material could result in deposition of amyloid. The deposition of the substance, aside from its exogenous (single or repeated injections of foreign substances) or endogenous origin (infectious processes, neoplasms, autoimmune diseases etc.) would be simultaneous with, or even following, the progressive renal disfunction. The final amyloid substance would represent the result of the elaborative processes carried out by the reticuloendothelial system. All the cellular phenomena observed in experimental amyloidosis could be interpreted as collateral attempts at degradation, elaboration and finally organization of the foreign substance deposited when different primary or secondary pathogenetic factors have produced an impairment of the renal function. The structural organization and assembly of amyloid into the so-called amyloid fibrils would represent the last stage of the elaborative and defensive mechanism in which the reticuloendothelial cells play a main role.

It was the purpose of this preliminary note to focus attention on the possible role played by the kidneys in experimental amyloidosis. The results submitted show that a primary renal damage can be recognized before any amyloid deposition in our strain of adult thymectomized mice. They lend support to the hypothesis that amyloid deposition depends on the condition of the kidneys whatever the origin of the amyloidogenic material.

Riassunto. Topi albini NMRI timectomizzati all'età di 2 mesi sviluppano una grave forma di glomerulonefrite evolvente verso la sclerosi. Una massiva infiltrazione di sostanza amiloide nella milza segue le alterazioni renali indicando che esiste uno stretto rapporto tra danno renale ed amiloidosi.

W. PIERPAOLI [16]

Schweizerisches Forschungsinstitut, Medizinische Abteilung Davos-Platz (Switzerland), 16th February 1967.

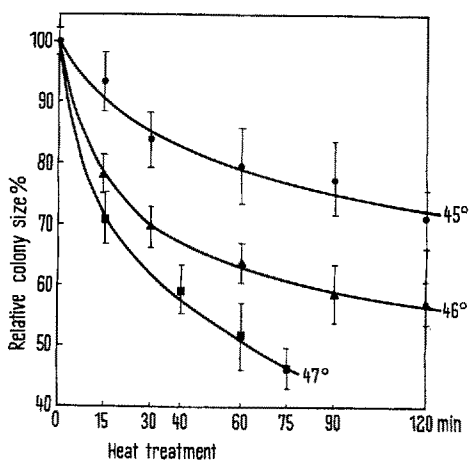
[16] Research fellow of the Italian National Research Council, Rome. This work was supported by the Swiss National Foundation for Scientific Research, Grant No. 3958.

Growth Inhibition in Mammalian Cells Exposed to Thermal Stress

The effects of thermal stress on cell populations are usually described in terms of heat-induced mortality. For example, if mammalian cells are held in suspension at 44 °–47 °C, viability in plating tests at 37 °C decreases exponentially, and at rates proportional to the temperature used[1,2]. These mortality curves are similar to the log-linear decline in survival seen with cell cultures exposed to X-rays[3,4] or UV-irradiation[5]. However, sublethal effects of exposure to elevated temperatures can also be seen. If the heat treatment is adjusted to yield a viable fraction of 10^{-5} or less, the colonies derived from single survivor cells may exhibit a marked diminution in size. A similar phenomenon ('small colony formation') has been described by SINCLAIR[6] in cultures of Chinese hamster cells exposed to X-irradiation; the reduced growth rate was mitotically transmissible and appeared to represent a stable change in cellular phenotype. It is accordingly of interest to determine whether or not the reduction in growth observed after heat treatment is based on a persistent defect in the cells concerned.

The experiments to be described were performed with a clonal line of pig kidney cells, using materials and methods that have been detailed previously[2]. Stock cultures were carried at 37 °C as monolayers in a nutrient (5CS199) made up of 5% new-born calf serum in Medium 199. For heat treatment, log phase cells were dissociated with 0.1% trypsin-versene and resuspended in 5CS199 at $1.0 \cdot 10^6$ cells/ml. These suspensions were then equilibrated with 5% CO_2 in air and immersed in a water bath adjusted to 45 °, 46 °, or 47 °C, ± 0.02 °. At graded intervals, samples of the suspension were removed and plated out in nutrient medium at 37 °C, using dilutions appropriate to yield well-separated survivor colonies from single cells. These assay cultures were maintained for 14 days in a humidified CO_2 incubator with 3 fluid changes. Petri dishes to be scored were stained with crystal violet and air dried.

A preliminary study was carried out to determine the extent of growth inhibition as a function of time-temperature treatments. The Figure provides a summary of these findings. The data show that reduction of size among survivor colonies depends on duration of thermal stress as well as the temperature level used. Although the decline in growth potential among survivor cells takes



Effect of heat treatment on average diameter of survivor colonies in recovery cultures at 37 °C. Each point is based on measurements of 30 colonies at 14 days of incubation.

[1] M. HARRIS, Expl Cell Res. *44*, 658 (1966).
[2] M. HARRIS, Expl Cell Res., in press.
[3] M. M. ELKIND, H. SUTTON and W. B. MOSES, J. cell. comp. Physiol. *58* (Suppl. 1), 113 (1961).
[4] G. COLUMBO and G. MARIN, Expl Cell Res. *29*, 268 (1963).
[5] H. H. LEE and T. T. PUCK, Radiat. Res. *12*, 340 (1960).
[6] W. K. SINCLAIR, Radiat. Res. *21*, 584 (1964).

place most rapidly during the initial phase of heat treatment, the kinetics of change are neither linear nor logarithmic. After prolonged thermal stress, the degree of growth impairment tends toward equilibrium levels. It should be noted that the induction of growth inhibition follows a time course that is different from the curve of thermal death for the cells concerned. The latter exhibits an initial shoulder with little or no mortality, followed by a logarithmic decline as previously noted. These findings suggest that mortality and growth inhibition may stem from separate effects of thermal stress.

Relative growth of heat-treated cells as survivor colonies

Clonal sublines of heated cells	Colony diameters, mm	
	First passage	Fourth passage
1	0.11	3.70 ± 0.12
2	0.11	4.00 ± 0.11
3	0.09	2.86 ± 0.11
4	0.11	3.18 ± 0.13
5	0.07	4.37 ± 0.18
6	0.12	3.24 ± 0.10
7	0.13	3.35 ± 0.11
8	0.14	3.09 ± 0.09
9	0.13	3.05 ± 0.08
10	0.18	3.74 ± 0.19
Averages	0.12 ± 0.03	3.46 ± 0.14
Unheated controls	3.02 ± 0.09	3.78 ± 0.13
P	< 0.001	> 0.10

First and fourth passage cultures incubated 12 and 14 days at 37 °C respectively. Mean values and standard error based on measurements of 30 colonies for each series.

Dwarf colonies were subsequently studied in serial passage, to see whether reduced colony size after heat treatment represents a stable variation. For this work, 10 microcolonies were selected at 12 days from recovery cultures that had been set up with cells exposed to 46 °C for 120 min. The diameter of each colony was determined with an ocular micrometer, after which the colony was removed with trypsin-versene and the suspension inoculated into a 3 oz. prescription bottle. Clonal sublines of cells obtained in this fashion were carried for 3 passages in serial subculture, and were then plated out in petri dishes. After incubation for 14 days, the average colony size was determined for each population. The Table gives the results of these determinations. It is clear that no significant difference in colony size can be demonstrated between heated and unheated cells after serial passage. Thus, 'small colony formation' after heat treatment appears to represent a non-genetic change, in contrast to the relatively permanent impairment that may follow X-irradiation. The cellular basis for growth inhibition by thermal stress remains to be determined[7].

Zusammenfassung. Erhöhter Temperatur ausgesetzte Schweinenieren-Zellen produzieren, bei 37 °C kultiviert, Mikrokolonien. Eine Reduktion in der Wachstumsrate ist proportional zur Dauer und Intensität der Hitzebehandlung und verliert sich im Laufe der weitern Kultivierung.

M. HARRIS

Department of Zoology, University of California, Berkeley (California 94720, USA),
5th January 1967.

[7] This work was supported by U.S. Public Health Service Research Grant No. GM-13692, and by an appointment as Research Professor in the Miller Institute for Basic Research in Science.

Studies on the Neurosecretory Cells in the Cerebral Ganglion of *Potamon magnum magnum* (Pretzman)

On the neurosecretory system of decapod crustaceans, a large number of morphological observations have been recorded[1-8]. These authors have attempted to classify the types of neurosecretory cells on the basis of the size of the cell, its way of discharging the product, the nature of the secretory granules and their position in the ganglion. It is apparent that work along these lines is needed, particularly in view of the fact that cytological differences in cell types often are parallel with differences in function. Further the importance of neurosecretory products in the physiology of many crustaceans is apparent from several recent reviews[9-11]. With this end in view, we have studied the neurosecretory cells in the cerebral ganglion of *Potamon magnum magnum* (Pretzman).

The neurosecretory groups of cells have been identified by using the Gömöri technique. Based on the shape, presence or absence of vacuoles in the cytoplasm, and the nature of secretion, the neurosecretory cells may be classified into 2 groups. One type of cell is large (20–45 μ) oval or polygonal with large central nucleus and vacuolated cytoplasm (Figures 1 and 2). These cells are with or without axons. They are mainly found in the anterior, ventral and lateral parts of the dorsal side of the ganglion. There are few cells of this type on the ventral side of the ganglion. The cytoplasm is basophilic, with a Nissl substance in a zone around the nucleus. The nucleus has

[1] M. ENAMI, Physiologia comp. Oecol. *3*, 23 (1949).
[2] M. ENAMI, Biol. Bull. mar. biol. Lab., Woods Hole *101*, 241 (1951).
[3] D. E. BLISS, J. B. DURAND and J. H. WELSH, Z. Zellforsch. mikrosk. Anat. *39*, 520 (1954).
[4] M. MIYAWAKI, Annotnes zool. jap. *28*, 163 (1955).
[5] J. B. DURAND, Biol. Bull. mar. biol. Lab., Woods Hole *111*, 62 (1956).
[6] R. PARAMESWARAN, Q. Jl microsc. Sci. *97*, 75 (1956).
[7] H. INOUE, Mem. Gakugei Fac. Akita Univ. Nat. Sci. *7*, 84 (1957).
[8] K. MATSUMOTO, Biol. J. Okayama Univ. *4*, 103 (1958).
[9] B. SCHARRER, in *The Hormones* (Ed. G. PINCUS and K. V. THIMANN; Academic Press, New York 1955), vol. 3, p. 57.
[10] J. H. WELSH, in *The Hormones* (Ed. G. PINCUS and K. V. THIMANN; Academic Press, New York 1955), vol. 3, p. 97.
[11] F. G. W. KNOWLES and D. B. CARLISLE, Biol. Rev. *31*, 396 (1956).