

immediately postmortem, then all the fibres in the biceps brachii muscle went into rigor mortis in a stretched position. A recent study on only the mouse biceps brachii muscle that entered rigor mortis in a stretched position indicated a significant negative correlation between muscle fibre diameter and the length of the sarcomeres¹⁵. This observation was not confirmed by the present study on similarly treated skeletal muscles from the mouse and the turkey.

The results of this study demonstrate the importance of limb position in determining the length of sarcomeres and the diameter of fibres in rigor muscles. This is an important consideration in the application of information on the dimensions of muscle fibres to an understanding of the anatomical function of skeletal muscles and in studies on muscle growth and development.

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Ultrastructural and X-ray microprobe comparison of gerbil and human pineal acervuli

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Summary. Only a morphological difference exists between gerbil and the human pineal acervuli. Neither qualitative nor quantitative differences in the chemical composition between both gerbil and human brain sand have been found. The mineral of the gerbil pineal acervuli is, as in the human, also hydroxyapatite.

One of the characteristics of the pineal body of Mongolian gerbils is a constant presence of calcareous deposits (acervuli, corpora arenacea, concretions or brain sand) found in all animals 11 weeks old and more^{1,2}. From this point of view, the pineal body of the gerbil is very similar to

that of the adult human. The organic matrix of the gerbil acervuli is composed of a carbohydrate, probably an acid mucopolysaccharide, complexed to a protein¹. The periphery of some calcifications shows an acid phosphatase or an esterase activity¹. Stained with alizarin red, the gerbil concretions give a positive reaction indicating the presence of calcium salts². This light microscopic finding is in good agreement with the observations of Palladini et al.³ concerning the human pineal acervuli.

A surprisingly great morphological and histochemical resemblance between human and gerbil pineal calcification has encouraged Japha et al.¹ to suggest that the latter should be used as an excellent model to explore the phenomenon of pineal calcification. In order to give more accuracy to this unexpected opportunity, it is necessary to compare the fine morphology and the mineralogical composition of the human brain sand with the gerbil pineal acervuli.

Material and methods. The pineal bodies of 5 male Mongolian gerbils (*Merio unguiculatus*) weighing 80 g were fixed by intracardial perfusion with 2% glutaraldehyde and 1% formol in 0.1 mol cacodylate buffer (720 mosmol; pH 7.2). After a brief rinsing in the same buffer, the pineal bodies were postfixed in 1% OsO₄ and embedded in Durcupan. Ultrathin sections were cut with a diamond knife, contrasted with uranyl acetate and lead citrate and observed in Zeiss EM 9A electron microscope. For the microprobe analysis, some pineal bodies were dehydrated and embedded without OsO₄ treatment in Durcupan. The 1000 Å thick sections of these blocks were mounted on nylon grids and analysed for 600 sec at 25 kV in a Hitachi HU-12 transmission electron microscope equipped with a Princeton Gamma Tech X-ray energy dispersive device.

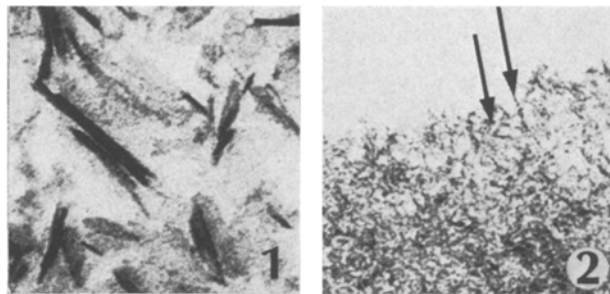


Fig. 1. Gerbil pineal calcification. A widely interspaced needle-like crystals can be seen. $\times 140,000$.

Fig. 2. Human pineal calcification. This material is composed of smaller and considerably more condensed crystals (arrows). $\times 140,000$.

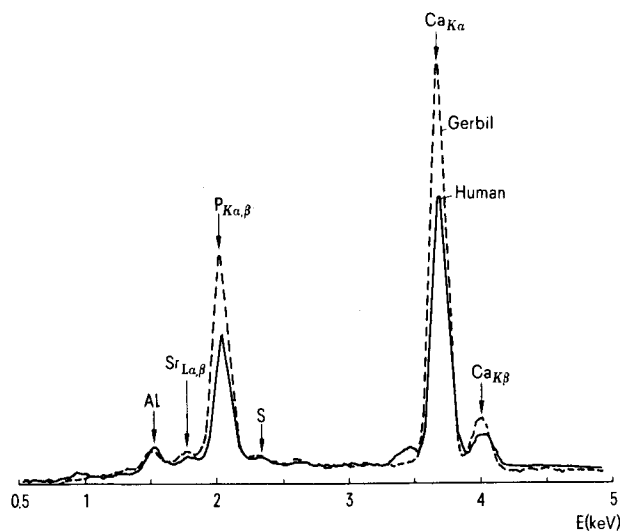


Fig. 3. X-ray microprobe analysis of gerbil (---) and human acervuli (—). Note an identically qualitative and quantitative composition. Aluminium peak corresponds to the specimen holder.

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To compare the chemical composition of the gerbil acervuli with the human one, a parallel microanalysis was made with concretions obtained from seven 65–75-year-old humans of both sexes prepared in an identical way as those from the animals¹⁰.

Results. The gerbil pineal concretions have a spheric form and measure 10–65 μm in diameter. They are mostly situated in vacuoles whose walls seem to be formed by a single clear cell. Under low electron microscope magnification, the acervuli show a very dark inner and peripheral clear zone. While a fine fibrillar material occupies the latter, the mineral of the concretions is localized in the dark inner zone. It is composed of randomly oriented irregular 300–1400 \AA long and about 35–70 \AA thick needle-shaped and widely interspaced crystals (figure 1). For comparison, the human acervular material is also composed of similar but smaller needles (500 \times 35 \AA). They are more regular and considerably more condensed than those in gerbil concretions (figure 2).

The X-ray energy dispersive microanalysis reveals a qualitatively identical mineral composition of both gerbil and human pineal corpora arenacea (figure 3). Their main elements are phosphorus and calcium with some traces of strontium, which frequently accompanies Ca in biological deposits⁴. For the sulphur peaks, it is difficult to say whether this element originates from sulfated mucopolysaccharides of acervular organic matrix or from the embedding medium. A semi-quantitative comparison of the Ca and P peaks in a gerbil and a human calculated after Russ⁵ indicates a relative difference less than 2.5%, which is considerably inferior to the error of the instrument used.

Discussion. Our transmission electron microscope analysis has shown that the human acervular mineral is composed of considerably smaller and much more condensed crystals than in the gerbil concretion. This morphological feature can be explained by the very great difference of the age of the minerals: while the gerbils were 4 months old, our human material came from 60–75-year-old patients. The confirmation of our hypothesis is found in a work of Boivin⁶: in a study of the experimental calciphylaxis of the connective tissue, he has demonstrated that the morphology of the hydroxyapatite changes during this process. Extrapolating his finding to our material, one can see that the youngest crystals found at the beginning of calciphylaxis correspond morphologically to those of the gerbil, and those observed at the end of the experiment have the identical ultrastructure as human acervular mineral.

As already known, the material of human pineal concretions is hydroxyapatite or carbonate apatite^{7–9}. The fact that the X-ray microanalysis has detected an identically qualitative and quantitative composition of human and gerbil corpora arenacea, suggests that the latter corresponds also to the bone mineral. On the other hand, the finding of the present study confirms that the suggestion of Japha¹ to consider the gerbil pineal acervuli as a model for analyzing the mechanism of pineal calcification under controlled conditions, is completely realizable.

- 10 With technical assistance of Miss Ch. Thommen and Mr P.-A. Milliquet. The authors wish to thank Dr T. Jalanti (C. M. E. Lausanne) for his help with the use of the X-ray microanalyzer.

Inhibition of transplanted mouse tumors by heterologous transfer RNA

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Summary. Injections of yeast tRNA to C57BL mice decreased takes and inhibited growth of syngeneic transplanted tumors. Mice remaining free of tumors as result of this treatment failed to develop tumors after challenge with 5×10^4 cells of the same tumor.

Recently we reported² that mice injected with transfer RNA (tRNA) showed changes in plaque-forming cells (PFC) for sheep red cells (SRC) and phagocytosis of rat red cells (RRC). Essentially, i.p. injections of yeast tRNA significantly depressed the numbers of PFC in C57BL and C3H mice immunized with SRC. Hepatic and splenic phagocytosis of ⁵¹Cr-labeled RRC was markedly reduced in tRNA-treated C3H mice. This report deals with effects on takes and growth of syngeneic transplanted tumors in mice injected with yeast tRNA, or tRNA derived from syngeneic liver or rat liver. Yeast tRNA was purchased from Sigma Laboratories (Cat. No. R2876). tRNA from mouse liver and liver of Lewis rats was prepared by using the technique of Kirby³ with minor modifications. Mice of strains C57BL/6 and C3H/eb, from 9 to 15 weeks old, were used. Transplanted tumors were derived from sarcomas induced in mice of the appropriate strain by s.c. injection of 0.6 mg of methylcholanthrene dissolved in 0.3 ml of olive oil. The tumors were passaged syngeneically by s.c. inoculation of 10^5 viable tumor cells suspended in 0.5 ml of phosphate buffered saline (PBS). Groups of control and experimental mice were matched, with experimental mice receiving 3 i.p. injections of tRNA dissolved in PBS during the week preceding inoculation of the tumor. Additional 3 injections per week of tRNA were continued throughout the experiment.

The table summarizes results of 7 experiments in which male C57BL mice were inoculated with sarcomas of 2 lines (T3 and T5). Inhibition of tumor growth in mice treated with yeast tRNA was observed in all experiments, as indicated by the lower values of tumor weight, expressed as percent of body weight. The differences were statistically significant, except for experiment VI; the p-value of 0.06 found in experiment II is of borderline statistical significance. Doses of tRNA per injection ranged from 100 to 600 μg . Experiment IV suggests a dose-related inhibition of tumor growth, inasmuch as 100 μg were ineffective, while 200 and 400 μg showed increasing effectiveness.

In addition to inhibiting tumor growth, yeast tRNA also reduced the number of takes of the tumors. As shown in the table, 58 out of 60 control mice developed progressively growing tumors, whereas this was the case for 65 out of 90 yeast tRNA-treated mice, with 25 remaining free of tumor. Analysis of the difference by means of the chi square test yielded a χ^2 of 14.5, corresponding to $p < 0.001$. Weights of liver and spleen, expressed as percent of body weight, tended to be directly proportional

- 1 This work was supported in part by a grant from the Stanley Johnson Foundation, Berne, Switzerland.
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