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 animals[10].

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 Å long and about 35–70 Å thick needle-shaped and widely interspaced crystals (figure 1). For comparison, the human acervular material is also composed of similar but smaller needles (500 × 35 Å). 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 deposits[4]. For the sulphur peaks, it is difficult to say whether this element originates from sulfated mucopolysacharides 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 Russ[5] 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 Boivin[6]: 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 Japha[1] to consider the gerbil pineal acervuli as a model for analyzing the mechanism of pineal calcification under controlled conditions, is completely realizable.

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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 reported[2] 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 ^{51}Cr-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 Kirby[3] 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

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Effect of yeast tRNA on transplanted tumors in male C57BL/6 mice

Experiment No.	Tumor (passage)[a]	Duration of experiments (days)	Group[b]	Dose of tRNA (μg)	Mice with tumors, total No. of mice	Tumor percent of b.wt (±SD)
I	T5 (3)	26	C	–	10/10	10.3 (7.0)
			IL	200	10/10	14.9 (10.6)
			Y	200	6/10	7.0 (6.6)[d]
II	T5 (5)	33	C	–	8/9	5.8 (3.5)
			IL	200	9/9	7.4 (4.3)
			Y	200	4/9	2.6 (1.8)[e]
III	T5 (7)	29	C	–	6/6	9.1 (5.0)
			RL	200	8/8	6.7 (4.4)
			Y	200	8/10	3.7 (1.7)[c]
IV	T5 (8)	22	C	–	9/9	7.8 (5.9)
			Y	100	7/8	7.3 (7.3)
			Y	200	8/9	4.4 (3.6)
			Y	400	6/8	3.5 (1.7)[d]
V	T5 (10)	24	C	–	10/10	3.5 (2.0)
			Y	300	9/10	1.3 (0.4)[c]
			Y	600	7/10	1.6 (0.9)[c]
VI	T3 (9)	35	C	–	7/8	7.6 (2.3)
			Y	500	4/8	4.2 (4.9)
VII	T3 (14)	21	C	–	8/8	6.8 (0.4)
			Y	500	6/8	2.1 (2.2)[d]

[a]Figure in parenthesis indicates passage of tumor. [b]C, control; IL, isogeneic liver tRNA; RL, rat liver tRNA; Y, yeast tRNA. [c]$p < 0.01$, [d]$p < 0.05 > 0.01$, [e]$p = 0.06$ (significance between control and experimental group).

to the size of the tumor. Accordingly, lesser degrees of hepatomegaly and splenomegaly were found in tRNA-treated mice without tumor, or tumors smaller than those in control mice.

In 2 experiments, yeast tRNA-injected mice, which failed to develop tumors, were challenged with a second inoculation of the same tumor without receiving additional injections of yeast tRNA. In experiment II, the 5 mice remaining free of tumor after the first inoculation developed progressive tumor growth after a second inoculation of 5×10^5 tumor cells. By contrast, no tumors developed in the 4 tumor-free mice of experiment VI challenged with 5×10^4 tumor cells.

In one additional experiment administration of yeast tRNA to female C57BL mice (8 controls, 8 experimentals; i.p. injections of 500 μg 3 times a week) caused significant inhibition of tumor growth: tumor percent of body weight, controls: 2.2 ± 1.0, experimentals: 1.0 ± 0.3; $p<0.01$.

In groups of male C57BL mice treated with tRNA of syngeneic liver (table, experiments I and II) or tRNA of rat liver (table, experiment III) there was no interference with take or growth of tumor. On the contrary, tumor weights in mice treated with syngeneic liver tRNA exceeded those of control mice, although the differences were not statistically significant. No adverse effects of treatment with tRNA were noted, and body weights of experimental mice did not differ significantly from those of controls. In 4 experiments, neither yeast tRNA nor syngeneic liver tRNA affected takes and growth of transplanted syngeneic tumors in C3H mice.

The work reported has demonstrated a systemic effect of yeast tRNA, expressed as inhibition of takes and growth of transplanted syngeneic mouse tumors. Mice remaining free of tumor, as a result of the treatment, were immune to challenge with the same tumor when small inocula were used (experiment VI), but they were not protected against a 10fold higher number of tumor cells (experiment II). Tumor-inhibiting activity was absent from tRNA of syngeneic liver or rat liver. C3H mice were not found to be susceptible to the yeast tRNA-induced inhibition of tumor growth.

We propose that the systemic biological effects of tRNA are based on interaction of tRNA with macrophages. Support for this hypothesis is provided by our observations on changes in phagocytosis and immune responses in mice treated with tRNA[2], and by previous studies demonstrating changes in phagocytic activity in mice injected with microsomes[4] or ribosomes[5]. The fact that yeast tRNA inhibited tumor growth in C57BL, but not in C3H mice, may be correlated with the higher reticuloendothelial activity[6,7] and stronger immune responses[8,9] distinguishing C57BL from C3H mice.

Geddes-Dwyer and Cameron[10] recently reported that growth of transplanted osteosarcomas in inbred rats was significantly inhibited when the tumor cells, prior to inoculation, were incubated in vitro with tRNA prepared from rat embryonic or mesenchymal tissue. By contrast, analogous treatment of tumor cells with tRNA derived from osteosarcomas, enhanced tumor growth. In line with the experimental design used by them, the authors attributed these effects to direct interaction of tRNA with tumor cells, although they could not exclude interaction of tRNA with non-tumor cells present in the suspension used for inoculation. Obviously, decision on the precise mechanism(s) responsible for the tumor inhibition observed by Gedder-Dwyer and Cameron[10] and in our system, requires acquisition of additional experimental data, including study of tRNA of other origins, investigations of various tumor-host systems, and use of purified species of isoaccepting tRNA.

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