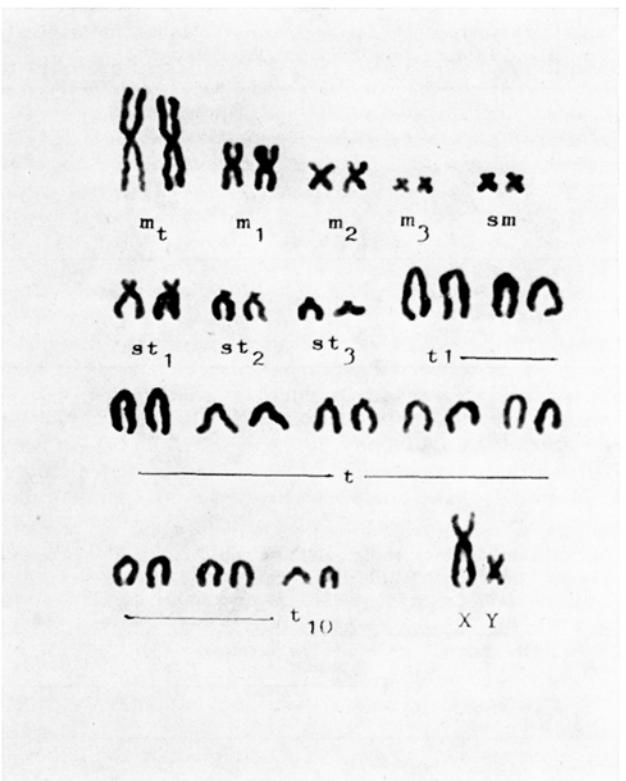


Fig. 1. Karyogram of a male *Suncus murinus* with a diploid number $2n = 38$. m, metacentric; sm, submetacentric; st, subtelocentric; t, acrocentric; $m_t$, 'translocation' metacentric.

The house shrew, *Suncus murinus* (Linnaeus), belongs to the family Soricidae (cf. ELLERMAN and MORRISON-SCOTT[3]). According to CHASEN[4] and MEDWAY[5], the Malayan form is *Suncus murinus murinus* (Linnaeus). The first report on the somatic chromosome number of *S. murinus* ( ≡ '*Crocidura murina*') seems to be that of TATEISHI (1937 and 1938, cited by BORGAONKAR[1]). The diploid number of 40 was subsequently confirmed by MANNA and TALUKDAR[6] and RAY-CHAUDHURI et al.[7] for the Indian taxon, YOSIDA et al.[8] for the Japanese taxon, and DUNCAN et al.[9] for the South Vietnamese taxon.

The present paper deals with the Malayan house shrew. Chromosome studies on 15 specimens of *Suncus murinus* collected in Kuala Lumpur and Petaling Jaya, Selangor (West Malaysia), revealed intra-population variation in diploid number. 3 karyotypic classes were recognized with $2n = 38$, 39 and 40 respectively (Figures 1, 2 and 3; Table I). Of the 15 specimens studied, 3 were found to possess a diploid number of 38, 9 with $2n = 39$, and 3 with $2n = 40$. The fundamental number, however, remained constant in all 3 karyotypes viz. N.F. = 56, and no variations or aberrations could be established within the same individual. Similarly, shrews of all 3 karyotypes

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