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Effect of PMSG on the development of the reproductive system in female mink. Huang Jianzhen, Zhen Yingkai, Bo Qingru. Code 5-3-M.


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Various feed levels for mink during reproduction. D. Mertin, K. Süvecova, E. Oravcova. Code 6-7-5-M.

Natural minerals in diets for mink. A.F. Kuznetsov, N.V. Mukhina, I.V. Barsov, A.A. Kuznetsov. Code 6-7-8-M.

Efficiency of feeding Arctic foxes of different sizes. N.A. Balakirev, E.M. Kollaeva, N.N. Loenko. Code 6-7-F.

Adsorbents in diets for mink. N.A. Balakirev, V.S. Snytko, E.A. Larina, V.M. Naumova. Code 6-7-M-F.

Levels of S1 (Silfen) and growth of young mink. A.M. Bespalov, A.L. Kiselev. Code 6-2-3-M.

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Melakril for pole cats. N.E. Chanaev. Code 7-6-O.


Biotin deficiency in foxes. G. Salyi, V. Sztojkov. Code 6-2-9-F.

High concentrations of vitamin A esters circulate primarily as retinyl stearate and are stored primarily as retinyl palmitate in ferret tissues. J.D. Ribaya-Mercado, M.C. Blanco, J.G. Fox, R.M. Russell. Code 6-3-0.


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8. Veterinary


The evaluation of Ipowet 5 and Ipowet aerosole (brompheninfos) effectiveness against external parasites (Sarcoptes scabiei v. canis and Chaetopsylla globiceps) in polar foxes. Kazimierz Sciesinski. Original Report. Code 9-12-F.

Comparative molecular analysis of plasmid-encoded tetracycline resistance genes in Staphylococcus lentus from mink and pigeons. Stefan Schwarz, Christiane Werckenthin. Code 8-9-4-14-M.


Characterization of chloramphenicol resistance genes in uropathogenic Staphylococci from mink. Stefan Schwarz, Silke Dieckmann. Code 8-9-4-14-M.


Role of alveolar type II cells and of surfactant-associated protein C mRNA levels in the pathogenesis of respiratory distress in mink kits infected with Aleutian mink disease parvovirus. Birgitte Vium, Bent Aasted, Søren Alexandersen. Code 9-M.


Sequence comparison of the non-structural genes of four different types of Aleutian mink disease parvovirus indicates an unusual degree of variability. E. Gottschalck, S. Alexandersen, T. Storgaard, M.E. Bloom, B. Aasted. Code 9-4-M.


Aleutian disease in the ferret. J.D. Stewart, N. Rozengurt. Code 9-O.


Production of mink enteritis parvovirus empty capsids by expression in a baculovirus vector system: a recombinant vaccine for mink enteritis parvovirus in mink. Jesper Christensen, Søren Alexandersen, Buchardt Bloch, Bent Aasted, Åse Utenthal. Code 9-M.


Mesocestoides canislagopodis (Rudolphi, 1810) (Krabbe, 1865) (Cestoda: Mesocestoididae) from Arctic foxes, Alopex lagopus (L.) in Iceland redescribed. B. Loos-Frank, K. Skirnisson, M. Eydal. Code 9-F.

Adrenal neoplasia in seven ferrets. L. Neuwirth, R. Isaza, J. Bellah, N. Ackerman, B. Collins. Code 9-O.


Experimental production and immunity characteristics of the mixed vaccine of botulismus (C type) and modified virus distemper vero cell of mink. K. Jin. Maopi Dongwu Siyang, Iss. 3, pp. 4-9, 1992. In CHIN. Code 9-M.


This course, the first of its kind in the Nordic countries, focuses on different research methods concerning hair and skin of fur bearing animals. The topics to be covered include paraffin technique, light microscopy, picture analysis with computer, immunological methods, cell culture, and skin and hair pathology. Demonstrations and laboratory work will be included. The guest lectures come from Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom and from Deutches Wollforschungsinstitut, Aachen, Germany. The course is aimed at postgraduate students and researchers from faculties of agriculture, natural sciences or veterinary medicine in the Nordic countries, the Balkans and Northwest Russia. The participants are expected to give a short presentation of their research work. The course language is English.

Organizers: Leena Blomstedt, Dept. of Biosciences, Div. of Animal Physiology, P.O. Box 17, SF-00014 University of Helsinki, Finland, Tel.: 1 358 0 191 7346, Fax: 1 358 0 191 7301, E-mail: Leena.Blomstedt@Helsinki.Fi. and Palle Rasmussen, Danish Institute of Animal Science, Dept. of Product Quality, Research Centre Foulum, P.O. Box 39, DK-8830 Tjele, Denmark, Tel.: +45 89 99 12 39, Fax: +45 89 99 19 19, E-mail: PVR%MH%Husdyr@shl.foulum.min.dk.

Deadline for applications: Send your informal application with a short curriculum vitae and description of your research interests to Leena Blomstedt by February 29, 1996.

Further information: Course organizers.
With optimism and still believing in the future of fur animal production, it is a pleasure to say goodbye to 1995 and welcome to 1996 which will hopefully only bring us good things.

1996 will at least be the year when The VIth International Scientific Congress in Fur Animal Production will be held in Poland, August 21-23.

This issue of SCIENTIFUR brings the First Announcement which has already been sent to all IFASA members appr. 10 weeks ago. Interested readers are asked - as soon as possible - to fill in and send the attached form (page 331) to: VIth IFASA Congress, c/o Marian Brzozowski, Polish Society of Animal Production, 9 Kaliska Street, PL-02-316 Warszawa, Poland. Tel/fax (4822) 22 17 23.

The Board of IFASA held the annual board meeting in Kazimierz, Poland, on September 21, 1995 and had a meeting with the organizing committee of the VIth IFASA Congress. The board was also attending a seminar on improvement of fur bearing animal production arranged by The Polish Society of Animal Science.

Before going into detail, the entire IFASA Board wish to thank our hosts for the invitation and the great hospitality extended to us during our entire stay. Our thanks especially goes to Prof.Dr. Grazyna Jezewska, Prof.Dr. Jozef Nurzynsky, University of Agriculture in Lublin, and to Dr. Józef Luchowiec, President of The Polish Society of Animal Production.

The conclusion of the common meeting between the arrangement committee and the IFASA Board was that the planning of the VIth IFASA Congress is progressing according to plan. It was also agreed that we must in cooperation ensure that all manuscripts sent to the Congress will be subject to proper scientific evaluation. A very crucial factor for the entire arrangement is to know the number of participants as early as possible. It is therefore our hope that the secretariat will receive the enrollment forms from as many participants as possible as soon as possible.

At the Board meeting, the accounts for 1994 and the budget for 1996 were confirmed and agreed upon. In this connection, the Board expressed their gratitude to CEFBA for the economic contribution to SCIENTIFUR. This contribution is the necessary basis for the future of IFASA and thereby also of SCIENTIFUR.

It was concluded that IFASA does not have the economic basis for a stimulation of activities in the working groups in the near future. It is, however, our hope that the working groups will establish closer contacts during the VIth Congress, and it will be appreciated if seminars etc. could be arranged in the name of IFASA, and if possible in cooperation with the Scandinavian Division for Fur Animals under The Scandinavian Association of Agricultural Scientists.

The future of SCIENTIFUR was discussed, and it was agreed that the possibility of going into electronic distribution shall be evaluated continuously.

The nomination and election of councillors and board of directors, which according to the constitution of IFASA shall take place in connection with the Congress every fourth year, was discussed in detail, and on the following pages all the information is given in the hope that responsible IFASA members will act according to the invitation given.

Back to the private backyard of SCIENTIFUR. We can now say goodbye to a very promising...
ial" year with a steady stream of abstracts and/or summaries of valuable reports published elsewhere as well as an increased number of original reports which have made SCIENTIFUR into the international leader in the publication of scientific reports on fur animal production. This is also reflected in the increase - even though it is modest - in the number of subscribers.

For 1996 we hope that this will be the year which brings SCIENTIFUR in front also regarding the number of subscribers.

In this connection it is hereby advertised that the Board of IFASA have decided that for a very low payment of copyrights we can make agreements with individual countries or language areas about the translation and distribution of SCIENTIFUR in the local language, perhaps supplemented by local information to the readers. If such a solution might have YOUR interest, please contact the editor.

Just as CEFBA is thanked for their considerable economic support, it is important for your editor to thank the individual organizations and persons who have made my job as editor free of problems and a great pleasure.

I hereby thank the Norwegian Fur Breeders Association for housing us as well as giving us access to the necessary facilities. Also the Danish Fur Breeders Association is appreciated for printing and binding SCIENTIFUR at a relatively low price, and the Danish Institute of Animal Science, Research Centre Foulum, DK, for showing their good will in making available the necessary facilities for the editor to finish each and every issue of SCIENTIFUR.

The personal staff around the editor, i.e. Jytte, Dorthe, and Hanne, as well as our language adviser Janne Hansen, have with their help in 1995 won an even greater place in my heart. Thank you again, and thank you all!

Also contributors and subscribers are asked to accept our sincere thanks for the cooperation in 1995.

WE WISH YOU ALL A MERRY CHRISTMAS AND A HAPPY AND PROSPEROUS NEW YEAR.

BEST REGARDS

[Signature]

YOUR EDITOR
Gunnar Jørgensen
NOMINATION AND ELECTION OF COUNCILLORS TO THE IFASA COUNCIL

As stated in the constitution of IFASA, councillors must be elected in each country according to the total number of IFASA members in the country in question (1-5 members = 1, 6-20 members = 2, and over 20 members = 3 councillors).

In the following list, the number of members and councillors as well as the names of councillors elected in connection with the Vth Congress in Oslo 1992 are stated:

<table>
<thead>
<tr>
<th>Country</th>
<th>Members*</th>
<th>Number of Councillors</th>
<th>Name of councillor(s)</th>
</tr>
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<tr>
<td></td>
<td>Nov. 95</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Argentina</em></td>
<td>2 (7)</td>
<td>1</td>
<td>Rafael Garcia Mata</td>
</tr>
<tr>
<td><em>Belgium</em></td>
<td>1 (0)</td>
<td>1</td>
<td>Not represented</td>
</tr>
<tr>
<td><em>Canada</em></td>
<td>4 (0)</td>
<td>1</td>
<td>Bruce D. Murphy**</td>
</tr>
<tr>
<td><em>Czech. Rep.</em></td>
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<td>1</td>
<td>Not represented</td>
</tr>
<tr>
<td><em>Denmark</em></td>
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<td>3</td>
<td>Christian F. Børsting, Vivi Pedersen, Jan Elnif</td>
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<tr>
<td><em>England</em></td>
<td>0 (1)</td>
<td>0</td>
<td>Not represented</td>
</tr>
<tr>
<td><em>Finland</em></td>
<td>9 (2)</td>
<td>2</td>
<td>Maija Valtonen</td>
</tr>
<tr>
<td><em>France</em></td>
<td>1 (1)</td>
<td>1</td>
<td>Jean Rougeot</td>
</tr>
<tr>
<td><em>Germany</em></td>
<td>1 (2)</td>
<td>1</td>
<td>Heinz Pingel</td>
</tr>
<tr>
<td><em>Greece</em></td>
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<td>1</td>
<td>Paschalis Ikonomidis</td>
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<td><em>Iceland</em></td>
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<td><em>Ireland</em></td>
<td>1 (1)</td>
<td>1</td>
<td>Alfred G. Foster</td>
</tr>
<tr>
<td><em>Italy</em></td>
<td>1 (2)</td>
<td>1</td>
<td>Not represented</td>
</tr>
<tr>
<td><em>Japan</em></td>
<td>3 (3)</td>
<td>1</td>
<td>Keiji Kondo</td>
</tr>
<tr>
<td><em>Korea</em></td>
<td>1 (7)</td>
<td>1</td>
<td>Deog-Oh Chang</td>
</tr>
<tr>
<td><em>The Netherlands</em></td>
<td>7 (1)</td>
<td>2</td>
<td>Wim Verhagen**</td>
</tr>
<tr>
<td><em>Norway</em></td>
<td>9 (3)</td>
<td>2</td>
<td>Adrian Smith, Anders Skrede</td>
</tr>
<tr>
<td><em>Poland</em></td>
<td>6 (6)</td>
<td>2</td>
<td>Stanislaw J. Jarosz**</td>
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<td><em>Romania</em></td>
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<td>1</td>
<td>Not represented</td>
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<tr>
<td><em>Russia</em></td>
<td>1 (7)</td>
<td>1</td>
<td>Alexander V. Taranin, Anatoly Koldaev</td>
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<tr>
<td><em>Slovakia</em></td>
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<td>1</td>
<td>Not represented</td>
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<tr>
<td><em>Spain</em></td>
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<td>0</td>
<td>Not represented</td>
</tr>
<tr>
<td><em>Sweden</em></td>
<td>4 (3)</td>
<td>1</td>
<td>Anne-Helene Tauson</td>
</tr>
<tr>
<td><em>USA</em></td>
<td>10 (8)</td>
<td>2</td>
<td>Le Grande C. Ellis, William L. Leoschke</td>
</tr>
</tbody>
</table>

*) Numbers in brackets are not cancelled and not paid memberships.

**) Board members. Have to be replaced. All councillors are elected for a 4 year period and can be replaced before the VIth Congress.
Names of councillors have to be approved within each country, and the secretariat of IFASA must be informed as stated in the paragraph regarding nominations for members of the IFASA Board.

If the secretariat of IFASA has not received nominations for councillors before July 1, 1996, the IFASA Board of Directors will appoint the councillor(s) in the country in question.

**NOMINATIONS FOR MEMBERS OF THE IFASA BOARD OF DIRECTORS**

According to the Constitution of IFASA, adopted at the meeting of IFASA Council on 13 August 1992, the members of the IFASA Board of Directors are elected by the councillors at the regular meeting held in conjunction with the IFASA Congress (20 August 1996 in Warszawa, Poland). An alternate is elected for each member at the same meeting. The members and alternates are elected for a period of four years.

The procedure for nominations is directed by the constitution. Nominations may be made by any individual member and must reach the secretary not later than 30 days prior to the election. Members wishing to nominate others for the post of President, Vice President or one of the three members of the board, should submit the names of individuals to the President, Prof. Einar J. Einarsson, Drøbakveien 23, N-1430 Ås, Norway, or fax +47 64 94 11 35, prior to 20 July 1996. A statement from nominees indicating their willingness to serve on the Board should be included in the written nomination.

The present board members are:

- **President**  
  Prof. Einar J. Einarsson, Norway
- **Vice president**  
  Editor Gunnar Jørgensen, Norway
- **Board member**  
  Prof. Bruce Murphy, Canada
- **Board member**  
  Prof. Stanislaw Jarosz, Poland
- **Board member**  
  Director Wim Verhagen, The Netherlands

Gunnar Jørgensen and Stanislaw Jarosz have asked not to be renominated.

**Alternate**

- Prof. Anders Skrede, Norway
- Prof. Maija Valtonen, Finland
- Prof. William Wehrenburg, USA
- Dr. Alexander V. Taranin, Russia
- Vacant
Morphology of mottling hairs in domesticated silver foxes

(*Vulpes vulpes*). Relation between the expression of the

*star* and the *mottling* mutations

*L.A. Prasolova, L.N. Trut*, E.B. Vsevolodov**

*Institute of Cytology and Genetics, Siberian Department
Academy of Sciences of Russia

**Institute of Experimental Biology, Kazakhstan*

Summary

The results of analysis of coat colour mutations arising in silver foxes during domestication are reported. Based on quantitative and qualitative analysis of the hairs showing the coat colour mutation *mottling*, it was concluded that a decrease in the eumelanin content in the cortex and medulla and irregular distribution of pigment granules along the hair shaft are the causes of hair mottling. From the results it is also concluded that there is a relation between the areas of the *star* and intensity of *mottling*.

Introduction

Clarification of the emergence of new forms of animals is a fundamental problem of the long-term experiment with domestication of the silver fox (*Vulpes vulpes*). Mutations affecting coat colour are good models for following the course from the gene to the outwardly manifest trait.

In the population of foxes domesticated at the Institute, genetically determined changes in coat colour in form of the *star* (unpigmented marks on the head) and the *mottling* (blotch from yellowish tinge to bright yellow on specific body areas) appeared (*Belyaev et al., 1979; Belyaev, Trut, 1986*). As known, there are many causes giving rise to changes in coat colour, starting from arisal of melanoblasts in the neural crest to formation of pigment granules in differentiated melanocytes and their distribution in hairs (*Searle, 1968; Prasolova et al., 1994*). The crosses we performed demonstrated that the *star* mutation is controlled by the semidominant autosomal S gene and that the embryonic mechanism determining the appearance of the *star* phenotype is a 1-2 day delay of the migration rate of the melanoblasts from the neural crest into the potentially depigmented areas of the body (*Prasolova, Trut, 1993*).

This study is concerned with a colour specific to many domestic animals characterized by yellowish *mottling*, blotches in strictly defined areas contrasting against a dark background. We have previously shown that the *mottling* is under the control of a single recessive gene, which is closely linked to the semidominant S gene determining the *star,*
and also that the functional activation of the e<sup>r</sup> gene contributes to the homozygous state of the S gene (Belyaev, Trut, 1986). Our present aim was to study the functional relationships between the phenotypic expression of the star and mottling, as well as to analyse the morphological structure of hair pigmentation under the effect of the e<sup>r</sup> mutation in comparison with that of standard colour in domesticated silver foxes.

Materials and methods

The study was carried out at the Experimental Farm of this Institute. Phenotypic expression of the mutations were estimated in silver foxes of the domesticated population showing both the star and the mottling mutations. Scores of the star were based on their areas and those of the mottling on the intensity of yellowish tinges of the coat against the background of lighter colour behind the ears, on the shoulder blades, sides and thighs (table 1). The value for interaction between the phenotypic expression of the star and the mottling was obtained by calculating the polychoric relation index; the significance of differences was tested by the chi-square test. Qualitative and quantitative analyses of pigment of hairs from mottling areas were performed using ESR (Electron Spin Resonance) methods (Vsevolodov et al., 1991). The ESR method is based on the direct proportionality between signal strength, I<sub>0</sub>, (recorded on paper tape of a spectrometer containing specially treated hair samples), and the number of free radicals contained in pigment. Using the black hairs from Karakul lambs as reference standard of the ESR signal samples, the I<sub>0</sub> values were calculated for samples of fox hairs. Melanin content was accepted as 100% in the control group of foxes of standard, silver black colour.

The relative content of melanin was calculated in groups with the yellow mottling of different intensities. Histological and cytological analyses of the mottling were performed according to routine methods. Skin samples were fixed in formalin and sections were stained with hematoxylin-eosin. Distribution of pigment granules along the entire hair was analyzed after its treatment with 0.3% trypsin solution for 20-30 min at room temperature, when air in the medulla was replaced by liquid and, as a result, pigment granules became well seen. The hairs were then washed, treated with alcohol, xylol and embedded in balsam.

Fig. 1a. The frequently observed variations in the size of the star mutation in tame foxes.
Fig. 1b. This figure allows one to compare the location and phenotypic expression of the *mottling* mutation in a tame fox pup and a dog.

Fig. 1c. Of particular interest are tame foxes, bearers of both the *star* and *mottling* mutations.
Table 1: Scores for the *star* and *mottling* mutations in tame foxes

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Score</th>
<th>Phenotypic expression of <em>mottling</em></th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single hairs</td>
<td>1</td>
<td><em>Mottling</em> areas of slightly yellowish tinge</td>
<td>1</td>
</tr>
<tr>
<td>0.25 - 0.45</td>
<td>2</td>
<td>Light yellow</td>
<td>2</td>
</tr>
<tr>
<td>0.50 - 1.50</td>
<td>3</td>
<td>Bright yellow</td>
<td>3</td>
</tr>
<tr>
<td>2.00 - 3.00</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 3.00</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean of the score values for the areas of the *star* in foxes with different degrees of phenotypic expression of the *mottling* mutation

<table>
<thead>
<tr>
<th>Phenotypic expression of <em>mottling</em> score</th>
<th>Score for area of the <em>star</em></th>
<th>Number of foxes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bright yellow <em>mottling</em></td>
<td>4.32 ± 0.25</td>
<td>31</td>
</tr>
<tr>
<td>2 Light yellow <em>mottling</em></td>
<td>4.02 ± 0.16</td>
<td>82</td>
</tr>
<tr>
<td>3 <em>Mottling</em> areas of slightly yellowish tinge</td>
<td>2.28 ± 0.18</td>
<td>79</td>
</tr>
</tbody>
</table>

Note: Significance of differences: P₁,₂ ns; P₁,₃>0.999; P₂,₃>0.999.

To analyse the distribution of medullar and cortical pigments granules, the hairs were embedded in paraffin, and transverse or longitudinal sections were made. Granules were isolated by hydrolysis in hydrochloric acid. Granules were photographed under the light microscope. They were measured on photographs, as shown in fig. 3.

**Results**

In the domesticated fox population, the occurrence of offspring showing different degrees of phenotypic expression of both the *star* and the *mottling* mutations was unexpectedly high (fig. 1a,b,c) (Belyaev, Trut, 1986). Our observations demonstrated that the phenotypic expression of the two mutations are linked in tame silver foxes showing both of these coat colour mutations. From the data of table 2 it follows that the areas of the *star* in the group of foxes with bright *mottling* is almost twice as large as those in the group with *mottling* areas of slightly yellowish tinge. The calculated K₂,₃ polychoric relation index between the phenotypic expression of the *star* and the *mottling* mutations in the same foxes (n=215), is +0.39 and highly significant (by the chi-square test, P>0.999). Thus, the data indicate that some genetic or epigenetic changes in the expression of the *mottling* lead to changes in the expression of the *star*.

We started our study on pigmentation by comparing the quality of melanin in hairs from areas of *mottling* and of standard hair. The entire spectrum of hair colour in animals and man is dependent on two distinct forms of melanin: black-brown eumelanin and yellow-reddish pheomelanin. Eumelanin and pheomelanin can be synthesized not only in the same animal, but also in the same hair, when differently coloured areas alternate. The chemical structure of eumelanin and pheomelanin is different because products of tyrosine oxidation are involved in the process of synthesis of eumelanin, while cystine and, consequently, sulphur, which is absent in eumelanin, is involved in the synthetic process of pheomelanin (Schakelford, 1948; Little, 1958; Searle, 1968; Silvers, 1979).
It has been suggested that synthesis of eumelanin is substituted by that of pheomelanin in areas of mottling. The suggestion appeared plausible, taking into account that paler colour was associated with yellowish tinge. Fig. 2 presents an example of qualitative and quantitative characteristics of pigmented areas with different degrees of yellowish mottling compared to standard silver black colour. It is seen that firstly, in all the groups with mottling areas, as well as in the standard group, the spectrum of the recorded ESR curve is the same as the one for eumelanin pigment, and the curve is different from the one for pheomelanin, having additional peaks, besides the major. It is also seen that the intensity of the $I_0$ signal, and, hence, melanin amount per unit of hair weight decreases from the control to the group with mottling areas. As intensity of the $I_0$ signal decreases, there is a concomitant increase in the intensity of yellow of mottling. The $I_0$ values, expressing the relative content of melanin in hairs from areas with different mottling scores, are compared for the control group (Table 3).

It is well known that hair colour depends not only on the type of melanin, but also on its amount. With the same type and amount of melanin, hair colour may vary because the granules may be of different sizes and differently distributed in it. Hairs are darker when the granules are uniformly and dispersely located than when lying as sparse clumps. It is also known that thick guard hairs are, as a rule, darker than thin underfur. Pigment granules from hairs from mottling and standard silver black areas are compared in fig. 3.
Table 3 ESR-spectrometric estimation of melanin content in the hairs of foxes with different coat colour pattern

<table>
<thead>
<tr>
<th>Colour</th>
<th>No. of samples examined</th>
<th>Intensity of ESR signal from the hairs of black Karakul lambs, %</th>
<th>Relative content of melanin compared to standard, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Underfur</td>
<td>Guard hair</td>
</tr>
<tr>
<td>1 Silver black</td>
<td>11</td>
<td>6.9 ± 0.3</td>
<td>11.0</td>
</tr>
<tr>
<td>2 Mottling areas of slightly yellowish tinge</td>
<td>10</td>
<td>4.5 ± 0.3</td>
<td>9.4</td>
</tr>
<tr>
<td>3 Light yellow mottling</td>
<td>12</td>
<td>3.4 ± 0.2</td>
<td>7.2</td>
</tr>
<tr>
<td>4 Bright yellow mottling</td>
<td>7</td>
<td>2.4 ± 0.2</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Note: Significance of differences: $P_{1,2}>0.999; P_{2,3}>0.99; P_{2,4}>0.999; P_{3,4}>0.99$.

Fig. 3. Melanin granules isolated from the hairs of foxes: a - bright yellow *mottling*; b - silver black. Approx. X 7000.

Pigment granules in *mottling* and silver black hairs are mainly ovoid. Almost rounded and irregular pigment granules are encountered more rarely. However, no significant differences in the content of pigment granules of different shapes were found between the groups. Pigment granules in hairs from *mottling* areas are significantly smaller than in hairs of standard silver black colour (Table 4). Pigment granules of hairs of different colour are all black.
Table 4  Sizes of pigment granules (µ) in mottling hairs and of standard colour

<table>
<thead>
<tr>
<th>Colour</th>
<th>No. of granules measured</th>
<th>Granules</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length</td>
<td>Width</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>x ± S.E.</td>
<td>cv</td>
<td>x ± S.E.</td>
<td>cv</td>
</tr>
<tr>
<td>1 Silver black</td>
<td>2029</td>
<td>0.773 ± 0.005</td>
<td>29.08</td>
<td>0.318 ± 0.002</td>
<td>28.16</td>
</tr>
<tr>
<td>2 Bright yellow</td>
<td>2056</td>
<td>0.686 ± 0.005</td>
<td>31.60</td>
<td>0.218 ± 0.002</td>
<td>36.86</td>
</tr>
<tr>
<td>3 mottling</td>
<td></td>
<td>&gt;0.999</td>
<td>&gt;0.999</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison of the pigment granule distribution demonstrated that the amount of both cortical and medullar granules is smaller in hairs from mottling than standard colour areas (fig. 4).

Fig. 4. Distribution pattern of pigmented cortical and medullar granules in the guard hairs from areas with silver black (a) and bright yellow mottling hairs (b). Magnification: X 300 (1); X 900 (2); X 500 (3).
The cortical granules are longitudinally oriented in hairs of standard colour. The closely lying side by side pigment granules have the appearance of chains. The medullar granules lie singly or as clumps on different planes. Clumps of pigment of different sizes occur more frequently in the medulla than cortex. The distribution of cortical and medullar granules in hairs from mottling and silver black areas is the same. Their amount is smaller, however, in the former. Not only is the total amount of pigment smaller in mottling compared to silver black hairs. Pigment granules are non-uniformly distributed along the length of mottling hairs (especially in underfur). In silver black foxes, the most intensely pigmented part is the top: it is devoid of medulla and its pigment granules lie closely adjacent. The middle part of the shaft is pigmented quite uniformly along its entire length both in the cortex and medulla down to the lower part of the shaft, where the amount of cortical and medullar pigment granules decreases in the mature hair. In the top of hairs showing mottling, scarce chains of pigment granules are scattered against a yellow background of unknown nature, and in the middle and lower parts of the hair pigmented areas alternate with unpigmented both in the cortex and medulla. This is in contrast to that which is observed in hairs of standard colour (fig. 5).

Discussion

The data we obtained on the relation between the areas of the star and intensity of mottling extend our results concerning the function of their relationship in tame foxes (Belyaev, Trut, 1986), suggesting that either the e' gene controlling mottling has a regulatory effect on the expression of the S gene, or that there may exist a common mechanism regulating the phenotypic expression of the two linked mutations.

Phenogenetic analysis of pigmentation of mottling demonstrated that its formation is not related to changes in melanin type: eumelanin is also synthesized in the mottling areas. There are no differences in the colour and shape of the pigment granules between mottling and standard coloured hairs. Pigment granules in mottling and standard coloured hairs have the species-specific ovoid, ellipsoid, or rounded shape suggesting that the morphology of melanocytes (presence or absence of processes) in mottling hairs is not substantially modified.

The dense clumps of pigment occurring in hairs of both colours are either dead melanocytes or those released from the hairs; there are, however, no significant differences in their amounts between mottling and silver black hairs. It should be noted that the significant differences in length and width of pigment granules, the wider variations in these characteristics in mottling compared to standard hairs, may evidence for impaired structure and function of melanosomes (Markert, 1956; Zvereva et al., 1976; Silvers, 1979; Konyukhov, 1990). As our studies demonstrated, formation of mottling is related to a decrease in the amount of produced cortical and the medullar pigment granules and its non-uniform distribution along the hair shaft, as well as in the cortex and medulla. What may be the nature of impaired pigmentogenesis in the mottling areas? It is difficult to give a straightforward answer.

Fig. 5. Distribution of pigment along the underfur from areas with silver black pigmentation (a) and bright yellow mottling (b). Magnification: X 500 (1); X 300 (2).
Because pigment granules are non-uniformly distributed along mottling hairs, it may be assumed that the synthesis of pigment providing the release of pigment granules during hair growth may be disturbed (Morris, 1967; Zvereva et al., 1976). The considerable decrease in the amount of pigment in mottling hairs is due to the cessation of migration of melanoblasts (Chen, Chavin, 1968; Bronner-Fraser, 1980; Prasolova, Trut, 1993), or to their impaired proliferation in the hair bulb because, as known, the degree to which melanin function is disturbed may be related to organismic factors and the position of melanocytes among the cells of the hair bulb (Chase, Mann, 1960; Prasolova et al., 1994). Identification of the genetic primary causes of coat colour mutations in silver foxes selected for tame behaviour is a major problem of studies concerned with the emergence of new forms of animals during the course of domestication.

Based on the obtained experimental data, it may be concluded that there is a relation between the phenotypic expression of the areas marked by the star and intensity of mottling. This conclusion supports and extends our previous results concerning functional relations between these coat colour mutations in tame foxes. Morphological analysis of pigmentation of mottling in tame foxes demonstrated that the formation of these mutations is due both to a decrease in eumelanin content in the cortex and medulla and irregular distribution of pigment along the hair shaft. These two mutations may also result from either structural disruption of the melanosomes, or the synthesis of pigment, or to the cessation of migration of melanoblasts from the neural crest.

Acknowledgements

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References


Energy economy and activity in farmed pine martens (*Martes martes*)

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Abstract

Energy economy of the pine marten (*Martes martes*) was studied by measuring the yearly variations in body weight, feed intake and activity. Feed intake was highest in the autumn, after which it decreased towards winter. Feed intake rose again in the spring but decreased at the onset of the breeding season in July. The body weights of both males and females varied seasonally (p<0.05), being highest in late spring and summer and lowest in mid-winter. Feed consumption (y) in males depended significantly (p<0.01) on the ambient air temperature (x) as follows: y=333 + 1.9037x. In females, no statistically significant dependence was found. Locomotor activity (y) depended significantly (p<0.05) on the ambient air temperature (x): y=491 + 6.755x. The animals were most active during the breeding season in June-August, being in locomotion 42% (605 ± 175 min) of the circadian 24-h. The least amount of movement was in March (25%; 360 ± 185 min). Locomotor activity was significantly (p<0.05) higher in large cages than in small ones. The circadian activity rhythm varied seasonally (p<0.05), but was not necessarily dependent on the daily light rhythm. Stomach volume was slightly larger than that of the other similar-sized mustelids. Sexual dimorphism was observed in some organ weights.

Introduction

For mustelids the requirements for heat production and loss differ considerably during winter and summer in subarctic conditions (Brown & Lasiewski, 1972; Korhonen et al., 1983; Harri & Korhonen, 1985). In addition to temperature variation, these animals must also adapt to wide fluctuations in light and nutritional rhythms which thus set special demands on their survival strategies. The pine marten (*Martes martes*) is a small-sized mustelid widely inhabiting the Northern Hemisphere. Because of its elongated slender body shape, its basal metabolic rate is rather high (Iversen, 1972; Schmidt-Nielsen, 1984). This species also has a pronounced sexual dimorphism. Thus males are larger and heavier than females (Moors, 1980). As concerns food habits, the pine marten is considered a generalist, varying its diets with seasonal abundance fluctuations (*Pulli-
Measurements
The animals’ health was continuously followed for 23 and 18 months, respectively. A known quantity of feed was given daily in excess and the uneaten feed was collected the next day in order to calculate the actual intake. The animals’ health was checked visually about once a week.

Seasonal changes in the behavioural activity of the males were recorded with video camera equipment (CCD video camera 7240, Bische UB-480 time-lapse tape recorder, Koyo monitor, Bische 12-300 infra-red light; 500 W). The locomotor activity of 8-10 males was recorded 6-7 days each month on which basis the average monthly locomotor activity was calculated. In addition, occurrences of the most common behavioural patterns were monitored during February 5-7 in 14 males. Observations were made visually through the window of an observation cabin situated 6 m from the animal cages.

The aim of the present study was to clarify seasonal changes in the energy economy of captive pine martens. Although energy economy in captivity does not necessarily correspond to that in the wild, captive conditions do provide comparatively better possibilities for carrying out detailed, continuous measurements and observations. Presumably, the basic physiological regulatory mechanisms are also operative in captive conditions, as indicated by earlier observations made on the polecat (Harri & Korhonen, 1985).

Materials and methods

Animals and general procedures
The experiments were carried out at the Fur Farming Research Station of Kannus in western Finland during 1990-93. Altogether 19 male and 16 female pine martens were used in the experiments. They were all originally captured wild. Before the experiments, the animals were housed in captivity for at least 3 years. Each animal was kept individually in farm cages measuring 35 cm wide x 70 cm long x 40 cm high under shed conditions. The animals were fed once a day at about 1 pm. The diet was composed of fresh mink feed (slaughter-house offal, fish, cereals) prepared by a central feed kitchen. Water was freely available from an automatic water dispenser.

Measurements
Body weights of males (N=10) and females (N=13) were continuously followed for 23 and 18 months, respectively. A known quantity of feed was given daily in excess and the uneaten feed was collected the next day in order to calculate the actual intake. The animals’ health was checked visually about once a week.

Altogether 33 carcasses (18 females, 15 males) were delivered by hunters for obductions during December 31 1990 - March 30 1991. Carcass and organ weights as well as stomach volume and the length of gastrointestinal tract were measured.

Statistical analyses were carried out according to the GLM-procedure in SAS using analyses of variance (ANOVA), Tukey’s Studentized Range (HSD) test and regression analysis. The results are expressed as mean ± SD.

Results
Changes in feed consumption
Seasonal changes occurred in the feed consumption of both sexes (p<0.05). Feed intake was highest during the autumn, after which it decreased towards winter. In spring, the intake was high again but decreased at the onset of the breeding season in July (fig. 1). In 1991 and 1992 the average consumption of the males was 325 ± 58 and 358 ± 37 g/animal/day, respectively. The consumptions for 1991 and 1992 expressed in energy values were 2077 ± 342 and 2318 ± 253 kJ/animal/day, respectively. The feed intakes of females in 1991 and 1992 were 277 ± 53 and 283 ± 38 g/animal/day, respectively. The corresponding energy values were 1735 ± 339 and 1824 ± 253 kJ/animal/day, respectively. Feed consumption (y) in males depended significantly (p<0.01) on the ambient air temperature (x) accord-
ing to the following equation: \( y = 333 + 1.9037x \). No statistically significant dependence was found in females.

**Fig. 1.** Seasonal changes in feed consumption (g/animal/day) of male (N=10) and female (N=13) pine martens during 1991-1992.

**Body weight changes**
Both sexes showed seasonal variation in body weight (p<0.05). Maximum values were typically found in the summer or early autumn and the minimum values during winter (figs. 2 and 3). In 1991 and 1992 the mean body weights for males were 1510 ± 180 and 1480 ± 190 g, respectively. The corresponding values for females were 1130 ± 170 and 1150 ± 220 g, respectively. Thus, the males were about 300-400 g heavier than the females.

**Fig. 2.** Seasonal changes in body weights (g) of males (N=10) pine martens. The data are expressed as mean ± SD.

**Circadian and seasonal variations in activity**
Fig. 4 gives the distribution of circadian activity for each hour as calculated from the total available data. It can be seen that the animals were most active during the morning hours. Peak activity was found between 10 and 11 am (33.9 ± 24.8 min/h). After feeding, at about 1 pm, the animals became rather inactive, resting mainly inside the nest boxes. Minimum activity was found between 4 and 5 pm (13.2 ± 21.5 min/h). In the evening, the animals resumed activity (at about 9 pm) being in locomotion until about 4 am. During the workday (8 am - 4 pm), the activity (23.8 ± 24.0 min/h) tended to be somewhat higher than outside the workday (19.6 ± 23.8 min/h).

**Fig. 3.** Seasonal changes in body weights (g) of female (N=13) pine martens.

**Fig. 4.** Circadian distribution of locomotor activity (min/h) in male pine martens (N=10).
As concerns seasonal variations (fig. 5), the animals were most active during the breeding season from June to August, when they were in locomotion 42% (605 ± 175 min) of the circadian 24-h. The least amount of movement (25% i.e. 360 ± 185 min/24 h) was in March. The mean locomotor activity of the year was 486 ± 180 min/24 h. Locomotor activity (y) depended significantly (p<0.05) on the ambient air temperature (x) as follows: y=491 + 6.755x. In addition, feed consumption (y) was in relation to locomotor activity (x) according to the following formula: y=288 + 0.1113x (p<0.05).

The activity results from visual observations of 14 males during February were rather similar (active 29% of the day) to the video recordings of 8 males (active 32% of the day) during the same time period. According to the visual observations, 60.2% of the day was spent inside the nest. The corresponding figures for sitting, standing and sleeping outside the nest were 2.9, 2.7, and 2.3 %, respectively.

The remaining 2.9% included other behaviours such as drinking, eating, self-grooming, defecating, urinating and stretching. The animals were observed to urinate and defecate 3 and 4 times per 24 h, respectively. The number of feeding episodes occurring with the same time interval was 10 on an average.

**Behaviour in large cages with alternative nests**

Table 1 summarizes the data on the alternative nest experiments. The use of the various nests varied among the animals. The most favored nests were wooden nest number 6 and styrofoam nest number 7. Styrofoam nest number 8 was not used at all. The animals did not prefer the top nest (number 1) instead of others. From 2 to 6 nest of 8 were used for defecation or as food storage, depending on the individual. However, no marked relation was found between nest dirtiness and amount of nest use.

Usage (% of total time studied) of the alternative nests by pine martens housed in a large enclosure (80 cm wide x 235 cm long x 60 cm high). 1=wooden nest at the top of the cage, 2-6=wooden nests in a row along the long side of the cage, 7-8=round styrofoam nests (inner diameter 20 cm, height 32 cm) along the shorter side of the cage. Styrofoam and top nests were on opposite sides of the cage. The wooden nests were 26 wide x 3cm 2 cm long x 36 cm high.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Time interval</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 1</td>
<td>27.5.-1.6.</td>
<td>2.0</td>
<td>9.2</td>
<td>6.1</td>
<td>9.1</td>
<td>9.3</td>
<td>4.5</td>
<td>13.5</td>
<td>0</td>
</tr>
<tr>
<td>Female 1</td>
<td>3.6.-9.6.</td>
<td>23.9</td>
<td>13.9</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>14.1</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Male 2</td>
<td>4.9.-9.9.</td>
<td>25.7</td>
<td>0.2</td>
<td>6.3</td>
<td>0.3</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Female 2</td>
<td>9.9.-15.9.</td>
<td>0.1</td>
<td>12.3</td>
<td>2.8</td>
<td>0.3</td>
<td>0</td>
<td>54.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Male 3</td>
<td>19.-24.10.</td>
<td>0</td>
<td>0</td>
<td>2.3</td>
<td>0.3</td>
<td>0</td>
<td>8.0</td>
<td>36.5</td>
<td>0</td>
</tr>
<tr>
<td>Female 3</td>
<td>23.-28.10.</td>
<td>0</td>
<td>0</td>
<td>56.5</td>
<td>0.8</td>
<td>0.3</td>
<td>0</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td>Male 4</td>
<td>14.-17.11.</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>9.6</td>
<td>23.0</td>
<td>0</td>
</tr>
<tr>
<td>Male 5</td>
<td>10.-12.12.</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>15.6</td>
<td>0</td>
</tr>
<tr>
<td>Female 4</td>
<td>3.-18.12.</td>
<td>0.9</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17.8</td>
<td>18.4</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>5.9</td>
<td>4.0</td>
<td>8.2</td>
<td>1.2</td>
<td>1.1</td>
<td>12.1</td>
<td>12.3</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
The mean locomotor activity of the animals in the large cage was 720 ± 213 min/24 h (males 795 ± 72 and females 627 ± 306 min/24 h). The activity of the males in the large cages was significantly (p<0.05) higher than that of the males in small cages (572 ± 32 min/24 h) during the same time period.

**Obduction results**

The main obduction data are presented in table 2. The length of the gastrointestinal tract was four times the body length in both sexes. Stomach volume was also the same order of magnitude in both sexes. Carcass weight and length of the males was significantly greater than that of the females. Sexual dimorphism was encountered in organ weights such as the kidneys, liver, heart and thyroid glands. However, the weights of adrenals, spleen, and thymus were about the same order of magnitude in both sexes.

**Discussion**

Observations on the activity pattern of the pine marten in the wild have revealed that this species is mainly active at night or twilight. In Finnish forest Lapland, for instance, the daytime movement of pine martens is only 1.3% of the total circadian activity budget (Pulliainen, 1980b). This nocturnal activity pattern is also typically found in other wild mustelid species (Gerell, 1969; Bäumler, 1973; Skirnisson, 1986). Under farm conditions, however, it has been noticed that activity patterns can differ from those in the wild, being in a looser relation to the environmental conditions but more fixed to feeding times and other farm work activities. Examples of a similarly changed activity pattern could be found in studies made on the mink (Klochkov, 1965, 1966; Gerell, 1969), the polecat (Korhonen et al., 1985) and the North American pine marten (Hawley, 1993). The present results show that such a modified pattern emerges in captive pine martens also. The highest activity levels were found between 8-12 am. After feeding time, at about 1 pm, the animals became rather inactive for several hours but resumed activity later on. Thus, the typical circadian activity pattern consisted of a system of short bursts of activity alternating with rest periods around the clock, as has been described in farmed polecats (Korhonen et al., 1985).

During the winter period January-March, our pine martens housed in small cages were active 433 ± 183 min/24 h.

**Table 2**

Comparison of carcass and organ weights (mean ± SD) of wild male (N=15) and female (N=18) pine martens. The carcasses were delivered by hunters from the eastern and western parts of Finland. (NS=not significant).

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass weight, g</td>
<td>975 ± 131</td>
<td>722 ± 55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carcass length, cm</td>
<td>46.7 ± 0.8</td>
<td>42.2 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gastrointestinal tract, cm</td>
<td>201 ± 15</td>
<td>170 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stomach volume, ml</td>
<td>89 ± 49</td>
<td>90 ± 27</td>
<td>NS</td>
</tr>
<tr>
<td>Adrenals, g</td>
<td>0.081 ± 0.022</td>
<td>0.068 ± 0.017</td>
<td>NS</td>
</tr>
<tr>
<td>Kidneys, g</td>
<td>7.5 ± 1.1</td>
<td>5.8 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spleen, g</td>
<td>2.5 ± 0.5</td>
<td>2.1 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Liver, g</td>
<td>33.7 ± 7.6</td>
<td>23.5 ± 6.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart, g</td>
<td>10.0 ± 1.0</td>
<td>7.7 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thymus, g</td>
<td>0.52 ± 0.43</td>
<td>0.42 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>Thyroid glands, g</td>
<td>0.079 ± 0.026</td>
<td>0.057 ± 0.013</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Testicles, g</td>
<td>0.41 ± 0.20</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Baculum, g</td>
<td>0.78 ± 0.36</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Uterus, g</td>
<td></td>
<td>0.69 ± 0.45</td>
<td></td>
</tr>
</tbody>
</table>
This is a much higher level than that described for the wild pine marten in Finland; Pulliainen (1983) noted that the pine marten travels 4.5 km daily in the winter. The walking speed of an animal of that size enables it to cover this distance in about one hour (Harri & Korhonen, 1985). The mean activity of captive beech martens during the winter is according to Hansen (1993) about 120 min/24 h. Correspondingly, captive polecats are active for about two hours during January-March (Korhonen et al., 1985). Furthermore, the activity level of farmed mink during the winter has been found to be 198 ± 67 min/24 h (Korhonen & Niemelä, 1993). Thus, it seems obvious that the activity level of our pine martens is rather high in comparison with the abovementioned examples. To what extent this reflects the welfare or physiological state of the animals remains unclear. It should be noted that the activity level of our martens housed in large cages was still markedly higher than that of the animals kept in the small cages. In Canada, Hawley (1993) mentioned that the transfer of martens from small to large cages produced behavioural changes. It was estimated that the animals became progressively more active with increased diurnal pattern. Hawley (1993) considered these changes to be positive with respect to well-being and productivity.

The lower critical temperature of the polecat or mink is close to +20-24°C (Korhonen et al., 1983). It can be estimated from the body size and shape that about the same holds true for the pine marten (c.f. Iversen, 1972; Brown & Lasiewski, 1972). Below that temperature the heat production of the pine marten increases linearly as the temperature decreases. It can be estimated that at +20°C, a typical temperature during the Finnish winter, pine martens must triple their basal heat production in order to maintain homeothermy. However, the measured energy intake of our pine martens was not dramatically different in the winter than in the summer. Taking into account the energy cost of the body weight change, winter cold does not seem to produce any detectable energy costs for pine martens provided a warm nest with dry bedding is available.

The stomach volume of the mink is 30-70 ml, depending on the sex, and can optimally hold about 75 g fresh feed at a time (c.f. Mink Production, 1985). Our results show that the pine marten has a greater stomach volume, i.e. about 90 ml for both sexes. Thus, the pine marten obviously can eat more at one time than the mink. The mean daily feed intakes of our male and female pine martens were 340 and 280 g/animal, respectively, divided into about 10 different eating episodes per 24 h. It has been observed that in the winter the mink eats 13 times on average in a 24-hour period, consuming 360 g of feed daily (Korhonen & Niemelä, 1993). The length of the gastrointestinal tract of the pine marten was found to be four times the body length. The other small mustelids such as the mink, ferret and polecat typically also have such a relatively short gastrointestinal tract (Sibbald et al., 1962). This means that the rate of food passage, during an average 3 hours, is considerably faster than that of most other monogastric species (Clemens & Stevens, 1980). A short digestive tract also means a high number of daily defecations.

The polecat, for instance, has been observed to have to leave the shelter of the nest for defecation and urination 4-7 times daily, even in the coldest winter weather (Korhonen et al., 1985). The same held true for our martens. It can therefore be concluded that the characteristics of the digestive tract significantly regulate the behaviour and energetics of mustelids, including the pine marten.

The observed seasonal changes in the body weights of the presently studied pine martens reflect the changes in seasonal energy demands and behavioural activity, and indicate that the body weight has various set point values during different seasons. Furthermore, the pattern of seasonal weight development is somewhat different than that in the mink or the polecat (Charlet-Lery et al., 1981; Korhonen et al., 1985). The body weights of the latter species are typically the highest in November-December, after which there is a marked decline in weight towards the approach of the breeding season in March-April. Their weights are lowest in mid-summer, after which they again start to increase. because the breeding season of the pine marten is from June to August. This sets the timing of the weight decrease and increase to a somewhat different rhythm as revealed by our results.
Acknowledgements

The authors would like to thank the staff of the Fur Farming Research Station for valuable assistance in carrying out these experiments.

References


Concentration of some trace elements in the fur of Greenland nutria during ontogenesis

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Summary
The content of some trace elements (Se, Co, V, Cr, As, Ti, Zr) in the fur of Greenland nutria during ontogenesis was studied by dispersive-roentgenfluorescent spectrometry. Fur samples were cut from two topological parts of the body (middle of back and middle of abdomen) of animals at the age of 60, 135, and 240 days. The obtained results were processed mathematically and statistically (M ± SD). Significant differences between sexes were observed in the concentration of Ti, Zr, As, Co and Cr.

Introduction
Several authors have dealt with the study of mineral composition in the organism of fur animals (Tjurnina, 1981; Saba et al., 1982; Berestov et al., 1984; Bialkowski and Saba, 1985; Mertin et al., 1990, 1991, 1992, 1993a,b, 1994a; Lohi and Jensen, 1991). The authors studied the concentration of mineral substances in the organism of fur animals in the above mentioned works. Buleca and Sviatko (1991a,b) dealt with the content of some macro- and microelements in the fur of nutria.

Mertin et al. (1994b,c,d) studied the concentration of mineral substances in the fur of standard nutria during the ontogenesis. The authors observed an uneven distribution of the studied substances depending on sex, age category and part of the body.

Material and methods
The experiment was performed in the Experimental Farm of Fur Animals of the Research Institute of Animal Production in Nitra. The animals were kept in one-floor cages with pools in a hall. They were fed pelleted feed mixture KK (producer AC Cataj) and they were given alfalfa and fodder beet as supplementary feed. The animals were clinically healthy and in requisite condition.

There were approximately 25 males and 25 females in the experiment depending on the age category. The experiment lasted eight months. Samples were
cut from two parts of body - middle of back and middle of abdomen - under halothane narcosis. One sample contained approximately 2 g of fur. Fur samples were collected in dependence on growth of the individual generations of fur, namely at the age of 60 days (juvenile fur), 135 days (moulting), and 240 days (fur maturity). Se, Co, V, Cr, As, Ti, Zr were determined from the fur samples by disperse-roentgenflourescent spectrometry (Tumanov and Stepanok, 1986). Concentration of these elements in the individual feeds was also studied (table 1).

Table 1  
Arithmetical means of studied mineral elements in feed components

<table>
<thead>
<tr>
<th>Element</th>
<th>Alfalfa</th>
<th>KK</th>
<th>Fodder beet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se (mg/kg)</td>
<td>0.090</td>
<td>0.033</td>
<td>0.071</td>
</tr>
<tr>
<td>Co (mg/kg)</td>
<td>0.060</td>
<td>0.070</td>
<td>0.160</td>
</tr>
<tr>
<td>Cr (mg/kg)</td>
<td>0.495</td>
<td>0.589</td>
<td>1.300</td>
</tr>
<tr>
<td>As (mg/kg)</td>
<td>0.072</td>
<td>0.056</td>
<td>0.093</td>
</tr>
<tr>
<td>Zr (mg/kg)</td>
<td>3.120</td>
<td>2.630</td>
<td>3.710</td>
</tr>
</tbody>
</table>

Table 2  
Concentration of some trace elements (mg/kg dry matter) in the fur of Greenland nutria during ontogenesis (x ± SD)

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Elements</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 B</td>
<td>Se</td>
<td>n = 16</td>
<td>n = 19</td>
<td>n = 16</td>
<td>n = 19</td>
<td>n = 16</td>
<td>n = 19</td>
<td>n = 16</td>
<td>n = 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>x = 0.201</td>
<td>0.184</td>
<td>0.231</td>
<td>0.172</td>
<td>0.139</td>
<td>0.126</td>
<td>0.355</td>
<td>0.392</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s = 0.049</td>
<td>0.070</td>
<td>0.088</td>
<td>0.117</td>
<td>0.060</td>
<td>0.038</td>
<td>0.134</td>
<td>0.129</td>
</tr>
<tr>
<td>60 A</td>
<td>Se</td>
<td>n = 15</td>
<td>n = 19</td>
<td>n = 15</td>
<td>n = 19</td>
<td>n = 15</td>
<td>n = 19</td>
<td>n = 15</td>
<td>n = 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>x = 0.198</td>
<td>0.217</td>
<td>0.245</td>
<td>0.235</td>
<td>0.145</td>
<td>0.127</td>
<td>0.335</td>
<td>0.398</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s = 0.089</td>
<td>0.079</td>
<td>0.149</td>
<td>0.153</td>
<td>0.080</td>
<td>0.064</td>
<td>0.127</td>
<td>0.121</td>
</tr>
<tr>
<td>135 B</td>
<td>Se</td>
<td>n = 21</td>
<td>n = 15</td>
<td>n = 21</td>
<td>n = 15</td>
<td>n = 21</td>
<td>n = 15</td>
<td>n = 21</td>
<td>n = 15</td>
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<tr>
<td></td>
<td></td>
<td>x = 0.228</td>
<td>0.190</td>
<td>0.212</td>
<td>0.231</td>
<td>0.155</td>
<td>0.132</td>
<td>0.385</td>
<td>0.354</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s = 0.086</td>
<td>0.047</td>
<td>0.101</td>
<td>0.102</td>
<td>0.056</td>
<td>0.054</td>
<td>0.126</td>
<td>0.016</td>
</tr>
<tr>
<td>135 A</td>
<td>Se</td>
<td>n = 21</td>
<td>n = 15</td>
<td>n = 21</td>
<td>n = 15</td>
<td>n = 21</td>
<td>n = 15</td>
<td>n = 21</td>
<td>n = 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>x = 0.208</td>
<td>0.219</td>
<td>0.167</td>
<td>0.244</td>
<td>0.142</td>
<td>0.127</td>
<td>0.123</td>
<td>0.377</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s = 0.090</td>
<td>0.106</td>
<td>0.049</td>
<td>0.123</td>
<td>0.054</td>
<td>0.034</td>
<td>0.116</td>
<td>0.121</td>
</tr>
<tr>
<td>240 B</td>
<td>Se</td>
<td>n = 8</td>
<td>n = 17</td>
<td>n = 8</td>
<td>n = 18</td>
<td>n = 8</td>
<td>n = 19</td>
<td>n = 8</td>
<td>n = 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>x = 0.235</td>
<td>0.157</td>
<td>0.209</td>
<td>0.178</td>
<td>0.143</td>
<td>0.115</td>
<td>0.467</td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s = 0.089</td>
<td>0.054</td>
<td>0.104</td>
<td>0.065</td>
<td>0.041</td>
<td>0.046</td>
<td>0.134</td>
<td>0.127</td>
</tr>
<tr>
<td>240 A</td>
<td>Se</td>
<td>n = 8</td>
<td>n = 19</td>
<td>n = 8</td>
<td>n = 18</td>
<td>n = 8</td>
<td>n = 20</td>
<td>n = 8</td>
<td>n = 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>x = 0.208</td>
<td>0.170</td>
<td>0.160</td>
<td>0.155</td>
<td>0.137</td>
<td>0.119</td>
<td>0.404</td>
<td>0.368</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s = 0.054</td>
<td>0.044</td>
<td>0.041</td>
<td>0.045</td>
<td>0.063</td>
<td>0.039</td>
<td>0.091</td>
<td>0.147</td>
</tr>
</tbody>
</table>
Results and discussion

The content of the studied trace elements in the fur of Greenland nutria is given in Table 2. The results show that the highest concentration in the fur of Greenland nutria absolutely expressed was with Ti (from 0.855 to 2.745 mg/kg d.m.), Zr (0.338-0.520) and Cr (0.323-0.467) and the lowest one with As (0.022-0.045 mg/kg d.m.).

There were significant differences only in the concentration of Ti (P<0.05) in the fur from back and Co (P<0.05) and Ti (P<0.01) in the fur from abdomen.

There were no significant differences between sexes in the concentration of the studied elements during the period of fur maturity. Significant differences were only with Ti (P<0.01), Se, Cr (P<0.05) in the dorsal region.

Fig. 1.

Fig. 2.
If we compare these results with the previous results of Mertin et al. (1994b) gained in the fur of standard nutria we can state that the differences between sexes in the concentration of the studied elements in the fur of Greenland nutria were less obvious. The highest concentration of the studied trace elements was with Ti, Cr, Zr, and the lowest one with As in the fur of both mutations. The distribution of the studied elements in the standard nutria was not as equal as in the Greenland nutria in depending on the sex and age category. The differences between sexes were significant in the content of Se, Co, Cr, and As (P<0.01) in the standard nutria at the age of 60 days. The content of the studied elements was approximately on the same level with our results.

The content of the studied elements was significant in the content of Se, As, Fe, and Co in the fur of both mutations. The distribution of the studied elements in the standard nutria was not as equal as in the Greenland nutria in depending on the sex and age category. The differences between sexes were significant in the content of Se, Co, Cr, and As (P<0.01) in the standard nutria at the age of 60 days. The content of the studied elements was approximately on the same level during the moultng season. Significant differences were observed only in the Co concentration. These findings correspond with our results. The most significant differences in the concentration of trace elements (Se, V, Zr, Co) in the fur of standard nutria were observed in the period of fur maturity compared with Greenland nutria.

References


Growth of standard and Greenland nutria from birth to the age 210 days

Vladimir Parkanyi, Dusan Mertin

The live weight growth of standard and Greenland nutria of both sexes from birth to age 210 days was observed. Standard nutria grew more intensively in comparison with Greenland nutria during the whole quiescent period. In the sexual maturation stage (150 days) the difference between both breeds was significant (standard weighed 3528 g, Greenland 3245 g), at the end of the observed period the difference was highly significant (4890 g against 3779 g).

The live weight of standard nutria females was lower by about 12% on average in comparison with males. The weight of Greenland nutria females was lower by about 10% in comparison with males.

Effect of castration on body growth of standard nutria

Vladimir Parkanyi, Dusan Mertin, Miroslav Oberfranc, Imrich Tocka

The experiments with castration of standard nutria were realized in two stages. In the first stage five castrates from age three months in the spring and summer periods were observed. In the second stage five castrates and five standard males from age three months in the summer, autumn, and winter periods were observed.

The animals were weighed and measured at weekly intervals. The results confirmed stagnation and retardation of castrate growth in comparison with control standard males of nutria from five months. This dependence is explicitly related to the period of sexual maturity of nutria of this age.

Castrates at six months achieved a live weight of 4078 g and a body length of 52.2 cm, while non-castrated standard males with their live weight of 5074 g and body length of 54.8 cm were suitable for skinning.

Effect of the method of litter weaning on the size and body weight of polar fox

Andrzej Filistowicz, Piotr Przysiecki

Polar fox litters born at approximately the same times with a similar average weight of kits were - after weaning and differentiated quantity (seven classes of litter numbers) - allotted into four equally large groups (52 kits from whole litters) with an equal number of males (23) and females (29) in each group. The authors used various methods of separating six-week old kits from their mothers.

The effect of method of weaning, number of litter, sex and weaning time on body weight on the 30th day after weaning, at the age of 16 weeks and the size and body weight at grading were studied. Foxes disengaged during weaning and deprived of any contact with their mothers were the most susceptible to stress, while the effect was the least in kits left - after being separated from mothers - in the cage of the dam and maintaining a complete visual, auditory and olfactory contact with their mothers in an adjoining cage for one month.

Prediction of dry hide length based on body weight and length of the wet hide of polar foxes

Andrzej Filistowicz, Piotr Przysiecki

An attempt was made to predict the length of dry hides on the basis of body weight at the age of 12, 16, and 20 weeks, body weight during the autumn assessment (grading), after slaughter, and the weight and length of wet hides of 74 male and 56 female
polar foxes. The authors indicate the influence of litter, origin and sex on all examined traits of the animals and the influence of birth date on the body weight at 12 weeks and the weight of wet hides of females. Of great cognitive value were equations assessing the length of dry hides made on the basis of measurements of body weight and the weight and length of wet hides.

Although these equations were very exact, they were of little practical value since they could not be used in the assessment of live animals. The exactness of equations assessing the length of dry hides only on the basis of measurements of body weight did not exceed 74%. Such equations may be useful in practice because the standard error in assessing these equations does not exceed 3.0-3.2% of the length of dry hides. Measuring the body weight of 20 week-old males and measurements of the body weight of females during the autumn grading made on the basis of live traits have been of the greatest significance in assessing the length of hides.


Metabolic profile of peripheral blood of silver fox at the beginning of the mating season

Vladimir Parkanyi, Dusan Mertin, Sonja Kassova

In silver fox females (n=40) the indices of the metabolic profile determined in blood from vena cephalica antebrachii at the beginning of the year (January 11th) were analyzed. The determined values of the metabolic profile were: erythrocytes 8.39·10^{12}.1^{1}, leucocytes 7.18·10^{9}.1^{1}, hemoglobin 19.91 g %, hematocrit 48.80 %, total protein 65.78 g.l^{1}, glucose 7.79 mmol.1^{1}, alkalic phosphatase 5.05 nkat.1^{1}, calcium 2.39 mmol.1^{1}, phosphorus 1.21 mmol.1^{1}, sodium 127.58 mmol.1^{1}, potassium 5.27 mmol.1^{1}. On the basis of the results from the metabolic profile it is possible to state that the treated females were healthy and in good breeding constitution.

Pol'nohospodárstvo 38, No. 9, pp. 719-722, 1992. In SLOV, Su. ENGL. 1 table, 6 refs. Authors' summary.

Analysis of live weight growth of silver foxes

D. Mertin, P. Fl'ak, V. Parkányi, I. Točka

We studied the live weight growth of silver foxes from age 30 to 180 days at the Department of Fur Animal Rearing of the Research Institute of Animal Production in Nitra during the years 1987-1989. The animals were clinically healthy and received a full-value nutrition.

The live weight of foxes ranged from 871 g to 942 g for males at the age of 30 days, and from 801 g to 919 g for females at the same age, and from 6319 g to 7186 g, and from 5200 g to 6583 g, respectively, in the period of the fur maturity, approximately at the age of 180 days.

We observed a significant influence of sex, and interaction of sex and age. Live weight growth estimated by means of linear regression showed the average daily gain at the age from 30 to 180 days expressed as a regression coefficient for males of 42 g, 34 g for females in 1987, 39 g and 38 g in 1988, and 33 g and 28 g in 1989. We also observed significant coefficients of the quadratic function in the years 1987 and 1989. The average live weight of males was 912 g at the age of 30 days, and 901 g of females, and 6815 g and 6387 g, respectively, at the age of 180 days irrespective of the years. We observed statistically significant differences between the years at the age of 90-180 days, and a highly significant sexual dimorphism at the age of 60-180 days, and the interaction of years x sex. The coefficients of the relative growth rate according to Minot irrespective of sex was R=1.507; and the coefficient of growth intensity according to Fisher k=0.0131.

The live weight at the age of 180 days was 7.3 times weight in the first month of age irrespective of sex. We estimated 95% confidence intervals of live weight on the basis of our results. The live weight growth of silver foxes was with some exceptions in agreement with other experimental material, and therefore the estimated confidence intervals will serve in the control of live weight growth.

Thermoregulatory responses to thermal stimulation of the preoptic anterior hypothalamus in the red fox (Vulpes vulpes)

J.J. Klir, J.E. Heath

The preoptic anterior hypothalamus (POAH) thermoregulatory controller can be characterized by two types of control, an adjustable setpoint temperature and changing POAH thermal sensitivity. Setpoint temperatures for shivering ($T_{\text{shiver}}$) and panting ($T_{\text{pant}}$) both increased with decreasing ambient temperature ($T_a$), and decreased with increasing $T_a$.

The POAH controller is twice as sensitive to heating as to cooling. Metabolic rate (MR) increased during both heating and cooling of the POAH. Resting temperature of the POAH was lower than internal body temperature ($T_b$) at all temperatures.

This indicates the presence of some form of brain cooling mechanism. Decreased $T_b$ during POAH heating was a result of increased heat dissipation, while higher $T_b$ during POAH cooling was a result of increased heat production and reduced heat dissipation. The surface temperature responses indicated that foxes can actively control heat flow from body surface. Such control can be achieved by increased peripheral blood flow and vasodilation during POAH heating, and reduced peripheral blood flow and vasoconstriction during POAH cooling.

The observed surface temperature changes indicated that the thermoregulatory vasomotor responses can occur within 1 min following POAH heating or cooling. Such a degree of regulation can be achieved only by central neural control. Only surface regions covered with relatively short fur are used for heat dissipation. These thermoregulatory effective surface areas account for approximately 33% of the total body surface area, and include the area of the face, dorsal head, nose, pinna, lower legs, and paws.


Estimation of glomerular filtration rate and evaluation of renal function in ferrets (Mustela putorius furo)

Maria I. Esteves, Robert P. Marini, Eva B. Ryden, James C. Murphy, James G. Fox

Three methods of determining glomerular filtration rate (GFR) were performed in adult ferrets, 9 months to 7 years old. Endogenous creatinine clearance was determined, using serum and urine creatinine values obtained during 24- and 48-hour collection periods from 27 ferrets housed in metabolic cages. Creatinine and radiolabeled inulin were administered to 12 female ferrets by constant IV infusion during isoflurane-induced anesthesia. Serial 20-minute urine collections, together with serum samples obtained at the midpoint of urine collection, provided measures for clearance calculations of these substances. Mean ± SD endogenous creatinine clearance in ferrets for metabolic cage collections was 2.50 ± 0.93 ml/min/kg of body weight. There were no significant differences between the 24- and 48-hour clearance rates. Mean inulin clearance was 3.02 ± 1.78, and mean exogenous creatinine clearance was 3.32 ± 2.16 ml/min/kg. Analysis of variance, using least-squared means adjustment, did not yield any significant differences between inulin and exogenous creatinine clearance rates. Exogenous creatinine clearance-to-inulin clearance ratio was 0.99 ± 0.46, and there was significant correlation between the 2 methods ($r=0.82$, $P=0.0001$). Significant body temperature effects on inulin or exogenous creatinine clearance were not found. Infused inulin clearance, the generally preferred method for GFR calculation in mammalian species, was significantly ($P=0.0069$) higher in younger (3.65 ml/min/kg) vs older ferrets (2.29 ml/min/kg). Results of this study indicate that inulin clearance is an adequate measure of GFR in ferrets as it is in other species. Compared with inulin clearance, exogenous creatinine clearance also provides a reliable estimate of GFR in ferrets.

Participation of the splanchnic nerves in the structure of the cranial mesenteric plexus in the coypu

Marian Langenfeld

Fig. 1. The cranial mesenteric plexus and the celiac plexus of the coypu (right side): 1 - cranial mesenteric plexus; 2 - cranial mesenteric artery; 3 - celiac plexus; 4 - celiac artery; 5 - aorta; 6 - greater splanchnic nerve; 7 - lesser splanchnic nerve; 8 - lumbar splanchnic nerves; 9 - sympathetic trunk; 10 - diaphragm; 11 - psoas major muscle; Th10-Th13 and Lr1-Lr3 - sympathetic trunks ganglia

The present study is a continuation of the research on the structure of the autonomic nervous system [3-8] and peripheral nervous system [1,2,9] in the coypu.

In the available literature no information was found on the participation of the splanchnic nerves in the structure of the cranial mesenteric plexus of the coypu.

1. Participation of the investigated splanchnic nerves in the structure of the cranial mesenteric plexus is individual in each specimen, irrespective of animal sex.

2. The greatest contribution to the structure of the cranial mesenteric plexus is of the lesser splanchnic nerve, 96.42% on the right and 92.85% on the left side of the body, next of the greater splanchnic nerve; 53.57% on the right and 57.14% on the left side and of the lumbar splanchnic nerves; 42.85% on the right and 35.71% on the left side.

Morphology of the stomach of the muskrat (Onatra zibethica L)

Hanna Jackowiak

The work presents the results of studies of partly and fully filled stomachs of 15 muskrats. Macro- and microscopic studies of the walls of these stomachs revealed morphological adaptations to changes in the size of the saccus caecus and the antrum pyloricum. The gland parts do not undergo any significant change in size.

The measurements given in the tables reflect changes in the length of stomach curvatures, characteristics of particular layers of the stomach wall in its various parts, folds of the mucosa and the submucosa, and bundles of the circular muscular layer.

"Shearing" of the skin cover

L.A. Burdel'

Analysis of the experimental results indicated that fur shedding (moulting) in mink is a result of a multi-faceted process of alimentary origin. It is primarily linked to underfeeding and dietary amino acid imbalance during the period of fur formation. Further studies were deemed necessary to confirm these conclusions.

Variable completeness of posttraumatic skin regeneration in warm-blooded animals

E.A. Efimov

It is generally accepted that skin regeneration in warm-blooded animals results in scar formation. Still we have found that skin regenerated at full-layered square skin wounds often resembles in its
structure normal skin rather than connective tissue scars. Morphological variability of the skin regenerates depended on the wound location and the animal species. Formation of the skin derivatives (hair follicles, sebaceous and sweat glands, skin folds) has been demonstrated. A classification of the skin regenerates has been proposed. It was repeatedly shown that the skin is able to give rise to organ-specific regenerates with the morphological features specific for the wound location.


Studies on multicomponent spectroscopic analysis of dye solutions

Marja Marjoniemi, Esa Mäntysalo

Absorbance measurements of dyes in solution are used in dye characterization, in setting up dyeing recipes, or in monitoring the progress of dyeing processes. Multicomponent spectroscopic analysis of dye solutions was performed in this research. The spectra of a set of dye solutions which contained either three or four different dye components were recorded. Several ordinary multivariate regression methods such as K-matrix, P-matrix, and Q-matrix least-squares regressions and factor based methods such as Principal Component Regression and Partial Least-Squares Regression were applied for the calibration, and the results were compared. The Partial Least-Squares method gave best results, especially in the case of four dyes. Conventional multivariate methods can not resolve severe overlapping of the spectra of the dyes in the visible region of light.

A 1:2 metal complex dyes were used in this research and the aging and the tendency to aggregate of the dyes were examined. The dyeing system used is made up of the primary colours yellow, red, and blue, plus a brown.

A VIS-spectrophotometer was applied for measuring the spectra of the dye solutions and L*a*b* values of some leather processing chemicals. The L*a*b* values were used when calculating colour differences ΔE for determining whether there would exist change in colour versus time. ΔE or gray scale are usually used for assessing change in colour when testing colour fastness of leather. When the value of ΔE is known, it is easy to quantify the change.


Preferences of farmed blue foxes for platforms, nest box and cage floor

Hannu Korhonen, Paavo Niemelä

A preference test system was devised to assess the preferences of farm-raised, juvenile blue foxes for six various types of resting platforms, including nest box roof, and for nest box and cage floor. The results showed that platform use was low since the test foxes preferred the cage floor. The amount of previous individual platform usage did not affect preference. However, foxes originating from groups with a high amount of previous platform use also had the highest amount of platform usage in the test situation. Of all the platforms, the nest box roof was preferred the most. Although the location of the platform in the present test situation was found to affect preference, it was difficult to finally separate the real effects of platform location and type.

No relationship was found between temperature and use of the platforms or nest box. On the basis of the present results we may conclude that platforms are not actually necessary for foxes during the winter period.


Immobilization of free-ranging red foxes (Vulpes vulpes) with tiletamine hydrochloride and zolazepan hydrochloride

Alejandro Travaini, Miguel Delibes

We evaluated Zoletil on free-ranging red foxes (Vulpes vulpes) in Spain. Twenty-two pup and 49 adult wild-caught red foxes (Vulpes vulpes) were immobilized with a combination of tiletamine hydrochloride and zolazepan hydrochloride in a 1:1
proportion (Zoletil). Mean (±SE) Zoletil doses were 10.57 (±0.41) mg/kg (range = 7.58-15.39 mg/kg, n=22) for pups and 10.51 (±0.33) mg/kg (range = 5.88-16.67 mg/kg, n=45) for adults. Mean induction and first recovery times for pups were 2.3 (±0.2) minutes (range = 1 to 5 minutes) and 35.5 (±3.28) minutes (range = 18 to 78 minutes), respectively. Mean induction and first recovery times for adults were 3.7 (±0.21) minutes (range = 2 to 8 minutes) and 35.4 (±2.22) minutes (range = 13 to 90 minutes), respectively.

We recommend Zoletil doses of 10 mg/kg for red foxes. For wild adult red foxes of unknown weight, an initial dose of 60 to 70 mg Zoletil should be administered. This dose should allow about 40 minutes of handling time.

Scent marking and social relationships in pine martens (Martes martes)

Michèle de Monte, Jean-Jacques Roeder

Scent marking was studied in pine martens (Martes martes) in female-female and male-female pairs. Results show that agonistically dominant individuals generally had higher scent marking frequencies. However, environmental familiarity can modify the social relationship, whatever the previous social experiences, and, consequently, marking activity.

Despite important intra- and interindividual variations, the subjects appeared to react to physical and social modifications by an increased marking rate. One factor affecting marking activity in both novel and established pairs was the activity level of the conspecific partner.

The data support the hypothesis that pine martens react in the same way to physical and social modifications, but with different response levels. The discussion focuses on possible functions of scent marking.

Polychlorinated biphenyl congeners in foxes in Germany from 1983 to 1991

S. Georgii, Ch. Bachour, K. Failing, U. Eskens, I. Elmadfa, H. Brunn

Red foxes served as a biological indicator for the temporal development of environmental contamination with polychlorinated biphenyls (PCB). The concentration of PCB congeners nos. 28, 49, 52, 101, 138, 153, and 180 were analysed in the body fat of 80 foxes (Canis vulpes) from Germany. The samples were from animals that had been submitted for examination in 1983, 1987, and 1991. Throughout this time period, a reduction was seen in the concentration of the highly chlorinated biphenyls 138, 153, and 180, whereas the concentration of the low-chlorinated congeners PCB nos. 28, 49, and 52 increased. No changes in contamination with congener 101 was observed. These results show a trend toward reduction of environmental contamination with highly-chlorinated biphenyls since 1983, while contamination with low-chlorinated congeners is apparently increasing.

An interesting observation is the disproportionately higher amount of 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180) over that of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) in body-fat samples from all foxes analysed. This phenomenon was also observed in 10 dogs examined as controls. Based on evidence from other authors of experimental toxicological studies on beagles, it appears that the fox possesses a similar cytochrome P450 isoenzyme that can degrade 2,4,5-trichlorosubstituted aromatic compounds. As a consequence, in canines, PCB 180, which is additionally meta-chloro substituted, is accumulated to a greater degree than is PCB 153.

The future of animal agriculture in Canada

Robert Blair, E. Edward Lister

Animal production makes effective use of the large land area of Canada that is not capable of producing crops for direct human consumption. It also pro-
vides a use of surplus cereal grain, not all of which can be exported. Trends in the dairy, beef, swine, egg, poultry meat, sheep, game and fur, and aquaculture sectors are described.


**Mediated chemocommunication and evolution in the mustelidae**

V.V. Rozhnov

The principal mode of chemocommunication in mustelids is mediated, involving the leaving of scent markers. The probability that various secretions (of specific skin glands, feces, urine) take part in mediated chemocommunication differs in different animals.

A comparative study of the patterns of scent marking in conjunction with a study of mustelid phylogeny reveals two principal pathways of development of marking behaviour related to the social life of these carnivores. The less social species (the main branch, Mustelinae) have established different specific methods of urine marking in order to transmit diverse information about individuals: species, sex, physiological state, etc. The more social species (the branch that includes the badgers, Melinae) exhibit behaviour involving the leaving of secretions of the subcaudal gland, transmitting information only about whether the individual leaving the mark is familiar or unfamiliar.

There are several stages in the evolution of the modes of urine marking: the leaving of urine along with feces (the "lavatories" arrangement), expansion of the area over which urine is left (spraying and the leaving of "urine trails"), the deposition of urine on a fatty base (fatty secretions of skin glands or secretions of a specific abdominal gland).

The main requirements for mediated chemocommunication are increased effectiveness of discovery of scent markers by nonspecific animals coupled with a more economical expenditure of water with the urine.

*Fig. 1. Marking behaviour in representatives of Mustela: a) urination while moving; b) urination during belly rubbing; c) rubbing with anal region; d) defecation.*

*Fig. 2. Marking behaviour of the pine marten and sable: a-d) rubbing with belly; e) urination standing up, with lateral body movements; f) defecation.*

*Fig. 3. Marking behaviour of the stone marten: a-d) urination while passing over an object; e) urination standing up; f) defecation.*

Fur biting in mink

Mogens Jørgensen

A case of fur biting in mink is described. 24% of the breeding animals were affected in March. From the finding of many hay mites and eggs in the fur, blood eosinofilia, dermal eosinofilic infiltration and clinical effect of antiparasitic treatment, it is most likely that fur biting and hair loss were provoked by hay mites.

Fig. 1. Et tilfælde af pelsgnav hos mink. Angrebet var forårsaget af hømiden (slægten Tyrophagus).


Tail-biting in mink (*Mustela vison*) is influenced by age at removal from the mother

G.J. Mason

Previous studies have shown that male mink (*Mustela vison*) removed from their mothers at seven weeks of age develop more tail-biting than males left with their mothers until six months. Mink in the wild do not damage their own pelts in this way, and such behaviour may well be an indication of chronic stress.

The aim of this experiment was to investigate further the causes of tail-biting by considering female young as well as male, and by lowering the age of which the 'late-weaned' mink were separated from their mother to 11 weeks, by which age their period of socialization should be complete. This was to generate results of more practical use to farmers, who cannot leave all young with their mothers until six months of age for reasons of space. Mink removed from their mothers at seven or eleven weeks of age did indeed differ in the incidence of tail-biting. 'Early-weaned' females were more likely than late-weaned females to have bitten their tails at six months of age. A similar result was evident as a trend for both sexes at ten months. Furthermore, at this age, some animals' tail tips were completely bald, and such animals were all early-weaned. Where provided with plastic drinker dishes, early-weaned animals were also more likely to chew these. Thus weaning age had long-lasting effects on a number of oral behaviour patterns. These results suggest that young animals predisposed to tail-bite might be diverted by the provision of other objects to chew, and that if problems of over-crowding are avoided, leaving mink kits with their mothers until 11 weeks might improve their welfare.


Immunocytochemical study of the growth hormone and prolactin pituitary cells in male and female suckling mink

Sergio Vidal, Pablo Sánchez, Albina Román, Lucas Moya

Adenohypophyseal growth hormone (GH) and prolactin (PRL) cells of suckling mink of both sexes at different stages of lactation were studied by the immunogold method and morphometry for electron microscopy. Hypophyses were perfused with fixative, postfixed in osmium tetroxide, and embedded in Epon 812. Ultrathin sections were labeled by the immunogold method with anti-human GH and anti-human PRL sera. Two PRL cell types were identified based on their morphology and the development of cytoplasmatic organelle. Significant differences in the granular sized of GH and PRL cells were found between males and females at different stages of suckling. The results suggest that the changes in secretion and storage in GH and PRL cells of suckling mink during lactation depend upon
sex and age, and that the granular polymorphism is not an unequivocal criterion for the identification of PRL cells in suckling mink.


Hematologic and serum chemistry reference values of adult brown mink

Douglas J. Weiss, William Wustenberg, Thomas J. Bucci, Victor Perman

Hematologic and serum chemistry reference values were determined for 160 12-month-old brown untamed captive mink (Mustela vison). Blood was obtained by jugular venipuncture after administration of ketamine and xylazine. There were no statistically significant differences between male and female mink. The packed cell volume, hemoglobin, and red blood cell count were 10 to 20% lower than previously reported for non-anesthesized mink. Serum glucose, alanine aminotransferase and aspartate aminotransferase values also were lower than previously reported values.


Biology and medicine of the domestic ferret: an overview

James W. Carpenter, Craig A. Harms, Lisa Harrenstien

In recent years, the domestic or European ferret (Mustela putorius furo) has become increasingly popular as a companion animal. This paper reviews our knowledge of the biology and physiology, nutrition, diagnostic procedures, restraint, and anesthetic procedures, and diseases of the ferret, and briefly outlines recommended preventive medicine procedures.


Reference intervals for insulin concentrations and insulin:glucose ratios in the serum of ferrets

F.A. Mann, S.L. Stockham, M.B. Freeman, C. Wagner-Mann, C.L. Besch-Wiliford, R.F. Nachreiner

Serum samples from 43 adult ferrets (Mustela putorius furo) of both sexes from three different sources were assayed for glucose using a glucose oxidase dry slide method and immunoreactive insulin (IRI) using a commercial radioimmunoassay kit. Ferrets from a separate research project (n=10) had significantly higher IRI and insulin:glucose (I/G) ratio than ferrets housed in a research animal supply facility (n=30) (P<0.05). The ferrets housed in the research animal supply facility were used to establish reference intervals for serum insulin concentration and I/G ratio. The resultant reference intervals were recorded in conventional units (glucose, 100-163 mg/dl; IRI, 4.6-43.3 µU/ml; I/G ratio, 3.6-34.1 µU/mg) and Système International units (glucose, 5.6-9.0 mmol/L; IRI, 33-311 pmol/L; I/G ratio, 4.6-44.2 pmol/mmol). Further work with the commercial IRI assay kit used in this study is necessary to determine the diagnostic serum IRI concentrations and I/G ratios in ferrets with insulin-secreting pancreatic tumors.

Analysis of the selection process in breeding of domesticated polar fox populations

S.N. Kashtanov

The purpose of this research was to analyse heterozygosity mechanisms in a domesticated population of Polar fox (Alopex lagopus L.). The polymorphism of the gene which codes the blood serum protein transferrin was used as a genetic marker. The level of the heterozygosity was studied in both the mature and young parts of the population.

The mature population is separated into subpopulations. Moreover, inbreeding leads to a decrease of heterozygosity in this part of the population.

On the other hand, selection can increase the number of heterozygous animals.

Genetika (Moskva) 29 (11): 1755-1757, 1993. 2 tables, 4 refs. In RUSS, Su. ENGL. Author's summary.

Insulin-like growth factor II in the mink (Mustela vison): determination of a cDNA nucleotide sequence and developmental regulation of its expression

T.J. Ekström, B.M. Bäcklin, Y. Lindqvist, W. Engström

Mink cDNA for insulin-like growth factor II (IGF-II) has been isolated by cDNA synthesis from bulk mRNA and subsequent PCR-screening. Positive clones were sequenced and analyzed. Analysis of a cDNA revealed that, compared with human, the mature mink IGF-II peptide contains 68 instead of 67 amino acids with a serine insertion at residue 40. With this exception, the homology between the human and mink mature peptide is 100% and is 94% between rat and mink. Comparing IGF-II transcripts in fetal and adult mink liver, fetal tissue contains three transcripts of 5.8, 4.5, and 4.0 kb. In contrast, adult liver expresses low levels of a 4.6-kb transcript.

Fig. 4. Predicted model structure of mink IGF II with the database structure of human IGF II overlaid

Seasonal prolactin secretion and its role in seasonal reproduction: a review

J.D. Curlewis

Fig. 1. Plasma prolactin concentrations, voluntary food intake, reproductive cyclicity and pelage moult in red deer hinds maintained under natural photoperiod and temperature and fed a pelleted food ad libitum. Data redrawn from Loudon et al. (1989).

Although all seasonal breeders show a seasonal change in prolactin secretion, there are continuously breeding species in which prolactin secretion is also under photoperiodic control. It is likely therefore that a seasonal pattern of prolactin secretion is only evidence of neuroendocrine sensitivity to changing photoperiod. Depending upon the species, this sensitivity to the seasonal changes in daylength may or may not be accompanied by seasonal changes in a biological endpoint such as seasonal reproduction or indeed other adaptations. Whether the seasonal change in prolactin secretion is an endocrine mediator of such adaptations remains in contention. Certainly in some species this signal does have a role in reproduction. For example, in species with an obligate seasonal embryonic diapause, the seasonal increase in prolactin can act as a luteotrophin (mink and western spotted skunk) or luteostatin (Bennett’s and tammar wallabies). In species where seasonal anoestrus or oligo- or azoosperma are due to a direct effect of photoperiod on gonadotrophin secretion, the seasonal changes in prolactin levels may also have been used by some species to influence seasonal reproduction. For example, the increase in prolactin in spring may be important in the golden hamster to augment increasing gonadotrophin concentrations and thus facilitate gonadal growth. The seasonal prolactin changes have also been causally linked with the
seasonal pelage moult cycle and may also be involved in another seasonal adaptation, the annual cycle of growth and metabolism. In contrast, there are species such as the domesticated breeds of sheep, in which a role for the seasonal change in prolactin secretion is yet to be identified or may not exist. In summary, present evidence supports the hypothesis that seasonal changes in prolactin secretion result from a neuroendocrine pathway which is common to all photoperiodic species, and that individual species have used this endocrine change in many different ways as a cue or mediator in the response to changing daylength.


Suprachiasmatic nucleus lesions abolish photoperiod-induced changes in the testis function and GnRH immunoreactivity in the mink, a short-day breeder

Daniel Maurel, Line Boissin-Agasse, Gisèle Roch, Serge Herbuté, Jean Boissin

Testicular activity (testis volume and plasma testosterone) and immunoreactive GnRH hypothalamic system were examined after suprachiasmatic nucleus (SCN) lesions in the mink, a short-day breeding mammal, whose sexual activity is inhibited by day lengths exceeding 10 h. In animals maintained under a natural photoperiod, SCN destruction performed during the period of maximum sexual activity (February) was shown to have no effect on onset of the testicular inactive period which begins at the end of winter and continues through spring. On the other hand, while gonadal activity began again at the end of autumn in intact animals, mink that had undergone SCN destruction remained sexually inactive until the end of the experiment period (February). The SCN could thus be crucial to the onset of sexual activity triggered by the reduction of day length, whereas onset of sexual inactivity is a spontaneous phenomenon. This was confirmed in a second experiment demonstrating that a short photoperiod (4L:20D), highly gonado-stimulatory in intact animals, had no effect on testicular activity after SCN destruction. An immunocytochemical study of the hypothalamic GnRH system (staining intensity and number of labelled perikarya and immunoreactive endings in the external layer of the median eminence) also showed consistent but very low rates of immunoreactivity and number of labelled perikarya and endings in operated animals.


Fig. 4. Effects of SCN lesion on the gonadostimulation triggered by a short-day (4 L:20 D) photoperiodic treatment. Animals were exposed to a 4 L:20 D photoperiod schedule after having been exposed to long-days (20 L:4 D) to interrupt the photorefractoriness. The results are compared to those obtained for intact or sham-operated animals maintained under the same photoperiodic conditions, and also to those obtained for intact, sham-operated or lesioned animals in natural photoperiod.
Age characteristics of reproductive functions in male silver foxes

L.V. Osadchuk

The fertility and hormonal function of testis were studied in young (one-year-old) and adult males of silver-black foxes having been bred over a long time for domesticated behaviour. It was shown that when selecting pedigree young stock, about one third of the animals did not take part in the first season of reproduction, and the fertility of the remaining young males was reliably lower than that of adults. The maximum hormone content in the blood of young males was found to occur before their most intensive mating time, while such a discrepancy is not observed in adults. Supposedly, the pertinent hormonal regulation mechanisms in young animals are not developed enough to control reproduction.

Sel'skokhozyaistvennaya Biologiya, No. 4: 26-31, 1992. 2 tables, 2 figs., 13 refs. In RUSS. Su.

English. Author's abstract.

Sex steroids in the plasma of polecats

N.K. Shul'gina, I.V. Belozerova, L.E. Pozdnyakova

Data are presented in graphs on the concentrations of oestradiol and testosterone in the plasma of polecats from weaning to slaughter at 7 months of age. The results are given separately for 6 groups of polecats in cages of different sizes (2-10 polecats per cage), and for the groups combined. For the latter, testosterone declined over the period from 1200 to approx. 300 pg/ml in males, and in females remained relatively constant at approx. 200-300 pg/ml. In both sexes, oestradiol declined from 30 pg/ml at weaning to approx. 5 pg/ml 3 months later, and then increased linearly to 30-50 pg/ml over the next 2 months. The age trends in hormone concentrations did not vary markedly among the 6 groups.


Periparturient behaviour of successfully reproducing farmed silver-fox vixens

Bjarne O. Braastad

As part of a project series aiming at understanding the reproductive behaviour of farmed silver foxes, this paper describes the normal variation in behaviour around parturition shown by successfully reproducing vixens. The behaviour of 19 vixens (eight primiparous, 11 multiparous) kept under traditional management conditions, was video-recorded inside the breeding box day and night, and analyzed in four phases from 24 h before delivery to 72 h after. Births were distributed uniformly around the clock. True litter size at birth was 3.8 (SD=1.7) for primiparous vixens and 4.7 (SD=1.5) for multiparous vixens. The period elapsing between individual births was 56 (SD=29) min. The parturition period lasted 192 (SD=87) min.

The time-budget of behaviour showed a pronounced individual variation in all phases, but with few differences related to parity. During the last 24 h prior to parturition, the vixens stayed in the breeding box 63% of the time. Two-thirds of this time was spent resting or sleeping. All vixens dug vigorously on the box floor, on average for a total of 102 min, mainly during the last hours before parturition. Vixens never lined the nest with fur tangles. The delivery of cubs seemed to be quite
easy, except for the first-born cub of some primiparous vixens. About 60% of the parturition phase was spent cleaning, grooming and inspecting cubs.

Fig. 3. Proportion of total observation time spent in various behaviour patterns, mean + SD. P, parturition period; numbers indicate days (24-h periods) before and after the parturition period. Note the three different scales. Significant differences between the first and the next 2 postparturient days are indicated, based on Wilcoxon matched-pairs test.

During the 3 postparturient days, vixens rested or slept 70% of the time, though an average sleeping bout lasted only 11 min. Vixens showed active cub-care, mainly consisting of grooming cubs while lying down, for 24% of the time during the first postparturient day and for 18% during the next 2 days. This behaviour showed no relationship to litter size, indicating that an average cub receives more care the smaller the litter size. The vixens were rarely seen to retrieve cubs or to bring them outside the breeding box. Vixens left their boxes for an average total of 50 min per postparturient day. The results showed that not only multiparous, but also primiparous vixens may show completely adequate behaviour around parturition.

The effect of slimming on embryo survival rate and the number of corpora lutea at 25 days of pregnancy in mink (Mustela vison)


70 yearling Scanblack breeding females (8 month old, weighing 1061 g) were randomly assigned to one of three treatment groups which were slimmed to a live weight of 787 (L), 878 (M) or 1071 g (H) during 54 days. Subsequently they were bred and at day 25 of pregnancy they were slaughtered and the number of corpora lutea, embryos and fetuses were counted. From this experiment it can be concluded that rearing conditions resulting in Scanblack breeding females of 787 g of live weight at the start of the breeding period will have a lower ovulation rate (16%) but comparable prenatal mortality rates as compared to females weighing 878 or 1071 g. Therefore reduction of litter sizes caused by slimming results from a depressed ovulation rate.

Application of new diagnostic techniques in animal reproduction: ultrasonography, laparoscopy and videendoscopy

Stefan Wierzbowski

Abstracts are given of 12 papers presented at a symposium held on October 14-15, 1993 in Parziewo, Poland. The papers covered results of work on mares, ewes, cows, bitches, cats and female ferrets and deer.

Effects of pregnant mare serum on the development of the reproductive system in female silver foxes

Huang Jianzhen, Bo Qingru, Zhen Yingkai

Thirty female silver foxes in anestrum were divided randomly into five experimental groups and one control group. Each animal in the experimental groups was injected with 1000 IU PMSG intramuscularly. The ovary and uterus were collected and their morphology and structure were studied in five stages after injection. The results showed that the ovary grew rapidly, the follicles of the ovary enlarged quickly and the uterus also developed quickly.


Isolation and cultivation of blastocyst-derived stem cell lines from American mink (Mustela vison)


Ten embryonic stem (ES) cell lines from mink blastocysts were isolated and characterized. All the lines had a normal diploid karyotype; of the ten lines studied, five had the XX and five had the XY constitution. Testing of the pluripotency of the ES-like cells demonstrated that 1) among four lines of genotype XX, X was late-replicating in three; both Xs were active in about one-third of the cells of line MES8, and analysis of glucose-6-phosphate dehydrogenase revealed no dosage compensation for the X-linked gene; 2) when cultured in suspension, the majority of lines were capable of forming "simple" embryoid bodies (EB), and two only showed the capacity for forming "cystic" multilayer EBs. However, formation of ectoderm or foci of yolk sac haematopoiesis, a feature of mouse ES cells, was not observed in the "cystic" EB. 3) when cultured as a monolayer without feeder, the ES cells differentiated into either vimentin-positive fibroblast-like cells or cytokeratin-positive epithelial-like cells (less frequently); neural cells appeared in two lines; 4) when injected into athymic mice, only one of the four tested lines gave rise to tumors. These were fibrosarcomas composed of fibroblast-like cells, with an admixture of smooth muscular elements and stray islets of epithelial tissue; 5) when the ES cells of line MES1 were injected into 102 blastocyst cavities and subsequently transplanted into foster mothers, we obtained 30 offspring. Analysis of the biochemical markers and coat colour did not demonstrate the presence of chimaeras among offspring. Thus the cell lines derived from mink blastocysts are true ES cells. However, their pluripotential capacities are restricted.

Embryonic stem cells derived from morulae, inner cell mass, and blastocysts of mink: comparisons of their pluripotencies


A characterization of cell lines that we derived from morulae (three lines), blastocysts (two lines), and the inner cell mass (ICM) is given. The karyotype of all the lines was normal; the genotype of four lines was XX, and four lines were genotypically XY. The pluripotencies and commitment status of the derived lines were estimated. First, there were not less than two-thirds of the cells in the populations of the lines derived from morulae and the ICM with both Xs active; 70-100% of the cells of the blastocyst-derived lines had one of the Xs in an inactive state. The activity of glucose-6-phosphate dehydrogenase (G6PD) in the lines (genotype XX) derived from morulae and ICM was found to be twofold higher than in lines with genotype XY, and G6PD activity was the same in the blastocyst-derived XX lines and XY lines. Second, when injected intraperitoneally into athymic mice, morulae and ICM-derived cells gave rise to simple and complex embryoid bodies (EB) resembling typical "cystic" mouse EBs. Third, when injected subcutaneously in athymic mice, the ICM- or morula-derived cells gave rise to typical teratomas containing derivatives of the three germ layers and components of organogenesis. Comparisons of cell lines of different derivations demonstrated that the pluripotencies of the ES cells derived from morulae or the ICM are higher than those of blastocyst derivation.


Polyteny in the cells of pre-implantation mink embryos

G.K. Isakova, T.G. Zybina, E.V. Zybina

Embryos were examined on the 17th, 20th, and 28th days after mating. On the 17th day most cells were the same size; 10% were nearly twice the size of the rest, and were presumed to be tetraploid. On the 20th day there was a large number of interphase nuclei in relation to embryo size, the nuclei ranging from "small" (presumably diploid) to "giant"; many nuclei (particularly giant nuclei) showed signs of endomitosis with clusters of polytene chromosomes.

On the 28th day there was much less variation in nucleus size than on the 20th day, with fewer gain nuclei and more fragmented nuclei. It is suggested that endomitosis and polyteny are the basis of the increase in diapausal blastocyst size. Evidence was also obtained for DNA and RNA synthesis by the embryo during diapause.


In vitro culture of silver fox embryos

H. Lindeberg, L. Jalkanen, R. Savolainen

This experiment was designed to establish in vitro culture methods for silver fox embryos in order to develop the methods for evaluation of the post-thaw viability of frozen embryos in future studies. Artificially inseminated silver fox females were killed humanely on predetermined days after insemination and oviducts and uteri were flushed for embryos.

The embryos were cultured in modified TCM 199 or in the same medium supplemented with silver fox oviductal tissue suspension for varying periods, from 6 days to 3 weeks. A total of 60 embryos was recovered. Only embryos beyond the 8-cell stage up to expanded blastocysts developed in vitro (28% of all embryos). Early stage blastocysts developed most reliably and were of the best quality.

Suspected thiamine deficiency (Chastek’s paralysis) in northern river otter (*Lutra canadensis*)

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Abstract

Chastek’s paralysis is a disease associated with the consumption of fish that contain thiamine inactivating factors commonly called thiaminase. The presence of thiaminase in the diet can inactivate thiamine (vitamin B₁) resulting in a thiamine deficiency. The disease has been reported in mink, foxes, and some marine mammals but apparently has not been previously reported in northern river otter (*Lutra canadensis*). Feeding captive river otter diets that contained 20, 40, or 60% carp (*Cyprinus carpio*) (wet weight of diet) supplemented with 50 mg thiamine hydrochloride/kg diet at mixing failed to prevent clinical signs suggestive of Chastek’s paralysis. These signs included decreased feed consumption, body weight loss, loss of coordination, and paralysis accompanied by occasional convulsions. The otter fed the 40 and 60% carp diets and one of the otter fed the 20% carp diet died or were euthanized. The other two otter in the 20% carp group responded to supplementation of the diet just prior to feeding with 100 mg thiamine hydrochloride/kg diet. Their feed consumption and body weights increased within a week of supplementation and they remained in good health for the remainder of the 26 week study.

Introduction

Thiaminase is a commonly used term for the net activity of heat-labile and heat-stable factors which inactivate thiamine (vitamin B₁) (*Fujita, 1954*). It is present in certain species of freshwater and marine fish and other aquatic animals (*Leonard, 1966*). Regular consumption of fish or mixed feeds that contain thiaminase can cause a nutritional disease called Chastek’s paralysis. The disease is characterized by a loss of appetite and malaise followed by incoordination, paralysis, convulsions, and death within two to three days. The disease was first described in fox on the Chastek Fur Farm in Glencoe, Minnesota in 1932 (*Gnaedinger, 1963*) and has since been reported in seals (*Geraci, 1972,*
Twelve wild caught, male northern river otter obtained from Louisiana were transported to the Michigan State University Fur Farm on January 20, 1991. The otter were weighed, examined by a veterinarian, immunized against mink virus enteritis and botulism, and given a booster for canine distemper, adenovirus, parainfluenza, and parvovirus (Vanguard 5, Norden Laboratories, Lincoln, Nebraska 68501). They were vaccinated at the time of capture (during December, 1990 and January, 1991) for canine distemper, adenovirus, parainfluenza, parvovirus, rhinotracheitis, calici virus, chlamydia, and panleukopenia (Galaxy 6 MHP and Eclipse 4, Solvay Animal Health, Mendota Heights, Minnesota 55118). The otter were housed individually outdoors in wire mesh cages (2.44 x 1.22 x 1.22 m) with attached wooden nest boxes (0.91 x 0.61 x 0.51 m) bedded with straw. They were provided the pretrial diet shown in Table 1 and drinking water ad libitum for 92 days.

The definitive study was initiated April 22, 1991. During the study, the otter (three per dietary group) were fed twice a day (in excess of what they would consume) diets that contained 0, 20, 40, or 60% carp (Table 1) taken from the mouth of the Saginaw River, Michigan by electroshocking. Carp from this area were used in the study because they contained substantial concentrations of environmental contaminants and could be readily obtained in sufficient quantities to conduct a long-term feeding trial (Heaton et al., 1995). Five samples of whole, raw, ground carp were submitted to the Michigan State University, Aquatic Toxicology Laboratory, for organochlorine pesticide and PCB analysis (Schwerts, 1982; Schmitt et al., 1985; Mora and Verbugge, 1991). The fish, poultry by-products, and liver used in the diets were ground and blended with the other dietary ingredients (Table 1) in a paddle mixer. The mixed diets were put into plastic containers (approximately 5 kg/container; enough feed for one day) and placed in a freezer (-18°C) until thawed at room temperature for approximately 18 hrs prior to feeding a portion of the feed to the otter. The remainder of the thawed feed was stored in a refrigerator at 2°C for approximately 6 hr and then fed to the otter. The otters were removed prior to each feeding.

Because carp have been reported to contain thiaminase (Gnaedinger and Krzeczowski, 1966; Leonard, 1966), 50 mg of thiamine hydrochloride/kg diet were added to the diets at mixing in an attempt to compensate for the thiaminase that would be inactivated by the thiaminase contained in the carp and provide adequate thiamine concentrations to meet the otters’ requirement for this vitamin. Green and others (1942a) reported that 10 mg of supplemental thiamine a day protected foxes from Chastek’s paralysis when fed a diet containing 20% whole uncooked carp. Thiamine was added to the diets rather than heating (82°C for a minimum of 5 min) the carp to destroy the thiaminase (Gnaedinger and Krzeczowski, 1966) because it was thought that cooking the fish might adversely affect the palatability of the diets (Gnaedinger, 1963) and/or alter the contaminants so that they would not be indicative of what was present in the environment. Following observation of clinical signs of suspected thiamine deficiency, samples of the control and 60% carp diets were submitted to national Environmental Testing, Inc., Chicago, Illinois, 60607 for thiamine analysis.
Table 1  Composition of diets

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Pretrial diet</th>
<th>0% carp (control)</th>
<th>20% carp</th>
<th>40% carp</th>
<th>60% carp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saginaw Bay carp</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Ocean fish scrap</td>
<td>40</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Cereal</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Poultry by-products</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Beef liver</td>
<td>6.5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Eggs</td>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>d-biotin (mg/kg)</td>
<td>0.11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thimine hydrochloride (mg/kg)</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Analysis (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>63.1</td>
<td>60.1</td>
<td>57.6</td>
<td>55.0</td>
<td>52.1</td>
</tr>
<tr>
<td>Crude protein</td>
<td>15.7</td>
<td>17.7</td>
<td>18.2</td>
<td>17.9</td>
<td>18.7</td>
</tr>
<tr>
<td>Crude fat</td>
<td>6.13</td>
<td>6.77</td>
<td>9.2</td>
<td>12.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>1.03</td>
<td>0.94</td>
<td>0.87</td>
<td>1.10</td>
<td>0.96</td>
</tr>
<tr>
<td>Ash</td>
<td>3.95</td>
<td>4.99</td>
<td>4.83</td>
<td>4.14</td>
<td>9.71</td>
</tr>
<tr>
<td>Thiamine hydrochloride (mg/kg, dry weight)</td>
<td>-</td>
<td>36.90</td>
<td>-</td>
<td>-</td>
<td>0.11</td>
</tr>
</tbody>
</table>

1  The fish, poultry, and liver were ground through 9.5 mm dies before mixing with the other ingredients
2  Cod, haddock, and flounder; Boston Feed Supply, Natick, Massachusetts 01760, USA
3  XK-40 Mink Cereal; XK Mink Foods, Plymouth, Wisconsin 53073, USA
4  Tyson Foods, Fort Smith, Arkansas 72901, USA
5  U.S. Biochemical Corp., Cleveland, Ohio 44122, USA
6  Analysis by Litchfield Analytical Services, Litchfield, Michigan 49252, USA
7  Dietary thiamine hydrochloride concentrations were determined in diets supplemented at mixing with 50 mg thiamine hydrochloride/kg after the appearance of clinical signs suggestive of thiamine deficiency but prior to additional dietary thiamine supplementation

During the study, the otters' body weights were recorded weekly and their feed consumption measured to the nearest gram for two consecutive days each week. According to the guidelines of the Michigan State University All University Committee on Animal Use and Care, any otter that lost 30% of its pretrial body weight was euthanized.

Results

All the otter gained body weight (mean ± SEM = 2.32 ± 0.24 kg) during the three month acclimation period and appeared to be in good health at the initiation of the study. The samples of carp that were analyzed for PCB and pesticide residues
contained 5.7 ± 0.24 (mean ± SEM; wet weight) ppm total PCBs (based on Aroclors 1242, 1248, 1254, and 1260) and ppb concentrations of numerous organochlorine pesticides including heptachlor, heptachlor epoxide, oxychlordane alpha chlordane, gama chlordane, lindane, dieldrin, endrin, aldrin, methoxychlor, t-nonachlor, endosulfan I, endosulfan II, p,p'-DDD, o,p'-DDD, p,p'-DDE, o,p'-DDE, p,p'-DDT (Davis, 1992; Heaton et al., 1995).

There was a marked decrease in feed consumption by the otter fed the diets that contained carp by the second week of the trial. This trend continued until day 46 when a thiamine deficiency was suspected in the otter fed carp based on clinical signs and 100 mg of thiamine hydrochloride/kg feed were added to the diets (including the control diet) just prior to feeding. Following supplementation of the diets with thiamine, feed consumption by the otter fed the 20 and 40% carp diets increased dramatically within a week (Table 2).

The otter body weights reflected the changes observed in feed consumption (Table 3). One otter fed the 60% carp diet escaped on day 30 and the other two on the 60% carp diet were euthanized on day 37 because they lost 30% of their pretrial body weight. The otter fed the 40% carp diet lost 24% of their body weight. One of the otter in the 20% carp group was euthanized on day 50 due to loss of body weight. The other two otter in the 20% carp group responded to the thiamine supplementation showing marked improvement in body weights that were again comparable to the controls by weeks 15-18 (Table 3).

Table 2

<table>
<thead>
<tr>
<th>Period</th>
<th>0% carp (control)</th>
<th>20% carp</th>
<th>40% carp</th>
<th>60% carp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks 1-2</td>
<td>907 ± 19.7</td>
<td>666 ± 52.9</td>
<td>617 ± 64.5</td>
<td>591 ± 31.4</td>
</tr>
<tr>
<td>Weeks 3-4</td>
<td>964 ± 18.9**</td>
<td>528 ± 67.6</td>
<td>499 ± 65.2</td>
<td>379 ± 52.3</td>
</tr>
<tr>
<td>Weeks 5-6</td>
<td>862 ± 63.6*</td>
<td>201 ± 58.7</td>
<td>229 ± 46.9</td>
<td>171 ± 54.6</td>
</tr>
<tr>
<td>Weeks 7-8*</td>
<td>920 ± 27.6</td>
<td>549 ± 118.9</td>
<td>428 ± 117.5</td>
<td></td>
</tr>
<tr>
<td>Weeks 9-10</td>
<td>780 ± 66.3</td>
<td>631 ± 36.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 11-14</td>
<td>824 ± 30.8*</td>
<td>579 ± 30.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 15-18</td>
<td>720 ± 30.3</td>
<td>596 ± 35.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 19-22</td>
<td>783 ± 30.0</td>
<td>477 ± 20.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 23-26</td>
<td>885 ± 31.6*</td>
<td>605 ± 23.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Feed consumption based on the mean of two consecutive days’ consumption per week
2 Mean ± SEM
3 N = 3 otter/group, unless noted otherwise
4 Means in the same row with the same letter superscript are not significantly different (P>0.05)
5 100 mg thiamine HCl/kg diet was added (day 46) to the control and 20% carp diets just before feeding for the duration of the study
6 N = 2 otter
7 N = 2 otters. Feed consumption for week 5 only
8 N = 2 otters. Feed consumption for week 7 only
Table 3  Body weight, body weight change, and percent body weight loss of male river otter fed various concentrations of carp

<table>
<thead>
<tr>
<th>Period</th>
<th>Body weight (kg)</th>
<th>0% carp (control)</th>
<th>20% carp</th>
<th>40% carp</th>
<th>60% carp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>9.16 ± 0.91a,b,c</td>
<td>9.28 ± 0.72a</td>
<td>9.14 ± 0.55a</td>
<td>9.36 ± 0.40a</td>
<td></td>
</tr>
<tr>
<td>Weeks 1-2</td>
<td>9.11 ± 0.57a</td>
<td>9.17 ± 0.49a</td>
<td>9.24 ± 0.34a</td>
<td>9.25 ± 0.18a</td>
<td></td>
</tr>
<tr>
<td>Weeks 3-4</td>
<td>9.02 ± 0.55a</td>
<td>8.66 ± 0.53a</td>
<td>8.38 ± 0.38a</td>
<td>7.65 ± 0.40b</td>
<td></td>
</tr>
<tr>
<td>Weeks 5-6</td>
<td>8.84 ± 0.64a</td>
<td>7.26 ± 0.21b</td>
<td>7.54 ± 0.62a</td>
<td>6.72 ± 0.20c</td>
<td></td>
</tr>
<tr>
<td>Weeks 7-8</td>
<td>9.08 ± 0.33a</td>
<td>6.92 ± 0.25g</td>
<td>6.90 ± 0.20a</td>
<td>7.65 ± 0.40b</td>
<td></td>
</tr>
<tr>
<td>Weeks 9-10</td>
<td>9.04 ± 0.29a</td>
<td>8.16 ± 0.65b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 11-14</td>
<td>8.90 ± 0.45a</td>
<td>8.17 ± 0.14g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 15-18</td>
<td>8.98 ± 0.47a</td>
<td>9.29 ± 0.44a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 19-22</td>
<td>8.36 ± 0.17a</td>
<td>8.98 ± 0.61a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeks 23-26</td>
<td>8.63 ± 0.92a</td>
<td>8.55 ± 0.55a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-0.53</td>
<td>-0.73</td>
<td>-2.24</td>
<td>-2.64</td>
<td></td>
</tr>
<tr>
<td>Percent loss</td>
<td>5.79</td>
<td>7.86</td>
<td>24.51</td>
<td>28.21</td>
<td></td>
</tr>
</tbody>
</table>

1 Body weights recorded once a week
2 Mean ± SEM
3 N = 3 otter/group, unless noted otherwise
4 Means in the same row with the same letter superscript are not significantly different (P>0.05)
5 100 mg thiamine HCl/kg diet was added (day 46) to the control and 20% carp diets just before feeding for the duration of the study.
6 N = 2 otter
7 N = 2 otter. Feed consumption for week 5 only
8 N = 2 otter. Feed consumption for week 7 only

After receiving the 40% carp diet for 41 days, one otter in the group exhibited an unsteady gait and loss of coordination followed by spastic paralysis and convulsions the next day. The convulsions lasted approximately 8 min. This otter was observed to experience another more severe convulsion (on day 43) and was euthanized. After 43 days on the trial, another otter fed the 40% carp diet was observed to experience a severe clonic convulsion and paralysis of the hind legs and died. The third otter in the group exhibited similar convulsions and paralysis on day 45 of the study.

In an attempt to treat the condition, the otter was administered 25 mg thiamine hydrochloride in 3 ml sterile physiological saline i.p., and 0.3 ml atropine sulfate (15 mg/ml) i.m. The atropine was administered to alleviate possible cholinergic stimulation. However, no beneficial response to the treatment was observed and the otter was euthanized after this condition persisted for more than an hour. During the episode, the otter experienced three convulsions that lasted about 2 min each. These convulsions were followed by jerking thrusts of the head and limbs about once every sec for approximately 30 sec. While experiencing a convulsion, all the otter appeared to be semiconscious. Their eyes were open and they salivated profusely. There were no vocalizations. Occasionally an otter would suddenly raise its head and throw it over its back as if gasping for air. These clinical signs, indicative of a thiamine deficiency, were not observed during numerous daytime observations of the otter fed 20 or 60% carp. Gross necropsy and histopathologic examination of the brain, liver, kidneys, heart, spleen, and adrenal and thyroid glands of the otter...
in the 20% carp group that was euthanized and those in the 40 and 60% carp groups failed to reveal any consistent lesions or alterations that were characteristic of a thiamine deficiency or PCB toxicosis (Green et al., 1942b; Okada et al., 1987; Roberts et al., 1978). The primary alterations seen in these otter were degenerative hepatic cellular changes consisting of infiltration of portal areas by lymphocytes, plasma cells, and macrophages. These areas were characterized by loss of hepatocytes, hemorrhages, and an accumulation of macrophages and some multinucleated giant cells (Langhans' type). One otter in each of the 40 and 60% carp groups had increased red pulp and decreased white pulp in their spleens. Analysis of samples of the control and 60% carp diets collected at the time the diets were fed to the otter but without supplementation with 100 mg thiamine hydrochloride/kg diet showed thiamine hydrochloride concentrations of 3.69 and 0.011 mg/100 g dry diet, respectively.

Discussion

It was originally thought that the decreased feed consumption and body weights of the otter fed the carp diets might be due to PCB toxicosis, as previous studies with mink have shown anorexia and decreased body weights to be early clinical signs of PCB toxicity (Aulerich et al., 1986). However, the clinical signs involving the nervous system exhibited by the otter fed 40% carp strongly suggested that the syndrome was due to Chastek's paralysis, since these symptoms have not been reported to be associated with PCB toxicity in other mammals but are characteristic of a thiamine deficiency in mink (Mustela vison) and foxes (Vulpes vulpes) (Green et al., 1942b, Okada et al., 1987). Other dietary causes of the syndrome seemed unlikely because the ingredients in the pretrial and treatments diets were similar, except for the high percentages of carp used in the treatment diets (Table 1).

The slight reduction in feed consumption of the controls noted during the trial may be due to the onset of warmer weather and a reduction in the energy requirement of the otter. The differences in feed consumption between the controls and otter fed 20% carp during the latter weeks of the study (following correction of the thiamine deficiency) are probably due, at least in part, to the higher energy (fat) content of the carp diet (Table 1).

Although a thiamine deficiency is often reversible with the administration of thiamine (Green et al., 1942b), the deficiency may have progressed to a non-reversible stage i the otter showing paralysis and convulsions prior to being treated with thiamine hydrochloride and atropine. The absence of characteristic lesions of Chastek's paralysis as noted in the otter examined histologically may not be uncommon. Okada and co-workers (1987) reported that fox cubs and adult female and kit mink failed to show microscopic lesions in the brain and heart and suggested that the disease progressed so rapidly that death occurred before characteristic lesions developed in these organs. The adult female mink in their study were exposed to the thiamine deficient diet that contained 60% raw fish for more than a month prior to the onset of clinical signs of the disease. Lesions and alterations commonly associated with thiamine deficient fox and mink include bilaterally symmetrical hemorrhages in the cerebrum, flaccid dilated right ventricles, cloudy and hemorrhagic myocardiums, and fatty degeneration of the liver (Green et al., 1942b; Okada et al., 1987).

To our knowledge, the thiamine requirement of river otter has not been determined. The thiamine requirement for young mink is reported to be 1.2 mg of thiamine hydrochloride per kg of dry feed and mature silver fox require 0.8 mg of thiamine hydrochloride per kg of dry feed (National Research Council, 1982). Thus, if the otter's thiamine requirement is similar to that of mink and silver fox, the control diet fed in this study would have met the otter's requirement for thiamine while the 60% carp diet would have been deficient in this vitamin. It is suspected that although the diets supplemented with 50 mg of thiamine hydrochloride/kg of feed at mixing originally contained more than adequate concentrations of thiamine, exposure of the vitamin to thiaminase present in the carp for several hrs following mixing before the diets were frozen and while the diets were thawing before consumption permitted sufficient biological inactivation of the thiamine to cause a deficiency. Supplementation of the control and 20% carp diets with 100 mg of thiamine hydrochloride/kg of feed just prior to feeding apparently provided the otter fed 20% carp with sufficient thiamine to meet their requirement and resulted in increased feed consumption and body weights.
within a week and they remained in good health for the remainder of the 26 week feeding trial.

It is doubtful that Chastek’s paralysis occurs in river otter living in the wild due to the diversity of their diet. However, based on the clinical signs observed in the otter fed carp in this study, the thiamine concentrations in the control and 60% carp diets, and the response of the affected otter to the addition of thiamine to the diets just prior to feeding suggests that Chastek’s paralysis can occur in captive river otter routinely fed diets that contain thiaminase. Thus, precautions should be taken to avoid this disease if thiaminase-containing species are a staple part of the diet. Species that contain thiaminase can be successfully fed if: (1) they are first heated (82°C for 5 minutes) to destroy the thiaminase, (2) supplemental thiamine is provided at feeding or as a separate food item, or (3) they are not fed thiaminase-containing fish every day. Leonard (1966) suggests that mink be fed thiaminase-containing fish no more than every other day or three days a week. Further restrictions may be necessary during lactation when an animal’s requirement for thiamine is greatest.

Acknowledgements

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Vitamin A metabolism in carnivores with special reference to fur bearing animals

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Semmelweisstr. 2, D-04103 Leipzig, Germany

Summary
Canines and mustelids transport vitamin A in the blood not only as retinol but predominantly as retinyl esters bound to lipoproteins. With regard to the tissue distribution extremely high levels of vitamin A were observed in the kidney of canines and mustelids. In the fox and the raccoon dog kidney levels were markedly higher (1066 and 1259 µg/g, respectively) compared to levels found in the liver (8 and 142 µg/g, respectively). This is discussed to be related to the excretion of vitamin A in the urine in some of these species.

Introduction
The importance of vitamin A has been recognized for decades. Additionally to vision, vitamin A is involved in fetal development as a morphogen (Hofmann & Eichele, 1994), in the regulation of the proliferation and differentiation of many cell types and in reproduction. Vitamin A deficiencies are associated with a decreased resistance of animals to infections. This might be caused by a breakdown of epithelial barriers or by changes in the immune system. The consequence for the animal is an increased incidence of diseases (Ross & Hämmerling, 1994). Until recently it has been common knowledge, that under physiological conditions vitamin A is transported in the blood as retinol bound to a specific carrier protein, the retinol-binding protein (RBP). In the postprandial phase chylomicron-bound retinyl esters can be detected in the plasma (Blomhoff et al., 1990). We were the first to show that, contrary to the assumption that lipoprotein-bound retinyl esters are responsible for the signs of vitamin A intoxication (Mallia et al., 1975; Smith & Goodman, 1976), in numerous species of carnivores a high percentage of retinyl esters is bound to plasma lipoproteins. In these species it seems to be a physiological way of vitamin A transport (Schweigert, 1988; Schweigert et al., 1990).

Material and methods
This report summarizes the results of two studies conducted on six dogs (Canis familiaris), four foxes (Alopex lagopus), and three raccoon dogs (Nyctereutes procyonoides) kept on a pelt farm in Finland as well as on six adult male grey seals (Halichoerus grypus) from Sable Island, N.S., Canada (Schweigert & Thomann, 1993) and nine male mink (Mustela vison) from a local pelt farm. Body fluids (blood plasma, urine) were first deproteinized with ethanol and then extracted with n-he-
xane. Tissues (liver, kidney, muscle, adipose tissue) were homogenized and extracted with a mixture of isopropanol and n-hexane (2:3, vol/vol) according to Radin (1981). Retinol and retinyl esters were separated and quantitated by high performance liquid chromatography (HPLC) which allows a detection limit below 5 ng (Furr et al., 1994). Lipoproteins were separated by selective precipitation with dextran sulfate (Burstein & Scholnick, 1973).

Results and discussion

Table 1 summarize the results on vitamin A in blood plasma, urine and different tissues. The results show that in all investigated species except the seal, vitamin A levels in plasma are much higher compared to humans, rodents and herbivorous species (Schweigert et al., 1991b). This is due to the high percentage of retinyl esters in plasma ranging between 75 and 96% of total vitamin A. Similarly to the situation found with hypervitaminosis A in rats and humans (Smith & Goodman, 1976), these retinyl esters are transported in blood plasma bound to lipoproteins. In dogs, foxes and raccoon dogs, 70 to 90% of retinyl esters (retinyl palmitate and stearate) are bound to the very-low (VLDL) and low density lipoproteins (LDL). Despite this, none of the investigated species showed signs of vitamin A intoxication as would have been the case in other species where plasma transport is highly regulated. Differences in tissue distribution of vitamin A and the secretion of large amounts of vitamin A in the urine as described by us for the first time not only in the dog (Schweigert et al., 1991a) might be viewed under the aspect of a nonspecific transport of vitamin A in plasma. In general, high levels of vitamin A found in the liver of dogs and mink correspond to values reported for other canines and mustelids (Berestov et al., 1984; Ribaya-Mercado et al., 1994). The liver is generally discussed to represent the main body storage organ for vitamin A (Blinhoff et al., 1990). Extrahepatic vitamin A represent less than 5% of the total body reserves. It is thus interesting to note that, except in seals, kidney levels are rather high compared to levels found e.g. in man (Raica et al., 1972). They correspond to levels found in cats and ferrets (Moore & Sherman, 1963; Ribaya-Mercado, 1994). In the fox and the raccoon dog the levels of vitamin A in the kidneys exceeds the level in the liver by far. In the fox, the lowest levels of vitamin A in the liver (8 μg/g) were associated with higher levels in the kidney (1259 μg/g). Although such low levels in the liver are generally regarded as a very severe form of vitamin A deficiency (Underwood, 1984), vitamin A in plasma was still high. This clearly shows that the nonspecific transport of vitamin A in blood plasma of canines and mustelids is not a sign of vitamin A intoxication but represents a physiological phenomenon.

Table 1  
Vitamin A (retinol equivalents as mean ± SD) in plasma and urine (μg/ml) as well as different tissues (μg/g tissue)

<table>
<thead>
<tr>
<th></th>
<th>mink</th>
<th>fox</th>
<th>raccoon dog</th>
<th>dog</th>
<th>seal</th>
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<tr>
<td>plasma</td>
<td>4.3 ± 1.8</td>
<td>10.1 ± 1.4</td>
<td>4.2 ± 1.0</td>
<td>4.0 ± 1.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>urine</td>
<td>nd</td>
<td>0.1 ± 0.01</td>
<td>2.2 ± 2.9</td>
<td>0.6 ± 0.4</td>
<td>nd</td>
</tr>
<tr>
<td>liver</td>
<td>886 ± 722</td>
<td>8 ± 3</td>
<td>142 ± 95</td>
<td>2060 ± 581</td>
<td>609 ± 395</td>
</tr>
<tr>
<td>kidney</td>
<td>318 ± 155*</td>
<td>1259 ± 294*</td>
<td>1066 ± 369*</td>
<td>509 ± 740*</td>
<td>8 ± 3*</td>
</tr>
<tr>
<td>skeletal muscle</td>
<td>-</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.4</td>
<td>3.0 ± 3.0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>adipose tissue</td>
<td>-</td>
<td>30 ± 8</td>
<td>15 ± 7</td>
<td>23 ± 13</td>
<td>45 ± 10</td>
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</table>

nd=not detected; *medulla; †total organ
It might be speculated that high levels of vitamin A in the kidneys are associated with the excretion of vitamin A (basically retinyl palmitate) with the urine, which is another peculiarity of vitamin A metabolism in canines. The ability to excrete up to 60% of the daily vitamin A intake with the urine might be regarded as protection against vitamin A intoxication.

In conclusion these unique results on vitamin A metabolism in canines and mustelids raise several questions regarding generally accepted mechanisms involved in vitamin A homeostasis. Canines and mustelids might represent valuable models to study the effects of nonspecific transported retinyl esters in plasma on a cellular and molecular level.

This article is based in part on data (dog, fox, raccoon dog, seal) published in German by Schweigert and Thomann 1993 in Monatshefte für Veterinärmedizin 48: 25-29.

References


The feeding of raw, fermented poultry by-products using mink as a model

H.A.P. Urlings, G. de Jonge, P.G.H. Bijker, J.G. van Logtestijn

In this study, the safety of fermentation as a method of preservation of raw animal byproducts used for animal nutrition was tested. Two feeding trials with mink, as a model for nonruminant animals, were carried out. In the first trial mink were given a fermented diet composed of raw poultry and fish byproducts supplemented with cereals, glucose, lactic acid, premix, and starter culture (Lactobacillus plantarum and Enterococcus faecium). These mink failed to deliver kits, and 7 of the 30 females in the test group died. At autopsy no specific cause of death could be diagnosed, although all the dead mink showed symptoms of cachexia. In a second trial, a group of mink kits, during the growth period, was given a diet composed of fermented poultry byproducts, just before feeding mixed with raw fish. The weight gain of the mink in the test group decreased statistically compared with that of the control group, mainly for the male members of the group. From the end of October until the beginning of November, during pelt priming, some mink showed symptoms of severe weight loss.

It is suggested that the measured increase of amino acid breakdown, and/or the acidic pH of the fermented diet, caused these unfavourable results. To examine the effect of the fermented diet on the gut flora, faecal samples were analyzed. The fermented diet changed the composition of the gut flora significantly. In the group that received the fermented diet the number of lactobacilli and the mesophylic aerobic count increased and the number of Enterobacteriaceae and enterococci decreased compared with the control group. In addition, the prevalence of salmonella decreased in the groups of mink fed the fermented diet. It is speculated that these beneficial effects on the gut flora could probably also be achieved in other nonruminant animals.


Proteolysis and amino acid breakdown of heated and irradiated poultry byproducts and muscle tissue

H.A.P. Urlings, N.G. Fransen, P.G.H. Bijker, J.G. van Logtestijn

As a result of intensification and centralization of poultry slaughtering, the amount of slaughter byproducts produced at a single location is increasing. These byproducts are rich in protein, fat, and vitamins and, therefore, constitute a potentially useful raw material for use as animal feed. To maintain the nutritive value of these byproducts they should be processed to minimize or eliminate degenerative changes that reduce the feed value of the product. In this paper amino acid breakdown in slaughter-fresh poultry viscera, heads, and breast meat is studied as a model. Initial amino acid breakdown in viscera was observed (also when bacterial growth was excluded by γ-irradiation), which resulted in high levels of total volatile N and cadaverine. Putrescine was produced only in viscera after bacterial proliferation. In heads and breast meat, no production of metabolites of amino acid degradation was observed as a result of initial enzymatic activity. It is concluded that during preservation of poultry byproducts not only bacterial proliferation, but also enzymatic breakdown of amino acids, must be prevented.


Evidence for a pancreatic lipase subfamily with new kinetic properties

Kenneth Tierstrup, Robert Verger, Frédéric Carrière

Several new members of the pancreatic lipase family have been reported recently, and amino acid sequence comparison reveals that this family can now be divided into three subgroups: (1) "classical" pancreatic lipases, (2) related proteins 1 (RP1), and (3) related proteins 2 (RP2) (Giller, T., et al. (1992) J. Biol. Chem. 267(23), 16509-16516).
Whereas "classical" pancreatic lipases are well characterized with respect to kinetic properties, i.e., interfacial activation and dependence on colipase in the presence of bile salts, the two latter subfamilies have been poorly investigated so far. The kinetic behaviour of a lipase from guinea pig pancreas differs, however, from that of "classical" lipases (Hjort, A. et al. (1993) Biochemistry 32, 4702-4707). This enzyme is highly homologous to RP2 lipases with the exception of a deletion in the so-called lid domain that regulates access to the active center of pancreatic lipases. We have now characterized a novel lipase from coypu (Myocastor coypus) pancreas. This enzyme, also belonging to the RP2 subfamily, possessed a full-length lid domain, but its kinetic properties are very similar to those of the guinea pig enzyme: (1) a high phospholipase activity, (2) the absence of interfacial activation, and (2) the absence of a colipase effect at high bile salt concentrations. Since both guinea pig and coypu pancreas produce a classical pancreatic lipase and no measurable phospholipase A2 activity, it is suggested that RP2 enzymes act as real phospholipases under physiological conditions. In fact, all RP2 lipases from other species might share phospholipase activity and fulfil new biological functions.


Organ distribution of vitamins A and E in carnivores

F.J. Schweigert, E. Thomann

Canides transport vitamin A in the blood not only as retinol but predominantly as retinyl esters bound to lipoproteins. With regard to the tissue distribution extremely high levels of vitamin A were observed in the kidney of canides. In the fox and the raccoon dog kidney levels were markedly higher (1066 and 1259 µg/g, respectively) compared to levels found in the liver (8 and 142 µg/g, respectively). This is discussed to be related to the excretion of vitamin A with the urine in these species. In other tissues vitamin A levels were only slightly higher compared to other species. With regard to vitamin E, the highest levels in canides were as well observed in the kidney, followed by the liver and adipose tissue.


Effect of protein and fat content in feed on plasma alanine-aminotransferase and hepatic fatty infiltration in mink

B.M. Damgaard, T.N. Clausen, P. Henriksen

Fig. 1. ALAT (U/l) in plasma in July (P>0.05), August (P<0.001), September (P<0.001), October (P<0.001), and December (P<0.001). Values are means and standard deviations.

The effect of the fat content in the feed on the development of fatty infiltration of the liver in the period from weaning until pelting was measured in two groups of male scanblack mink (Mustela vison) fed 20% and 45%, respectively, of metabolizable energy (ME) from protein. Furthermore, plasma activity of alanine-aminotransferase and the content of specifically chosen clinical-
chemical variables in the blood were measured. At pelting time in December, the liver weights were absolutely and relatively heavier in relation to body weight and had a considerably higher fat content at 20% of ME from protein than at 45% of ME from protein. From August to pelting time, the activity of alanine-aminotransferase in plasma was higher at a low protein level than at a higher protein level in the feed. It is concluded that the content of protein and fat in the feed affects the incidence of hepatic fatty infiltration in mink. In the growth period, it is possible, based on plasma activity of alanine-aminotransferase, to select animals with histological fatty infiltration of the liver.


Vitamins in the formation of hair cover (mink)

In mink that had lost fur there was a slight sign of niacin deficiency. Feeding the mink on a feed mixture supplemented with a vitamin preparation containing thiamin, riboflavin, vitamin C, Benfor-thiamin and Biometek M corrected the disorder in the vitamin metabolism by increasing the blood concentrations of total thiamin and its coenzyme, thiamin diphosphatase 11 and 38%, respectively, and decreasing transketolase reaction by 11%. Fur growth was restored after correcting the vitamin deficiencies.


Paprin autolysate
G.S. Taranov, A.S. Fedoseev

Mink in 4 groups were fed a basal diet with or without paprin autolysate 1.7, 3.4 or 5.1 g/100 kcal diet. In its chemical composition, Paprin autolysate did not differ from natural Paprin. At the end of the period, average liveweight of males and females was 2080 and 1160, 2000 and 1196, 2000 and 1170, and 2080 and 1160 g, respectively. Of the pelt produced, 52.2, 55.5, 44.3 and 44.3% was particularly thick. It was concluded that paprin autolysate can be added to the diet for rearing mink to provide up to 30% of the dietary digestible protein.


Various feed levels for mink during reproduction
D. Mertin, K. Súvegova, E. Oravcova

We studied the effects of various feed levels (flushing) on the reproduction parameters of primiparous female mink. A trial was performed in the Division of Fur Animal Rearing of the Research Institute of Animal Production in Nitra under experimental conditions. The trial involved 26 standard female mink (13 experimental and 13 control). The trial II was conducted in the field under practical conditions on the Farm of Fur Animals at Stará Myjava. The trial involved 197 standard female mink (98 experimental and 99 control mink). The feed ration was identical in both groups as regards energy and quantity. Its reduction started in the experimental groups in February. Between February 19-28, the feed ration was at a minimum. The experimental females were fed ad libitum in both trials from the beginning of March. Mating started on March 4 by the system 1-7-8 and 1-7. Live-born animals were recorded up to three days. In addition to reproduction parameters we also studied live weight. Live weight was balanced in the experimental conditions in both groups. A significant difference was found in live weight close to the time of mating (P≤ 0.01). In our experiments we found a positive effect of flushing on the reproduction parameters of mink. We recorded a significant increase in the number of liveborn animals in the experimental group per experimental (difference of 2.54 kits) as per kittened female (difference of 0.94 kits). In the practical conditions we recorded a tendency towards an increasing number of kits in the experimental groups in both cases.

**Natural minerals in diets for mink**

A.F. Kuznetsov, N.V. Mukhina, I.V. Barsov, A.A. Kuznetsov

Vermiculite, added to a diet given to mink, increased their natural resistance to viral infection, decreased feed toxicity in the digestive tract due to its absorption activity and decreased, or prevented, the incidence of hepatosis. Vermiculite decreased feed cost per unit production and feed waste.


**Bakterin-SL and feed supplements**

G.D. Katsy, T.M. Stoikevich, Yu.A. Manannikov, S.A. Kashchenko

Bakterin-SL, a dried bacilli preparation, given to mink during the suckling period and again in August, acted as a therapeutic and a preventive, stimulating growth, increasing body resistance to infection and favourably influencing the structure and condition of the fur.

In further feeding trials, a crystallized, water-insoluble mass of microorganisms, given as a part of the basal diet to provide up to 32% digestible protein, helped to reduce feeding costs of young mink without reducing the pelt quality.


**Efficiency of feeding Arctic foxes of different sizes**

N.A. Balakirev, E.M. Kollaeva, N.N. Loenko

Offspring of large or small pairs of Arctic foxes were separated into groups according to liveweight, sex, age, and size. They were fed diets containing digestible protein 7.5, 8.5, or 9.5 g/100 kcal metabolizable energy (ME), without or with ionol (an antioxidant) and an enzyme preparation, amino-subtilin, at 20 and 50-80 mg/100 kal ME, respectively. In autumn some foxes in the group given protein 9.5/100 kcal ME received 50% additional dry feeds. Young foxes from large parents had higher growth rates from 2 to 3 months old than did those from small parents, regardless of diet composition. Average pelt size for all groups was 23.5 cm².

Offspring from large parents had a slightly higher feed utilization efficiency based on unit area of pelt produced. Diets containing protein 7.5 and fat 5.2 g/100 kcal ME, compared with those with protein 8.5 or 9.5 g/100 kcal ME, produced pelts of average size 24.2 cm² and gave the least feed cost/unit of pelt size.


**Adsorbents in diets for mink**

N.A. Balakirev, V.S. Snytko, E.A. Larina, V.M. Naumova

In mink and Arctic foxes, zeolite added to the diet significantly increased body weight and pelt size and decreased the incidence of acute tymipnites and gastroenteritis.


**Levels of S1 (Silfen) and growth of young mink**

A.M. Bespalov, A.L. Kiselev

100 brown mink aged 180 days were divided into 5 groups and given feed without or with the antioxidant S1 (Silfen) at 0.005, 0.01, 0.02, or 0.04%.

Mean liveweight at 60, 120, and 180 days old was not significantly different among the groups. In blood serum there was no difference in total protein. Residual nitrogen and total lipids tended to be lower and phospholipids greater in the experimental groups than in the controls. Inclusion of antioxidant in the diets had a positive effect on albumins, transferrin, ceruloplasmin and globulin frac-
tions. In the liver intensity of lipid peroxidation was lower and vitamin E greater in the experimental groups. Best results were with 0.02% antioxidant.


Comparison of fibre digestion and digesta retention time between nutrias (Myocaster coypus) and guinea pigs (Cavia porcellus)

Ei Sakaguchi, Akemi Nabata

Fig. 3. Digestibilities of crude protein and metabolic body size (kg$^{0.75}$) in (a) leaf-eared mice, (b) hamsters, (c) degus, (d) rats, (e) guinea pigs, (f) rabbits, (g) nutrias and (h) maras.

(1) Digestibilities of feed, and transit and retention time of fluid and particle digesta marker were measured in nutrias (Myocaster coypus) and guinea pigs (Cavia porcellus) fed a diet containing 50% alfalfa.

(2) The digestibility of fibre was higher in the nutria, along with the longer retention time of digesta.

(3) The liquid and particle markers were similarly excreted, suggesting no separation mechanism in the gastrointestinal tracts of both the animals.

(4) The apparent digestibility of protein in the nutria was superior to the guinea pig and other small hindgut fermenters, suggesting that the contribution of coprophagy to protein nutrition of nutrias is significant.


Melakril for pole cats

N.E. Chanaev

Polecats were fed from 2 months old on a basal diet without or with Melakril (melatonin in a biodegradable polymer).

Average pelt size was 8.2 and 7.6 dm$^2$, respectively, and 45.6 and 51.2% of the pelts were defective.

Average body weight at 3 months old was 1726 and 1522 g. Melakril increased the digestibility of protein and fat.


Plant feeds in the diets for raccoon dogs

Yu.S. Zabolotskikh

Grass meal and Conifer meal added to the basal diet of raccoon dogs to provide 15 and 5% dietary metabolizable energy (ME), at the expense of animal protein, without and with the enzyme preparation Amilosubtilin had no adverse effect on blood composition and increased fertility.

It was recommended that during pregnancy the daily diet of raccoon dogs should include grass meal 5 to 10 and conifer meal up to 3 g/100 kcal ME, and after whelping the dogs should be given grass meal 10 to 30 g each daily, increasing the amount gradually to 80 g. The conifer meal was prepared from spruce or pine cut before the spring sap flow.

Evaluation of methods to certify the "premium" quality of Chilean fish meals

J.J. Romero, E. Castro, A.M. Díaz, M. Reveco, J. Zaldívar

Chile produces fish meals of excellent quality for which the international market pays premium prices. A series of experiments were conducted to define a methodology to certify this "premium" quality. Twenty-seven fish meal samples originating from 6 different plants and properly identified with respect to catch composition, lapses between catch and processing and chemical composition of prime materials and final product were submitted to the following tests: biogenic amines, available lysine, in vitro digestibility, in vivo protein digestibility in rainbow trout and a chick biototoxicological test. Also, 5 samples were analyzed in Norway for digestibility in mink, and several pertinent chemical analyses. Protein digestibility determined with rainbow trout, preceded by selected chemical analysis, appeared to be the most efficacious method. This procedure has had good repeatability and was well correlated with other indicators and with mink digestibility values. It can be organized as a routine procedure with reduced cost and time commitment. At least one third of the meals tested proved to be of superior quality.


Effects of supplemental dietary sodium chloride and restricted drinking water on mink


Thirty-six male mink were fed diets that contained 0, 1, 2, or 4% supplemental salt (sodium chloride) and were given drinking water ad libitum for 7 days. Three mink on each diet were then placed on ad libitum, 50% ad libitum or 25% ad libitum drinking water for the next 14 days. Ad libitum water consumption was directly proportional to the salt content of the diets. Feed consumption was inversely related to the level of dietary salt, although water restriction had a greater effect in reducing feed consumption than did the supplemental salt. The clinical signs of salt toxicity-water restriction observed were increased thirst, mild dehydration, decreased feed consumption, decreased body weight, rough coat, crusty nose and eyes, irritability in the early stage, and lethargy in the later stages. In general, serum and urinary sodium and chloride ion concentrations increased with increasing dietary salt concentrations. Expressed as a percent of brain weight, liver, spleen, kidney and heart weights of mink fed supplemental salt were less than the control weights. Adrenal gland weights increased in response to water restriction.

Brain sodium concentrations were not affected by salt supplementation when drinking water was provided ad libitum. However, restricting drinking water generally resulted in increased brain sodium concentrations. Mild to moderate micro- or macrovesicular vacuolar changes were observed in the livers of some mink fed each level of dietary salt, but were especially prominent in the mink restricted in drinking water.


Biotin deficiency in foxes

G. Salyi, V. Sztojkov

On a farm with 160 silver and 402 blue foxes the appetite of the foxes decreased gradually during a period of 1.5-2 months and the majority of animals showed mild or severe conjunctivitis. Dried discharge was found around the eyes and occasionally also in the angle of mouth.

The nostrils and pads were dry, swollen and cracked. Alopecia and discolouration of the fur developed as common signs partly due to long hairs falling out and partly due to depigmentation of the fur. After biotin treatment (0.5 mg/day/animal) the appetite improved rapidly.

The treatment was effective; animals with conjunctivitis and skin alterations recovered. As a result of the 2-month biotin treatment the long hairs grew again, but could not mature by the time of furring. In development of biotin deficiency the consumption of boiled eggs, rancid slaughterhouse by-pro-
ducts and a rabbit feed containing sulfonamides, as well as lack of feeding of vitamins was suspected.


High concentrations of vitamin A esters circulate primarily as retinyl stearate and are stored primarily as retinyl palmitate in ferret tissues

J.D. Ribaya-Mercado, M.C. Blanco, J.G. Fox, R.M. Russell

Objective and methodology: We determined the kinds and amounts of vitamin A compounds (retinol and various retinyl esters) circulating in serum and stored in liver and other selected tissues of ferrets, using high-performance liquid chromatography.

Results: The concentration of total retinyl esters in serum (43 ± 1 μmol/L, mean ± SEM) was 25 times greater than that of retinol (1.7 ± 0.2 μmol/L). In serum, 56% of retinyl esters was retinyl stearate, 33% was retinyl palmitate, and 5% was retinyl oleate. In contrast, in liver, vitamin A was stored primarily as retinyl palmitate (51%); smaller amounts of retinyl oleate (19%) and retinyl stearate (16%) were found. In kidneys, adrenals, small intestine, adipose tissue, skin, stomach, and eyes, retinyl palmitate was also the predominant retinyl ester, followed by retinyl stearate. In colon, lungs, and bladder, equal amounts of retinyl palmitate and retinyl stearate were observed. Other retinyl esters present in smaller amounts in most of these tissues were retinyl oleate, retinyl linoleate and/or -myristate, retinyl heptadecanoate, retinyl arachidonate, and retinyl laureate.

Conclusions: Thus, the primary form of vitamin A that circulates in the blood of ferrets is retinyl stearate, whereas the primary storage form of the vitamin in tissues is retinyl palmitate. Concentrations of total vitamin A in ferret serum and other tissues were 3-73 times greater than those reported for their corresponding human tissues.


Use of fresh brown Livex for feeding young raccoon dogs

S. Jarosz, B. Barabasz, O. Szeleszczuk, P. Gogol

The authors intended to determine the possibility of using Livex in feeding doses for young yenots as well as its effect on digestibility, the level of some physiological indices and production traits. Fresh brown Livex was introduced into the feed for yenots in gradually increasing quantities: 15, 30, and 45% as compared to the control. Investigations on digestibility showed a good digestibility of nutrients, especially protein (89.7 and 92%) in the groups containing an increased proportion of Livex. In these groups the authors also observed improved palatability and appetite. The results of the physiological investigations (haematology, transaminase activity) did not show deviations from the norms accepted as proper for this species. The addition of 45% Livex resulted in a slight decrease in the qualitative indices of hair cover which may suggest a negative effect of large quantities of Livex.

Distemper giant cell pneumonia in mink

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Summary

Acute distemper in mink has frequently been reported as a systemic infectious disease easily diagnosed by histopathological methods. Four mink with non-specific clinical histories were sent to our laboratory for post-mortem diagnosis. After histopathological examination the most important lesion identified was a giant cell pneumonia. A similar lesion has been described in measles, caused by a virus closely related with canine distemper virus (CDV). This condition allowed us to suspect a CDV infection in the studied animals, which was confirmed using immunohistochemical methods. The present paper describes the findings of that uncommon course of CDV infection in mink.

Introduction

Canine distemper (CD) is a systemic disease of carnivores. It is caused by a Morbillivirus closely related to measles, and rinderpest viruses. Distemper in mink was first reported by Shaw (1933). Depletion of lymphocytes, diffuse interstitial pneumonia, inflammation and/or demyelination of the central nervous system, and intracytoplasmic or intranuclear inclusion bodies are normally the most important pathology of sick animals (Appel, 1987). The disease is usually controlled in mink farms by attenuated live virus vaccination. Outbreaks of acute canine distemper in mink have been reported in Spain both in unvaccinated (Nieto et al., 1992) as well as in Aleutian disease virus-infected animals (Nieto et al., 1993). Infection was systemic with histopathological findings similar to the classic picture of CD in other species, such as dogs (Appel, 1987) and foxes (López-Peña et al., 1994). Measles giant cell pneumonia is an uncommon condition in humans infected by the measles virus (Enders et al., 1959). Defects in the immune system predispose to this condition which is frequently reported as associated with immunosuppressing diseases such as HIV-infection, leukaemia or lymphoma (Nadel et al., 1991; Harboldt et al., 1994; Hervas et al., 1990). A remarkable feature in its histology is the presence of multinucleated giant cells containing both intranuclear and intracytoplasmic inclusion bodies (Hervas et al., 1990). The objective of the present report is to describe lesions of mink with an atypical pattern of distemper. Distemper was suspected by the presence of syncytia in lung and lymphatic tissues and confirmed by immunohistochemistry.

Materials and methods

Four five to seven-month-old mink were sent to the Veterinary School of Lugo in the autumn of
1993 for post-mortem examination. The animals came from a farm where the mothers had been routinely vaccinated against distemper. The sick animals had not been vaccinated and were negative to the Contre-electro-immuno-phoresis test against Aleutian disease virus. Clinical signs were depression, loss of appetite and death. Samples of all organs were taken at the necropsy, fixed in 10% buffered formalin and embedded in paraffin. Three μm sections were stained with hematoxilin and eosin for histopathology. Immunolabelling of CDV in sections was made by means of the labelled streptavidin-biotin technique (LSAB kit, DAKO Corp., Carpinteria, USA) using a monoclonal antibody against the NP protein of CDV as the primary antibody (Dr. C. Örvell, Stockholm, Sweden). As positive controls we used sections taken from a dog with canine distemper; as negative controls we employed sections of an unvaccinated mink with no clinical signs of disease.

Results

Pulmonary congestion and oedema but no other significant findings were seen at the necropsy. Histological examination of the lungs showed generalised congestion and oedema. Discrete foci of pneumonia with infiltrations of mononuclear cells and multinucleated giant cells were seen (fig. 1). Multinucleated giant cells had variable sizes and shapes, homogeneous eosinophilic cytoplasm and variable numbers of nuclei (fig. 2). No inclusion bodies were observed in either the bronchial or bronchiolar epithelium nor in the multinucleated giant cells.

In lymph nodes and spleen there was an obvious depletion of lymphocytes in secondary follicles. Multinucleated giant cells without inclusion bodies were present in these organs, too. These syncytia were located in subcapsular sinuses, cortical zone and medullar lymphatic cords.

Inclusion bodies were not found in the epithelia of any organ, and no lesions were seen in the central nervous system.

Canine distemper viral antigen was demonstrated in syncytial cells by immunohistochemistry in lung and lymph nodes as well as in macrophages and lymphocytes (fig. 3). In the lung, immunolabelling was concentrated in the areas where the damage was more severe. Epithelial and nervous cells showed no uptake of immunolabelling.
Discussion

Distemper in mink has been described as a systemic disease with lesions in the respiratory, nervous and lymphatic systems (Nieto et al., 1992). Our results present an atypical morphology and immunohistochemical pattern of CDV infection in mink, demonstrating a focal giant cell pneumonia, and syncytia formation in lymph nodes. The sick animals had not been vaccinated, although their mothers were immunised. Four-week-old kits born of vaccinated mothers are protected against canine distemper virus (Hansen and Lund, 1972). Maternal antibodies interfere with vaccination of kits until 11 weeks after birth. Thus, kits of vaccinated mothers cannot be vaccinated until this age (Hansen and Lund, 1972). In these cases, the animals were not protected against distemper because they were 5-7 months old and still unvaccinated. Syncytia in CDV infected animals have been reported as an aid to the diagnosis of distemper. They are found most frequently in the brain and anterior uvea of the eye and occasionally observed in lungs and lymph nodes (Summers and Appel, 1985). They appear in dogs 15 days postinfection (Summers and Appel, 1985). They apparently derive from the coalescence of hyperplastic alveolar cells or from the reticulo-endothelial system (Summers and Appel, 1985; Harboldt et al., 1994). Their formation is associated with the presence of a fusion factor within the envelope glycoproteins of CDV (Fisher and Bussell, 1977). However multinucleated giant cells in distemper are always found with other lesions such as diffuse interstitial pneumonia, encephalitis and inclusion bodies (Summers and Appel, 1985). The cases we report had no evident nervous lesions and inclusion bodies were not apparent. Moreover, the lungs showed a focal pneumonia which was different from the diffuse interstitial pattern described in typical acute canine distemper. The immunolabelling of CDV antigen in tissues is now accepted as a helpful method for the definitive diagnosis of distemper (Miry et al., 1983; Hewicker et al., 1990). In the present report CDV antigen was identified in the syncytia, macrophages and lymphocytes of the pneumatic areas and in mediastinal lymph nodes and spleen, whereas we did not observe it in other lymphoid or epithelial tissues. This supports the suggestion that the respiratory system provides the best environmental conditions for the multiplication of CDV (Gorham, 1960). A giant cell pneumonia caused by measles virus has been reported in humans (Enders et al., 1959). Histopathological lesions are variable and depend to some extent on the immune competence of the host, as well as on the duration of the disease. In immune compromised patients, classic giant cell pneumonitis, with readily demonstrable intranuclear inclusions is present. The lungs of non-immune compromised patients lacked the above pattern and displayed a spectrum of less specific findings ranging from organised diffuse alveolar damage to interstitial pneumonia with giant cells, but without viral inclusions (Radoycich et al., 1992). Aleutian disease virus (ADV) is the most important immunosuppressing agent in mink. The animals we studied were not known to be immune compromised and were negative to ADV. Moreover we were unable to find inclusion bodies in the nucleus or cytoplasm of giant cells. This fits the descriptions in measles infected, but not immunocompromised humans (Radoycich, 1992). There are reported differences in the strains of CDV with regard to their biological behaviour, although only one serotype is presently recognised (Reculard and Guillot, 1972; Summers et al., 1984). It may be possible that the atypical distemper we have reported here is due to a variant strain of CDV. Unfortunately, no virus isolation was performed.

In conclusion, the pathology of the CDV infection in the mink we have described consists of a focal giant cell pneumonia with close similarities to that described in human measles virus infection. The presence of syncytia was an important indication of distemper, the diagnosis of which was confirmed by immunolabelling.

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References


The evaluation of Ipowet 5 and Ipowet aerosole (bromphenvinfos) effectiveness against external parasites (Sarcoptes scabiei v. canis and Chaetopsylla globiceps) in polar foxes

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Summary

The aim of the investigation was to evaluate Ipowet 5 and Ipowet aerosole effectiveness against external parasites of polar foxes: itch mite - Sarcoptes scabiei v. canis and flea - Chaetopsylla globiceps.

A high effectiveness of a 0.5-1% aqueous emulsion of Ipowet 5 applied 5 times against scabies was observed.
Ipowet 5 applied twice in the form of a 0.2% aqueous solution and Ipowet aerosole completely prevent polar fox flea infection.

Introduction

A phosphororganic compound from the enolophosphates group - bromphenvinfos - originally synthesized in Poland was subjected to detailed toxicological analysis and metabolic investigations. These investigations allowed its further application in the therapeutic forms against external parasites of animals.

It had a high therapeutic effectiveness in controlling warbles (Hypoderma bovis) in the autumn and spring (Melophagus vinus and Bovicola ovis), pediculosis (Haematophinus suis) and sarcoptic mange (Sarcoptes scabiei v. suis) in swine.

Ipowet 5 and Ipowet aerosole had already been used in domestic animals to control fleas (Ctenocephalides canis and Ctenocephalides felis) in dogs and cats, sarcoptic mange (Sarcoptes scabiei v. canis) in dogs, Demodex canis in dogs and ticks (Dermacentor pictus and Ixodes ricinus) in dogs.

No effects of bromphenvinfos in the therapeutic doses on the histoenzymatic reactions were observed. It did not significantly cause any anathomopathologic and histopathologic changes in the organs and tissues of animals.

The results of the investigations of the residues of Ipowet 5 and Ipowet 25, preparations based on bromphenvinfos, in sheep tissues, were used to establish the withdrawal period which amounts to 7 days (Sciesinski, 1994).
The residues in the tissues of young cattle after the application of Ipowet 5 (brompheninfos) in the therapeutic doses against external parasites were not high which allowed us to establish the withdrawal period as 7 days (Juszkiewicz et al., 1975; Kroczynska, Kroczynski, 1981).

The withdrawal period (after the brompheninfos application) in milk was established as one day (Kroczynska, Kroczynski, 1981; Sciesinski, 1994). Brompheninfos is quite quickly eliminated from the tissues of pigs and thus no withdrawal period is suggested after the application of Ipowet 5 and Ipowet 25 (Kroczynska, Kroczynski, 1981; Sciesinski, 1978).

No histomorphologic nor histoenzymatic changes were observed in animals which were treated with similar doses when controlling external parasites (Sciesinski, 1994).

Invasions of external parasites cause great economic losses in foxes resulting from smaller body weight gains and poorer pelt quality.

The aim of the work was to investigate brompheninfos in its therapeutic forms against external parasites in polar foxes.

Material and methods

The effectiveness of Ipowet 5 and Ipowet aerosole was evaluated in relation to the following external parasites in polar foxes; itch mite, Sarcoptes scabiei v. canis, and flea, Chaetopsylla globicieps. Sarcoptic mange was observed in places which are predilective for that parasite, i.e. on the head, in the ear region and in some animals also on their sides.

The identification of the itch mite was performed using the method of potassium lye described by Stefanski, and Zarnowski (1968a) and Stefanski (1989b). Fleas were observed on foxes in numbers from a few to several dozen on one animal.

The evaluation was performed on 82 polar foxes aged from 8 months to 3 years. The animals originated from private and cooperative farms.

Results and discussion

The insecticide effectiveness of 0.5-1% Ipowet 5 aqueous emulsion was tested against sarcoptic mange in polar foxes. Five times repeated rubbing with 0.5-1% solution completely prevents sarcoptic mange (see Table 1, page 321).

The twice repeated application of 0.2% Ipowet 5 aqueous emulsion prevent flea infection (Table 1). The aerosole form of brompheninfos, Ipowet aerosole, was used in polar foxes against fleas.

After a single spraying the effectiveness amounted to 90% and after the twice repeated procedure to 100%. No side effects were observed in the treated animals after the application of the preparations.

Conclusions

1. Brompheninfos - Ipowet 5 - shows high effectiveness against itch mite in polar foxes.
2. Ipowet 5 and Ipowet aerosole completely prevents flea infection.

References

Stefanski, W. 1968b. parazytologia weterynaryjna PWRiL, Warszawa.
Table 1: External parasite control in polar foxes using Ipowet 5 and Ipowet aerosole (bromphenvinfos)

<table>
<thead>
<tr>
<th>Treated animals</th>
<th>Applied preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite species</td>
<td>Number of animals</td>
</tr>
<tr>
<td>Sarcoptes scabiei v. canis</td>
<td>8</td>
</tr>
<tr>
<td>Sarcoptes scabiei v. canis</td>
<td>6</td>
</tr>
<tr>
<td>Chaetopsylla globiceps</td>
<td>20</td>
</tr>
<tr>
<td>Chaetopsylla globiceps</td>
<td>18</td>
</tr>
<tr>
<td>Chaetopsylla globiceps</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 1 continued

<table>
<thead>
<tr>
<th>Applied preparations</th>
<th>Number of animals</th>
<th>Number of applications</th>
<th>Results of treatment</th>
<th>Average % effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose mg/kg b.w.</td>
<td>Method of application</td>
<td>Number of applications</td>
<td>Number of animals</td>
<td>Completely cured</td>
</tr>
<tr>
<td>7.5</td>
<td>rubbing</td>
<td>5 times every 3 days</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>rubbing</td>
<td>5 times every 3 days</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>spraying</td>
<td>once</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>spraying</td>
<td>twice every 10 days</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>spraying</td>
<td>twice every 10 days</td>
<td>30</td>
<td>-</td>
</tr>
</tbody>
</table>
Comparative molecular analysis of plasmid-encoded tetracycline resistance genes in Staphylococcus lentus from mink and pigeons

Stefan Schwarz, Christiane Werckenthin

A total of 39 Staphylococcus lentus isolates - 18 from mink and 21 from pigeons - were investigated for their resistance to antibiotics of the tetracycline family.

Resistance to tetracycline, but not to minocycline (Tc'Mn'-phenotype) was observed in 2 (11.1%) of the mink strains and in 6 (28.6%) of the pigeon strains. Combined resistance to tetracycline and minocycline (Tc'Mn'-phenotype) could not be detected in the mink strains, whereas 2 (9.5%) of the S. lentus isolates from pigeons exhibited this type of tetracycline resistance. The resistance genes associated with the Tc'Mn'-phenotype were shown to be located in 8 S. lentus isolates on small plasmids ranging in size between 4.5 and 7.5 kbp. Restriction endonuclease mapping enabled a differentiation of these phenotypically homologous plasmids from S. lentus to belong to two families of staphylococcal Tc' plasmids. Subsequent hybridization experiments with specific gene probes identified 7 of the 8 Tc' plasmids from S. lentus to carry the staphylococcal resistance gene tet(K). The remaining plasmid carried a tet(L) gene which is commonly found in bacilli. The two minocycline-resistant S. lentus strains from pigeons possessed the chromosomally-encoded resistance gene tet(M) which had been identified to be a part of streptococcal and enterococcal transposons.


Macrolide-lincosamide resistance in Staphylococcus lentus from mink and pigeons

Stefan Schwarz, Christiane Werckenthin, Erika Nussbeck, Heidrun Meyer

So far, resistance to macrolide- and lincosamide antibiotics has been described in strains of Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus intermedius and Staphylococcus hyicus. The respective resistance genes (erm) were shown to be located on small plasmids (ermC) or to be part of chromosomally integrated transposons (ermA, ermB). Up to now, very little is known about macrolide-lincosamide resistance (ML') in Staphylococcus lentus.

Resistance to macrolide-lincosamide antibiotics occurred in 3 (16.7%) of 18 S. lentus isolates from mink and in 7 (53.8%) of 13 S. lentus isolates from pigeons as revealed by the agar diffusion test. Plasmids encoding ML' could be identified by protoplast transformation in 2 of the 3 mink strains and in 2 of the 7 pigeon strains. Both ML' plasmids from the pigeon strains exhibited sizes of 2.4 kbp and resembled in their restriction maps the small ermC-encoding plasmids previously isolated from other staphylococcal species of human and animal origin. However, one of these plasmids specified inducible resistance, while the other plasmid conferred constitutive resistance to ML-antibiotics. With their sizes of 3.9 and 8.3 kbp, the two plasmids from the mink strains were larger than ML' plasmids commonly found in staphylococci. On the basis of their restriction maps, they exhibited no homology to one another, nor to previously described staphylococcal ML' plasmids. Both ML' plasmids of S. lentus from mink mediated constitutive resistance to ML-antibiotics.


Characterization of chloramphenicol resistance genes in uropathogenic Staphylococci from mink

Stefan Schwarz, Silke Dieckmann

Staphylococcal urinary tract infections are among the major causes of losses in mink. So far, little is known about the staphylococcal species associated with these infections and their resistance to antibiotics. In this connection, the antibiotic resistance of staphylococci isolated from mink and from their environment was investigated. A total of 75% of
the staphylococci from mink and 64% of the staphylococci from environmental sources were identified biochemically to belong to the species *Staphylococcus lentus*. These *S. lentus* isolates proved to be more resistant to antibiotics than isolates of *S. xylosus* and *S. intermedius* also found in the samples. Only *S. lentus* isolates exhibited resistance to the antibiotics chloramphenicol and neomycin which had been extensively applied. Among the *S. lentus* isolates, those from the mink were more often resistant to ampicillin, chloramphenicol, kanamycin, neomycin and streptomycin; they also demonstrated multiple resistance at a higher frequency than the *S. lentus* isolates from the environment. Further analysis of chloramphenicol resistance (Cmr), observed in 78% of the *S. lentus* isolates, revealed that the respective resistance genes were located on small multicopy plasmids ranging in size from 3-6 to 4.6 kbp. Restriction endonuclease mapping and Southern blot hybridization with specific gene probes allowed the differentiation of four different Cmr plasmid types.

**Molecular cloning of a mink prion protein gene**

H.A. Kretzschmar, M. Neumann, G. Riethmüller, S.B. Prusiner

Transmissible mink encephalopathy (TME) is a rare disease which is presumably transmitted to ranch-raised mink from scrapie-infected sheep orf and bovine spongiform encephalopathy-infected cattle products. Although the infectious agent of TME has not been isolated, there is circumstantial evidence that TME is caused by prions. The experimental host range of TME includes sheep, cattle, monkeys and hamsters. However, TME has never been transmitted to mice. Since experiments in transgenic animals have shown that the prion protein (PrP) gene modulates the susceptibility, incubation time and neuropathology of prion-induced disease, we have started to analyse the mink PrP gene. PrP as deduced from a genomic DNA sequence consists of 257 amino acids and overall shows similarity of 84 to 90% with the sequences of the PrPs of other mammalian species. It remains to be determined whether these differences in the primary structure of PrP will explain the peculiar host range of TME.


**Role of alveolar type II cells and of surfactant-associated protein C mRNA levels in the pathogenesis of respiratory distress in mink kits infected with Aleutian mink disease parvovirus**

Birgitte Viuff, Bent Aasted, Søren Alexandersen

Neonatal mink kits infected with Aleutian mink disease parvovirus (ADV) develop an acute interstitial pneumonia with clinical symptoms and pathological lesions that resemble those seen in preterm human infants with respiratory distress syndrome and in human adults with adult respiratory distress syndrome. We have previously suggested that ADV replicates in the alveolar type II epithelial cells of the lung. By using double in situ hybridization, with the simultaneous use of a probe to detect ADV replication and a probe to demonstrate alveolar type II cells, we now confirm this hypothesis. Furthermore, Northern (RNA) blot hybridi-
zation showed that the infection caused a significant decrease of surfactant-associated protein C mRNA produced by the alveolar type II cells. We therefore suggest that the severe clinical symptoms and pathological changes characterized by hyaline membrane formation observed in ADV-infected mink kits are caused by a dysfunction of alveolar surfactant similar to that observed in respiratory distress syndrome in preterm infants. However, in the infected mink kits the dysfunction is due to the replication of ADV in the lungs, whereas the dysfunction of surfactant in preterm infants is due to lung immaturity.


Expression of Aleutian mink disease parvovirus capsid proteins in a baculovirus expression system for potential diagnostic use

Wai-Hong Wu, Marshall E. Bloom, Bradley D. Berry, Michael J. McGinley, Kenneth B. Platt

A 2.3-kb cDNA clone encoding Aleutian mink disease parvovirus (ADV) structural proteins VP1 and VP2 was inserted into the polyhedron gene of autographa californica nuclear polyhedrosis virus (AcNPV) and expressed by the recombinant virus, AcADV-1, in Spodoptera frugiperda-9 cells. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and western immunoblot analysis (WIA) indicated that synthesis of both VP1 and VP2 was being directed by AcADV-1. Fluorescence microscopic examination of AcADV-1-infected S. frugiperda-9 cells indicated that the recombinant protein was present within the nucleus of the cells, and electron microscopic examination of these cells revealed the presence of small particles 23-25 nm in diameter.

Structures resembling empty ADV capsids could be purified on CsCl density gradients, thus indicating that the ADV proteins were self-assembling. The antigenicity of recombinant VP1 and VP2 was evaluated by WIA. Sera collected from 16 mink prior to infection with ADV did not react with VP1 and VP2. Ten sera collected from mink with counter current immunoelectrophoresis (CIE) titers greater than 4 log2 reacted with VP1 and VP2 in WIA. Two of 6 sera with CIE titers of 4 and 1 of 14 sera with CIE titers <4 reacted with the recombinant proteins. These results suggest that baculovirus recombinant ADV capsid proteins may be useful as diagnostic antigens.


Sequence comparison of the non-structural genes of four different types of Aleutian mink disease parvovirus indicates an unusual degree of variability

E. Gottschalk, S. Alexandersen, T. Storgaard, M.E. Bloom, B. Aasted

The present work shows that at least four different sequence types of Aleutian mink disease parvovirus (ADV) are present in ADV isolates from mink. We here report the nucleotide sequences of these four types of ADV from nucleotide 123 to 2208 (map unit 3 to 46). This part of the genome encodes three non-structural (NS) proteins of ADV. Comparison of the deduced amino acid sequences of these NS proteins showed that the ADV proteins are much less conserved than the NS proteins from other members of the autonomous group of parvoviruses.

In general, we found that the middle region of the ADV NS-1 protein was relatively well conserved among the types, while both the amino- and carboxy-terminal ends of the protein had higher amino acid variability. Interestingly, the putative NS-3 protein from type 3 ADV is truncated in the carboxy-terminal end.

The molecular evolutionary relationship among the four types of ADV was examined. This analysis, taken together with the unusually high degree of variability of the ADV types, indicates that the ADV infection in mink is likely to be an old infection compared to the other parvovirus infections or, alternatively, that ADV accumulates sequence changes much faster than other parvoviruses.

Transmissible mink encephalopathy species barrier effect between ferret and mink: PrP gene and protein analysis

Jason C. Bartz, Debbit I. Mckenzie, Richard A. Bessen, Richard F. March, Judd M. Aiken

Experimental infection of transmissible mink encephalopathy (TME) in two closely related mustelids, black ferret (Mustela putorius furo) and mink (Mustela visa), revealed differences in their susceptibility to the TME agent. When challenged with the Stetsonville TME agent, a longer incubation period was observed in ferrets (28 to 38 months) than mink (4 months). Western blot analysis of ferret and mink prion proteins (PrP) demonstrated no detectable differences between the proteins. Northern blot analysis of ferret brain RNA indicated that PrP mRNA abundance is similar in infected and uninfected individuals. We amplified the PrP coding region from ferret DNA using the polymerase chain reaction and compared the deduced amino acid sequence of the ferret PrP gene with the mink PrP gene. This comparison revealed six silent base changes and two amino acid changes between mink and ferret: Phe → Lys at codon 179 and Arg → Gln at codon 224, respectively. These changes may indicate the region of PrP that is responsible for the species barrier effect between mink and ferret.


Pathogenesis of Aleutian mink disease parvovirus infection: Effects of suppression of antibody response on viral mRNA levels and on development of acute disease

Søren Alexandersen, Torben Storgaard, Nerry Kamstrup, Bent Aasted, David D. Porter

We suppressed the B-cell development and antibody response in mink by using treatment with polyclonal anti-immunoglobulin M (anti-IgM) to study the effects of antiviral antibodies on development of Aleutian mink disease parvovirus (ADV)-induced disease in more detail. Newborn mink kits were injected intraperitoneally with 1 mg of either anti-IgM or a control preparation three times a week for 30 to 34 days. At 21 days after birth, groups of mink kits were infected with the highly virulent United isolate of ADV. At selected time points, i.e., postinfection days 9, 13, 29, and 200, randomly chosen mink kits were sacrificed, and blood and tissues were collected for analyses. The efficacy of immunosuppressive treatment was monitored by electrophoretic techniques and flow cytometry. Effects of treatment on viral replication, on viral mRNA levels, and on development of acute or chronic disease were determined by histopathological, immunoelectrophoretic, and molecular hybridization techniques. Several interesting findings emerged from these studies. First, antiviral antibodies decreased ADV mRNA levels more than DNA replication. Second, suppression of B-cell development and antibody response in mink kits infected at 21 days of age resulted in production of viral inclusion bodies in alveolar type II cells. Some of these kits showed mild clinical signs of respiratory disease, and one kit died of respiratory distress; however, clinical signs were seen only after release of immunosuppression, suggesting that the production of antiviral antibodies, in combination with the massive amounts of free viral antigen present, somehow is involved in the induction of respiratory distress. It is suggested that the antiviral antibody response observed in mink older than approximately 14 days primarily, by a yet unknown mechanism, decreases ADV mRNA levels which, if severe enough, results in restricted levels of DNA replication and virion production. Furthermore, such a restricted ADV infection at low levels paves the way for a persistent infection leading to immunologically mediated disease. The potential mechanisms of antibody-mediated restriction of viral mRNA levels and mechanisms of disease induction are discussed.


Aleutian disease in the ferret

J.D. Stewart, N. Rozengurt

An outbreak of Aleutian disease in a colony of ferrets used for biomedical research which began in December 1992 in a research institute in London, UK, is reported. Three animals were clinically
affected with signs of posterior ataxia and incoordination and 12 (including the 3 affected animals) of 20 animals tested were positive for Aleutian disease. PM examination of one of the clinically affected animals detected histological signs typical of the disease. This is the first report of the disease in ferrets used for biomedical research in the UK.


Aleutian mink disease parvovirus infection of mink macrophages and human macrophage cell line U937: Demonstration of antibody-dependent enhancement of infection

Hiroyuki Kanno, James B. Wolfinbarger, Marshall E. Bloom

Aleutian mink disease parvovirus (ADV) infects macrophages in adult mink. The virulent ADV-Utah I strain, but not the cell culture-adapted ADV-G strain, infects mink peritoneal macrophage cultures and the human macrophage cell line U937 in vitro. However, preincubation of ADV-G with ADV-infected mink serum enhanced its infectivity for U937 cells.

The enhancing activity was present in the protein A-binding immunoglobulin G fraction in the serum, but F(ab')2 fragments failed to enhance the infection. On the other hand, the same sera inhibited ADV-G infection of Crandell feline kidney (CRFK) cells. Although U937 cells were not fully permissive for antibody-enhanced ADV-G infection, ADV mRNA expression, genome amplification, and protein expression were identical to those found previously for ADV-Utah I infection of U937 cells. Preincubation of ADV-Utah I with soluble protein A partly inhibited the infection of U937 cells but did not affect infection of CRFK cells.

In mink peritoneal macrophages, preincubation with the infected mink serum did not make ADV-G infections. However, the infectivity for mink macrophages of antibody-free ADV-Utah I prepared from the lungs of infected newborn mink kits was enhanced by ADV-infected mink serum. Moreover, protein A partly blocked ADV-Utah I infection of mink macrophage cultures. These results suggested that ADV-Utah I enters mink macrophages and U937 cells via an Fc receptor-mediated mechanism. This mechanism, antibody-dependent enhancement, may also contribute to ADV infection in vivo. Furthermore, since ADV infection in mink is characterized by overproduction of anti-ADV immunoglobulins, antibody-dependent enhancement may play a critical role in the establishment of persistent infection with ADV in vivo.


Production of mink enteritis parvovirus empty capsids by expression in a baculovirus vector system: a recombinant vaccine for mink enteritis parvovirus in mink

Jesper Christensen, Søren Alexandersen, Buchardt Bloch, Bent Aasted, Åse Utenthal

The VP-2 gene of mink enteritis parvovirus (MEV) was amplified by the polymerase chain reaction using MEV DNA isolated from the faeces of a naturally infected mink. Subsequently the VP-2 gene was cloned into a baculovirus expression vector. Recombinant baculoviruses were isolated and the MEV VP-2 gene product was characterized after expression in Sf9 insect cells. The MEV VP-2 product had the same size as that reported for the wild-type MEV VP-2 protein and was recognized by convalescent sera from MEV-infected mink and a panel of monoclonal antibodies reactive to MEV. Furthermore, the VP-2 protein was able to form parvovirus-like particles, which had haemagglutinating properties comparable with the wild-type MEV. The cloned VP-2 gene was sequenced and only five nucleotide differences were found after alignment with the known sequences of the MEV type 1 and type 2 isolates.

Surprisingly, the VP-2 gene encoded a valine and a tyrosine at amino acid positions 232 and 234, identical to the situation found in MEV type 1, but at position 300 there was a valine which is a determinant of MEV type 2. Immunization of mink with approximately 40000 haemagglutinating units of
recombinant MEV VP-2 induced a measurable antibody response as tested by haemagglutination inhibition. Furthermore, the immunized mink did not excrete virus and did not develop clinical disease upon challenge with a virulent isolate of MEV.


Modernization of serotyping methods for Pasteurella multocida in nutria

R.A. Kanymov, E.M. Agaeva

The first high-molecular pasteurella fractions, found by gel-chromatography, possessed the typical specificity. In epizootic pasteurella strain typing, isolated from nutria, was found that the acute disease form induced type B; and the chronic disease, types B and D.


Diagnosis and therapy of colibacillosis in blue foxes

Zhu Qitai, Yang Xianjin, Wang Weizhi

A disease broke out in a blue fox farm located in Lianyungang city in April 1991. It was characterized by diarrhoea, septicaemia and purulent uteritis-vaginitis abortion in female foxes. 15 female foxes were affected and 7 died. The mortality rate was 46.67%. Several isolates were cultured from uterus, gastrointestinal, spleen and facial samples. They were identified by standard biochemical tests. The serotypes were O₁₆,K₂₂α₁₈ (ETEC), O₂₂:K₁₉ (ETEC), O₂₆:K₆₀ (ETEC). The disease was controlled by I.M. gentamycin 800 mg per day per fox.


Electron-microscopical observations of kidneys of coypu experimentally infected with Escherichia coli

P.A. Mkhargrdzeli

The nephrocytes can serve as a suitable model for studies of common regularities of experimental coli-bacteriosis courses in coypus.


Evaluation of serum estradiol concentrations in alopecic ferrets with adrenal gland tumors

Robert A. Wagner, David P. Dorn

Fig. 1. Typical appearance of the dorsal, bilaterally symmetric alopecia in the tail and tail-base region of a ferret with an adrenal gland tumor
Seventeen ferrets were examined because of progressive bilaterally symmetric alopecia that was nonpruritic. Dermatologic and endocrinologic testing were used to determine the cause of the alopecia. Resting cortisol, testosterone, and thyroxin concentrations and results of ACTH stimulation tests were found to be within reference range limits established for this species. High serum estradiol concentrations were found to be a reliable indicator of adrenal cortical neoplasia in these ferrets.


*Mesocestoides canislagopodis* (Rudolphi, 1810) (Krabbe, 1865) (Cestoda: Mesocestoididae) from Arctic foxes, *Alopex lagopus* (L.) in Iceland re-described

B. Loos-Frank, K. Skinnison, M. Eydal

![Fig. 3](image)

*Mesocestoides canislagopodis* is re-described and figured from the intestine of *Alopex lagopus* from Iceland. The parasite was found to be the most common helminth in the intestines of 50 *A. lagopus* caught in the western and northern parts of Iceland in 1986 and 1987. Prevalence of infection was 72%. Between 1 and 6000 worms were recovered from infected hosts with a mean intensity of 502. Nine foxes harboured more than 500 worms and 6 foxes (1 to 11 years old, mean = 4 years) carried more than 1000 worms. 92% of foxes 2 years old and over were infected, but only 36% of puppies and yearlings were infected. Most of the worms were found in the small intestine, but occasionally they were recovered from the colon.


**Adrenal neoplasia in seven ferrets**

L. Neuwirth, R. Isaza, J. Bellah, N. Ackerman, B. Collins

Medical records from 7 ferrets presented to the VMTH with histologically confirmed adrenal neoplasia were reviewed. Three neutered female ferrets had adrenal cortical adenoma; four ferrets (2 neutered females, 2 neutered males) had adrenal cortical carcinoma. Ultrasound identified unilateral enlargement or abnormal shape of the adrenal gland in all ferrets. Only 1 ferret had adrenomegaly on abdominal radiographs. Adrenomegaly was identified in 1 ferret by magnetic resonance imaging (MRI). All ferrets were treated by adrenalectomy followed by a tapered dose of prednisone. Surgical complications were limited to fatal hemorrhage from the caudal vena cava in 1 ferret. Remission of clinical signs occurred in all 6 ferrets which survived surgery. The time of follow up varied from 3 to 16 months. The 3 ferrets with adrenal adenomas were still alive 3 to 7 months after surgery. All 3 ferrets with adrenal carcinoma developed metastasis and were euthanized from 2 to 16 months after surgery.

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INTRODUCTION

The VIth International Scientific Congress in Fur Animal Production will be held in Warszawa, Poland, August 21 to 23.

The Congress is arranged by INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION (IFASA) and POLISH SOCIETY OF ANIMAL PRODUCTION.

ORGANIZING COMMITTEE
Prof. dr Grażyna Jeżewska – Chairman,
Andrzej Frindt, Jerzy Sławoń, Marian Brzozowski,
Danuta Dąbrowska, Ewa Krawczyk.

SCIENTIFIC COMMITTEE
Prof. dr Andrzej Frindt – Chairman,
Stanisław Jarosz, Stanisław Niedźwiadek, Marek Switonski, Andrzej Filistowicz, Grażyna Jeżewska,
Witold Scheuring.

SCIENTIFIC PROGRAMME
The scientific programme will consist of oral reports and posters. The papers will cover topics according to the 5 working groups of IFASA.
1. Breeding, reproduction and genetics.
3. Pathology and diseases.
5. Fur properties.

This invitation includes call for titles and abstracts prior to January 30, 1996.
The manuscripts are to be received in Warszawa prior to March 15, 1996.
Further information about the scientific programme will be announced later, together with instructions to the authors. There will be given invited papers during the Congress.

COUNCIL MEETING
The Council and the Board of IFASA will have their meeting on August 20, afternoon.

SOCIAL PROGRAMME
Reception will be given on August 21, evening.
There will be a sightseeing on August 21, afternoon.
Will be organized post congress tours.

REGISTRATION
Please fill in the preliminary registration form and return it immediately by post or fax.
PRELIMINARY PROGRAMME

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COSTS

The registration fee will be approx. 180,-USD for IFASA members and 220,-USD for non-members, accompanying persons - 90,-USD.

The participants can select accommodation in a range of hotels listed in Second Announcement, “C.B. International Ltd.” was able to negotiate substantially lower promotional prices for all participants.

INFORMATION

For all information related to the Congress please contact:

VIth IFASA Congress
c/o Marian Brzozowski
Polish Society of Animal Production
9 Kaliska street
02-316 Warszawa
tel/fax (4822) 22 17 23

VIth International Scientific Congress in Fur Animal Production

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Polish Society of Animal Production
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