these animals as in intact control rats. This observation, although not directly relevant to the present subject, is mentioned, since it must be considered paradoxical. It might have been expected that adjuvant arthritis in hypophysectomized rats would develop more severely than normally, due to the decreased production of glucocorticosteroids. That the opposite result was obtained can perhaps be correlated to the observation of TAUBENHAUS and AMROMIN[6] that hypophysectomized rats fail to develop significant amounts of granulation tissue in response to turpentine. They associated the phenomenon with lack of pituitary growth hormone.

The above observations, indicating that the suppressing effect of estrone on adjuvant arthritis is not dependent on the presence of adrenals or ovaries, establish that this result can be considered to be the direct effect of estrone or of its metabolites. This conclusion, however, does not explain whether the effect of estrone is based on its anti-inflammatory properties[7], or on its possible immunologically suppressing effect[8], or on something else.

Zusammenfassung. Die Adjuvans-Arthritis konnte durch Östrontherapie gehemmt werden auf gleiche Weise bei intakten Ratten wie bei Ratten nach bilateraler Adrenalektomie, Ovariektomie oder nach Adrenal- und Ovariektomie. Bei hypophysektomierten Tieren (ohne Östrontherapie) war das Krankheitsbild bedeutend milder als bei intaken Kontrollratten.

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Experimental Amyloidosis and Renal Disease

The numerous investigations on the pathogenesis of experimentally induced or spontaneous amyloidosis of laboratory animals have clearly failed so far to indicate a common origin of the infiltrative process. Several recent reviews on amyloidosis have summarized the present knowledge of the disease and indicated a few valuable hypotheses on the pathogenesis[1-4].

The chemical, histochemical and electron-microscopic investigations of the composition and structure of the amyloid substance[5,6] have given a fairly complete picture of the mechanism of amyloid deposition, although they did not reveal the initial primary factors which bring about the build-up of amyloid.

Immunological studies carried out in recent years[7,8] have ruled out the participation of a classical immune reaction leading to the formation of casein-induced amyloidosis in rabbits and mice; neither has an autoimmune process been demonstrated as a cause of amyloidosis[9].

The data of TEILUM[10] and BATTAGLIA[11] give no definite answer about the cellular or extracellular origin of amyloid. Indeed, the presence of reticuloendothelial cells and their dynamic, phagocytic and possibly secretory activity in locations of amyloid infiltration or formation may well have nothing to do with the production of amyloid but may simply represent a normal defence mechanism to eliminate the amyloidogenic material probably derived from elsewhere.

The different techniques employed to induce amyloidosis in laboratory animals and the remarkable species differences to amyloidogenic treatment demonstrate additionally that, if there is a common etiological factor, it has not yet been recognized.

We may thus ask whether there is any evident pathological alteration which constantly accompanies the pre-amyloidotic and the amyloidotic state, and which could help us to recognize a common primary pathogenetic mechanism in experimental amyloidosis.

It is the purpose of this communication to submit the hypothesis that renal damage might be a possible primary cause of amyloidosis in laboratory animals, and to give some experimental evidence for this hypothesis.

This consideration is based on numerous data from the literature and on observations in casein-induced[8] amyloidosis in mice and in amyloidosis observed in adult thymectomized mice[12]. An extremely severe glomerulonephritis or renal sclerosis is consistently found in casein-induced amyloidosis in mice. Additionally, the high frequency of amyloidosis in some strains of inbred mice which develop spontaneous glomerulonephritis[13-14] is strongly in favour of this hypothesis

On the basis of the former findings [8,12], and to test the hypothesis that amyloid deposition depends at least partially on the actual functional conditions of the kidneys of the experimental animals used, the following experiments were performed.

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50 outbred Swiss albino mice (NMRI) raised in this aboratory were thymectomized at 2 months of age. The operation was performed under Nembutal anaesthesia and the thymus was removed by suction. 50 littermate controls were sham-operated. All animals were fed ad libitum. Beginning 60 days after the operation, and thereafter, at intervals of 1 month, 2 mice and 2 sham-operated controls were killed. Kidneys, liver, and spleen were fixed in 10% neutralized formalin and stained with haematoxylin-eosin and metachromatically with methyl-violet. The histological results have shown that, from 3 months after thymectomy, the mice developed a severe and progressive glomerulonephritis while no amyloid deposits could be revealed in the organs examined. Animals killed from 4–8 months after thymectomy show a progressive nephritis and renal sclerosis with parallel massive amyloid deposits in the spleen. The nephritic process definitely precedes the first manifestations of amyloidosis in the spleen. No sign of amyloidosis or glomerulonephritis has ever been observed in the organs of the littermate sham-operated control mice.

It thus seems clear that the amyloid infiltration appears after the onset of glomerulonephritis and proceeds parallel to the evolution of glomerulonephritis to the final sclerotic stage. The character of nephritis (Figure 1) resembles strongly the supposed autoimmune process in NZB/Bl mice as described by HELYER and HOWIE[13] The later appearance of amyloidosis of the spleen (Figure 2) probably does not represent a simultaneous or even the same autoimmune process but is presumably the result of renal impairment and sclerosis bringing deposition of the optically amorphous material. This does not exclude the possibility that, in these experimental conditions, the amyloid substance may also derive from the supposed autoimmune process of the kidneys. However, we suggest that the impaired and deficient glomerular filtration is, if not the direct, the main cause of amyloid deposit, irrespective of the origin of the material.

It must be pointed out that in this outbred strain of mice (NMRI) neonatal thymectomy results in nearly 100% death with wasting disease. The strain used in former experiments on casein-induced amyloidosis[9] had given a very low incidence of the wasting syndrome after neonatal thymectomy and no spontaneous amyloidosis. The correlation between amyloidosis and tendency to post-thymectomy syndromes is therefore quite evident in the NMRI strain used in the present experiments.

It is difficult to bring together into a single primary pathogenic process of the kidneys all the different conditions of human primary, hereditary, and secondary amyloidosis, paramyloidosis and experimental amyloidosis of laboratory animals, although there is a strikingly high percentage of cases in all kinds of amyloidosis in which a renal involvement is established or can be suspected[3,15].

It can be suggested that the functional incapacity of the kidneys to eliminate endogenous or exogenous

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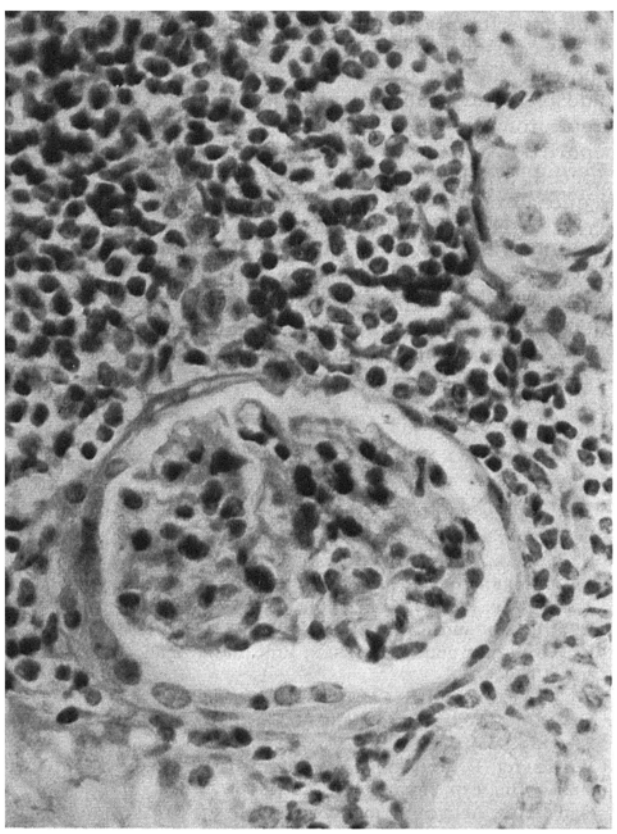

Fig. 1. Glomerular lesion in a NMRI mouse thymectomized as adult and killed 7 months after thymectomy. Irregular thickening of the glomerular basement membrane. Endothelial proliferation with hypercellularity. Massive periglomerular parvicellular infiltration. Haematoxylin and eosin, × 400.

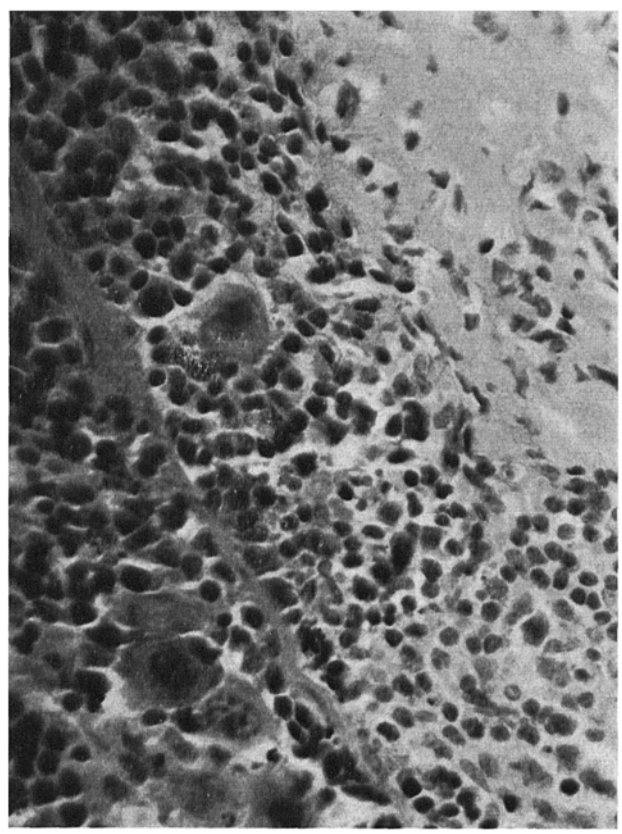

Fig. 2. Perifollicular aspect of the spleen of a NMRI mouse thymectomized as adult and killed 7 months after thymectomy. Numerous perivascular macrophages and massive amyloid deposit. Haematoxylin and eosin, × 400.

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.

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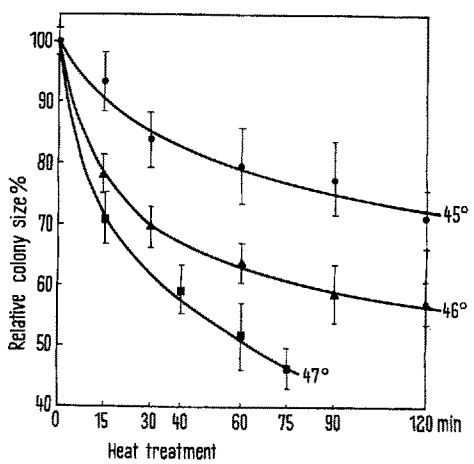
Growth Inhibition in Mammalian Cells Exposed to Thermal Stress

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.

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