Suppression of Adjuvant Arthritis by Estrone in Adrenalectomized and Ovariectomized Rats

It has been demonstrated in a previous work[1] that treatment with estrone inhibits the development of adjuvant arthritis in intact rats and greatly ameliorates the symptoms of the disease already developed before estrone treatment. In order to elucidate the mechanism mediating this estrone effect, we have studied the influence of this hormone on adjuvant arthritis in rats after adrenalectomy and/or ovariectomy. It has been established that estrogens may stimulate the production of glucocorticosteroids in man[2] as well as in the rat[3]. The estrogen-induced influence on adjuvant arthritis could thus be attributed to increased glucocorticosteroid secretion. The effect could also be influenced by cyclic variations in endogenous estrogens. In the present experiments these possible mechanisms have been excluded by preceding bilateral adrenalectomy or ovariectomy. One group with extirpation of both adrenals and ovaries is included to exclude completely the presence of endogenous estrogens. An attempt was also made to study the hormonal effect in hypophysectomizedts ra.

Female Spraque-Dawley rats weighing 160–180 g were used. Arthritis was induced by injecting 0.6 mg of heat-killed dried *Mycobacterium phlei* in 0.1 ml of paraffin oil into the left hind foot pad. Development of arthritis was observed daily and the degree of arthritis was scored macroscopically as described previously[1] with the exception that the injected foot was excluded from the results, since the reaction at the injection site does not necessarily have the same significance as disseminated arthritis[4]. Maximum score is thus 80/rat. Removal of the endocrines was performed according to D'AMOUR and BLOOD[5] in combined nembutal-ether-anaesthesia, 2–3 weeks prior to beginning the estrone treatment. Adrenalectomized animals were maintained on s.c. injections of desoxycorticosterone acetate, 1.0 mg/rat given in 1.0 ml of sesame oil, twice per week. Estrone (kindly supplied by Messrs. N. V. Organon, Oss, Netherlands) was administered s.c. in a dose of 1.0 mg/rat in 1.0 ml of sesame oil twice a week. The control animals were treated with sesame oil.

The suppressing effect of estrone on adjuvant arthritis was of similar degree in all 4 groups studied, that is, in intact rats and in rats after adrenalectomy or ovariectomy or both. Therefore only the results of 2 groups are graphically presented (Figures 1 and 2).

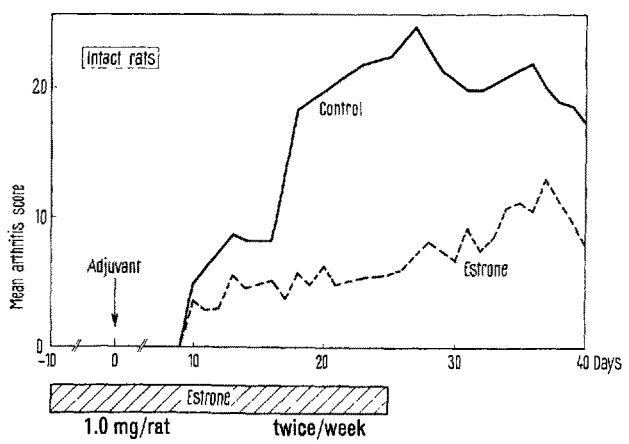

Fig. 1

It was our purpose to repeat the experiment on hypophysectomized animals, but they could not withstand the estrone treatment; most of those treated with the hormone had already succumbed before the injection of adjuvant. The sesame oil treated hypophysectomized rats endured the arthritis almost in a normal way. Figure 3 shows that a considerable milder disease developed in

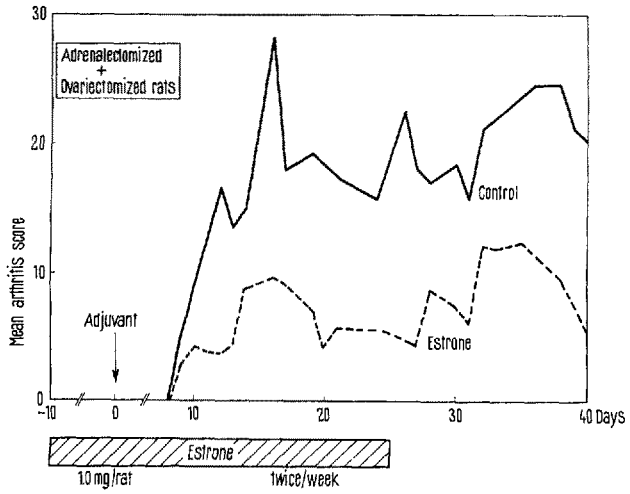

Fig. 2

Figs. 1 and 2. Effect of estrone on the development of adjuvant arthritis in intact rats and in rats after adrenalectomy and ovariectomy. Each curve is based on observation of 9–12 rats.

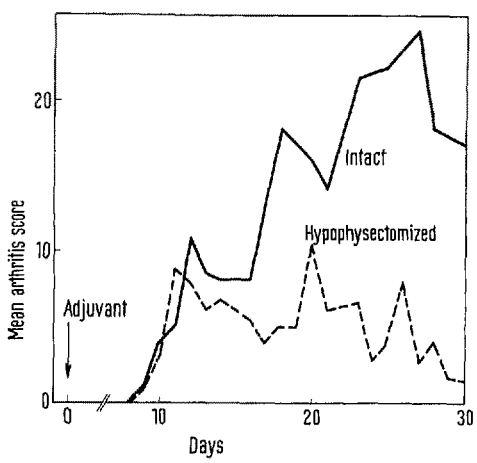

Fig. 3. Development of adjuvant arthritis in hypophysectomized rats. The group included 8 animals, of which 3 died, on the 14th, 24th and 25th days, respectively, after the adjuvant injection. The group of intact control animals consisted of 11 rats.

[1] P. TOIVANEN, K. MÄÄTTÄ, R. SUOLANEN and R. TYKKYLÄINEN, Medna Pharmac. exp., in press.
[2] S. D. GRANT, F. CH. PAVLATOS and P. H. FORSHAM, J. clin. Endocr. Metab. *25*, 1057 (1965).
[3] J. I. KITAY, Endocrinology *73*, 253 (1963).
[4] J. R. WARD, R. S. CLOUD, E. L. KRAWITT and R. S. JONES, Arthritis Rheum. *7*, 654 (1964).
[5] F. E. D'AMOUR and F. R. BLOOD, *Manual for Laboratory Work in Mammalian Physiology* (University of Chicago Press, Chicago, Illinois 1954).

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.

P. TOIVANEN, HELINÄ SIIKALA, P. LAIHO and T. PAAVILAINEN

Department of Medical Microbiology, University of Turku (Finland), 7th March 1967.

[6] M. TAUBENHAUS and G. D. AMROMIN, J. Lab. clin. Med. *36*, 7 (1950).
[7] E. M. GLENN, W. L. MILLER and C. A. SCHLAGEL, Recent Prog. Horm. Res. *19*, 107 (1963).
[8] P. TOIVANEN, Annls Med. exp. Biol. Fenn. *45* (1967), in press.

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.

[1] A. S. COHEN, Int. Rev. exp. Path. *4*, 159 (1965).
[2] E. CLERICI and M. L. VILLA, Sperimentale *115*, 314 (1965).
[3] H. A. AZAR, A. Rev. Med. *17*, 49 (1966).
[4] G. D. SORENSON, W. A. HEEFNER and J. B. KIRKPATRIK, Meth. Achiev. exp. Path. *1*, 514 (1966).
[5] A. S. COHEN and E. CALKINS, Nature *183*, 1202 (1959).
[6] E. P. BENDITT and N. ERIKSEN, Proc. natn. Acad. Sci. U.S.A. *55*, 308 (1966).
[7] W. PIERPAOLI and E. CLERICI, Experientia *20*, 693 (1964).
[8] E. CLERICI, W. PIERPAOLI and M. ROMUSSI, Pathologia Microbiol. *28*, 806 (1965).
[9] E. CLERICI, P. MOCARELLI, W. PIERPAOLI, L. PROVINI and M. L. VILLA, Clin. exp. Immunol. *1*, 425 (1966).
[10] G. TEILUM, Acta path. microbiol. scand. *61*, 21 (1964).
[11] S. BATTAGLIA, Klin. Wschr. *39*, 795 (1961).
[12] Unpublished experiments.
[13] B. J. HELYER and J. B. HOWIE, Lancet *2*, 1026 (1963).
[14] F. M. BURNET and M. C. HOLMES, in *The Thymus in Immunobiology* (Ed. R. A. GOOD and A. E. GABRIELSEN; Harper and Row, New York 1964), p. 656.