ated liver cells receive a 'normal' enzyme content. Becoming older and moving to the vena centralis, this enzyme content changes, depending on age and diet.

Zusammenfassung. Nachweis, dass nach fettreicher Diät Glykogen hauptsächlich in der Zone 1 des Rappaportschen Leberacinus abgelagert wird, was durch den Abbau von Enzymen, die in den Zonen 2 und 3 am Glykogenaufbau beteiligt sind und durch die physiologische Regeneration in der Zone 1 verursacht wird.

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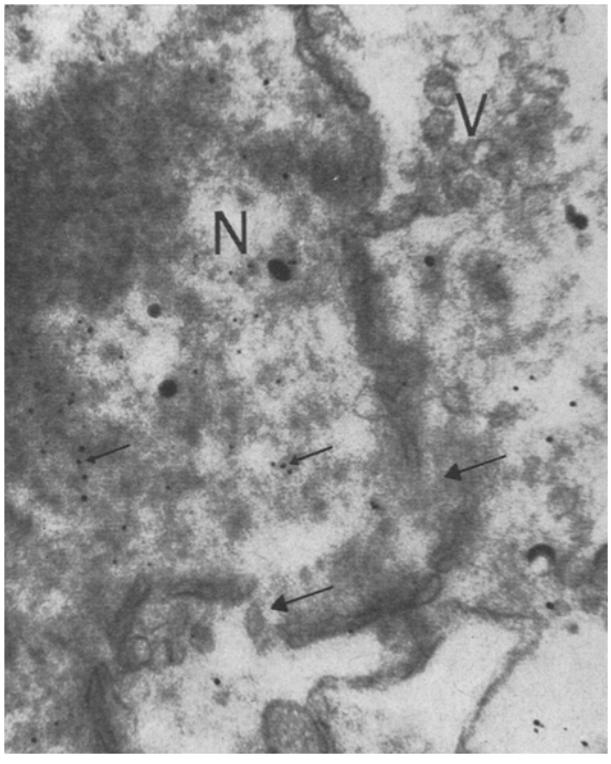
The Effect of Temperature on Nuclear Permeability

It has been shown that the transfer of RNA from the nucleus to the cytoplasm is a temperature dependent process[1–3]. These studies, however, do not distinguish between the effect of temperature on the processing of RNA within the nucleus and its effect on the permeability of the nuclear envelope to macromolecules. In this investigation the action of temperature specifically on the exchange process was studied by injecting colloidal gold particles, coated with polyvinylpyrrolidone, into the cytoplasm of the multinucleated amoeba *Chaos chaos*. The injected cells were incubated at different temperatures and the intracellular distribution of the particles determined with the electron microscope. Since these particles are inert, and not altered by temperature dependent changes in cell metabolism, their ability to enter the nucleus should depend primarily on the characteristics of the nuclear envelope, assuming that diffusion within the cytoplasm is not rate limiting. Furthermore, both colloidal particles and ribonucleoproteins cross the nuclear envelope through central channels within the nuclear pores[4,5]. Thus, variations in the uptake of gold particles should reflect changes in the properties of the pathways used for naturally occurring substances.



An electron micrograph of a permeable nucleus following incubation at 2 °C. The gold particles (small arrows) are concentrated in the nucleoplasm (N). Breaks in the nuclear envelope are apparent (large arrows). Small vesicles (V), possibly fragments of the envelope, are frequently associated with permeable nuclei. In this instance the vesicles are restricted to the cytoplasm, but they have also been seen in the nucleoplasm.

The experiments were performed on well-fed, interphase amoebae. The prodecures for culturing the cells, preparing colloidal gold, microinjection, and electron microscopy have been described in previous reports[4,6]. Two gold fractions were used; one contained particles ranging from 30–170 Å in diameter (L-fraction), and the second contained 25–55 Å particles (S-fraction). The amoebae were injected at room temperature (approximately 25 °C) and left at that temperature or rapidly transferred to an incubator set at 34°, 10°, or 2 °C. The cells were fixed in OsO_4, 30 or 50 min after injection, and subsequently sectioned and examined with the electron microscope. Gold particles were counted and measured in adjacent regions of nucleoplasm and cytoplasm according to the methods described earlier[7].

The results of the 50 min experiments performed with the L-fraction are shown in Table I, A. The percent of the total particle count present in the nucleoplasm, decreased as the temperature was lowered from 34° to 10 °C. The decrease from 34° to 25 °C is statistically significant ($P < 0.025$), as is the decrease from 25° to 10 °C ($P < 0.001$). Surprisingly, when the temperature was dropped to 2 °C there was an increase in the concentration of particles in the nucleoplasm, and the results are not significantly different from those obtained at 25 °C ($P > 0.5$). Closer examination of the cells incubated at 2 °C showed that there were 2 separate populations of nuclei. This was not the case at higher temperatures. In one population (non-permeable nuclei), consisting of approximately 70% of

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Table I. Particle distribution

Temperature (°C)	No. of amoebae	No. of nuclei	Total volume examined (μm^3) [a]	No. of particles in cytoplasm	No. of particles in nucleoplasm	Percent of total count in nucleoplasm	High and low value for individual amoebae (%)
A) 50 min experiments – L-fraction							
34	5	44	71	8,171	5,997	46	51–35
25	11	64	78	11,058	4,959	32	47–16
10	7	63	100	10,226	586	8	15–2
2	5	50	82	6,347	3,856	32	37–10
B) 30 min experiments – L-fraction							
25	6	37	59	4,676	1,176	21	27–13
2	6	52	84	5,217	217	3	7–1
C) 50 min experiments – S-Fraction							
25	5	40	64	10,544	11,175	51	63–48
10	5	23	35	4,211	2,037	32	41–26

[a] Half of each value represents nucleoplasm and half cytoplasm.

Table II. Size distribution of particles in nuclei (%)

Temp. (°C)	Number of particles measured	0–35 Å	35–70 Å	70–105 Å	105–140 Å	> 140 Å
34	1000	1	52	42	5	0
10	600	2	56	41	1	0

the nuclei, the concentration of gold particles in the nucleoplasm was only 3% of that in the cytoplasm. The remainder of the nuclei were permeable, and actually contained higher concentrations of gold than the cytoplasm. The latter finding can be explained by the fact that the envelopes of the permeable nuclei contained localized regions in which breakdown of membranes could be observed (Figure). Non-permeable and permeable nuclei were frequently found adjacent to one another in the same cell, indicating that the temperature dependent variations in gold uptake are limited by the nuclear envelope, rather than by variation in the migration rates of the colloidal particles within the cytoplasm. Table I, B gives the results of experiments in which the cells were incubated at 25° and 2 °C for 30 min. At this time interval the differences in nuclear uptake are significant ($P < 0.001$), and at 2 °C only about 2% of the nuclei were permeable, demonstrating that there is essentially no membrane breakdown during the first 30 min at low temperature.

One explanation for the observed decreases in nuclear permeability is that lowering the temperature effectively reduces the size of the central channels within the pores. To test this hypothesis the size distributions of the L-fraction particles present in the nuclei following incubation at 10° and 34 °C for 50 min were determined (see Table II). There is a difference in the number of particles at the upper limit of the permeability range, but it is unlikely that this could account for the differences in the uptake rates since only a small percentage of particles are involved. Further evidence that lowering the temperature does not simply exclude larger particles, but also alters the exchange rates of small particles was obtained by studying the uptake of S-fraction particles at 25° and 10 °C (see Table I, C). Although this fraction contains particles which are well below the size range (105–140 Å) in which variations in exclusion were detected, there is still a highly significant difference in nuclear uptake ($P < 0.001$).

In conclusion it has been shown that the relative rates of exchange of particulate material across the nuclear envelope decreases as the temperature is lowered from 34° to 10 °C. One might expect similar temperatures effects for the exchange of ribonucleoprotein, since this substance utilizes the same pathways as colloidal gold. When the cells were incubated at 2 °C there were indications of nuclear envelope breakdown. Although it could be shown that the nuclear envelope represents the temperature dependent barrier, it could not be determined from the available data whether physical or chemical processes are effected. It was evident however, that the observed differences in the concentration of gold within the nucleoplasm were not due simply to changes in the dimensions of the central channels.

Zusammenfassung. Die relative Transportgeschwindigkeit von kolloidalem Gold durch die Membran des Zellkerns verringert sich, wenn die Temperatur von 34° auf 10 °C herabgesetzt wird. Ein ähnlicher Temperatureffekt darf für den Transport von Ribonukleoproteinen angenommen werden, da diese Substanzen denselben Weg benützen wie kolloidale Teilchen. Werden die Zellen bei 2 °C inkubiert, so machen sich Anzeichen einer Zerstörung der Kernmembrane bemerkbar. Die beobachteten Konzentrationsunterschiede von kolloidalem Gold innerhalb des Kernplasmas konnten nicht durch Grössenänderungen der Zentralkanäle in den Kernporen erklärt werden.

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