Anti-inflammatory activity of the acid carboxypeptidase on carrageenin-induced paw edema is summarized in Table II. The acid carboxypeptidase showed a more potent anti-inflammatory activity than α-chymotrypsin and bromelain. In the analysis at 2, 3 and 4 h after injection of carrageenin (Table II), the log dose response curves for the acid carboxypeptidase were linear within the limit of experimental error. However, in the analysis at 1 h after injection of carrageenin, the log dose response curve was not linear. This phenomenon seems to indicate the slow absorption of the acid carboxypeptidase which has a molecular weight of 51,000. These findings seem to suggest that the acid carboxypeptidase hydrolyzed bradykinin in vivo. No appreciable adverse reactions, such as hemorrhage and ascites, were observed at the doses given.

Summary. The acid carboxypeptidase from *Penicillium janthinellum* catalyzed the rapid release of arginine, and the slow release of phenylalanine, proline, serine and glycine from the carboxy-terminal of bradykinin at pH 4.15 to 4.8. Anti-inflammatory activity of the acid carboxypeptidase seems to suggest that the enzyme hydrolyzed bradykinin in vivo.

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Interaction Between Dehydroepiandrosterone, Glucose-6-Phosphate Dehydrogenase, and Cyclic Adenosine-3',5'-Monophosphate in Neoplastic and Normal Human Mammary Tissue

Only recently, the apparent interaction between dehydroepiandrosterone (DHEA), 3β-hydroxy-5-androsten-17-one, glucose-6-phosphate-dehydrogenase (G-6-PDH, EC 1.1.1.49), and cyclic adenosine-3',5'-monophosphate (c-AMP) in normal and diseased subjects has been reported[1]. Although in vitro a distinct activation of c-AMP phosphodiesterase (EC 3.1.4.17) by DHEA and DHEA sulfatide could be demonstrated, this direct effect seems to be secondary to the indirect inhibition of said enzyme by DHEA and its sulfatide via the G-6-PDH system[2]. Here, the allosteric inhibition of G-6-PDH by DHEA and DHEA sulfatide[3] probably leads to the accumulation of G-6-P, which in turn is known as a competitive inhibitor of c-AMP phosphodiesterase[4]. Hence, the influence of DHEA, its endogenous or synthetic sulfoconjugates, as well as of other steroidal G-6-PDH inhibitors upon the growth of cell cultures from normal and neoplastic human tissue[5,6] eventually may also be ascribed to such regulatory effects. Furthermore, this hypothesis is supported by the observation that DHEA inhibits the mitosis of cultured human lymphocytes[7]. Based on these experiments, DHEA, G-6-PDH activity, and c-AMP were determined in normal and neoplastic human mammary tissue.

Material and methods. In the course of mastectomy, due to suspected mammary cancer, samples were removed from neoplastic and adjacent normal tissue and immediately submitted to histological inspection. For determination of total DHEA and G-6-PDH activity, one tissue aliquot was weighed and homogenized in 1.0 ml 0.9% sodium chloride/0.025% EDTA per 200 mg of wet tissue. After centrifugation for 10 min at 5000 *g*, the supernatant was decanted and assayed for total DHEA[8], G-6-PDH[9], and soluble proteins[10]. A second aliquot was homogenized in 1.0 ml 6% trichloroacetic acid per 50 mg of wet tissue and the homogenate centrifuged for 10 min at 10,000 *g*. Following decantation, the supernatant was diluted with 0.1 ml 0.1 *N* hydrochloric acid per ml and extracted with 4 volumes water-saturated ether. The ether extract was discarded and the aqueous phase subjected to the assay of c-AMP[11].

Results and discussion. Whereas the G-6-PDH activity in 7 samples of cancer tissue averaged 421 ± 241 mU/mg soluble proteins, the enzyme activity in all corresponding normal tissues did not even reach 25 mU/mg protein. Conversely, the concentration of total DHEA in cancer tissue, as compared to the normal tissue, was found to be decreased by 56.6 ± 13.6%. The individual concentrations, however, varied between 0.8 and 6.1 μg/mg soluble proteins in cancer tissue and between 1.8 and 12.3 μg/mg protein in normal tissue, thus preventing a direct statistical evaluation of tissue levels. From such findings it might be concluded that in cancer tissue the lack of DHEA or its endogenous sulfoconjugate, e.g. DHEA sulfatide, results in a considerable rise of intracellular G-6-PDH activity. Relatively low concentrations of DHEA in cancer patients have already been reported by SONKA et al.[12]. According to the aforementioned concept, the

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depletion of G-6-P by an unrestricted activity of G-6-PDH should be correlated with reduced concentrations of c-AMP in cancer tissue. Indeed, by comparison of cancer tissue and corresponding normal tissue, the concentration of the cyclic nucleotide in the former tissue turned out to be decreased by 69.7 ± 16.0%. Again, the individual values of either group exhibited substantial variations with levels between 0.13 and 2.30 pMol/mg cancer tissue and 0.55 and 3.98 pMol/mg normal tissue. Therefore any interaction between DHEA, G-6-PDH, and c-AMP under physiological conditions, suggested in general for some metabolic diseases, may very well also pertain to special tissue. Still, it remains to be seen to what extent DHEA or its sulfatide participate in the regulation of intracellular c-AMP levels, and hence in cell propagation[13].

Summary. When total DHEA, G-6-PDH activity, and c-AMP were determined in human neoplastic mammary tissue and corresponding normal tissue the G-6-PDH activity in the former tissue greatly exceeded that found in normal tissue. On the other hand, a remarkable decrease of total DHEA and c-AMP could be detected in cancer tissue, hinting at the participation of DHEA in the intracellular regulation of G-6-PDH and c-AMP levels.

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Ligand-Leakage in Affinity Chromatography: a Second Note on the Mathematical Approach

Experimental evidence[1] prompted Gribnau and Tesser[2] to derive a leakage-function that describes the hydrolytic release (above pH 5) of bioaffinity ligands attached to Sepharose, Sephadex or cellulose by the CNBr method.

In a note to their paper, Thöni[3] pointed out that the half-lives and the time course of ligand release can be calculated conveniently from tabulated χ^2-values using the well-known relation between the cumulative probability function of the Poisson-distribution and the χ^2-distribution.

The derivation of the leakage function of Gribnau and Tesser rests on 4 basic assumptions: a) at time $t = 0$ all ligand molecules are attached to the matrix by the maximal number of bonds, n, b) the cleavage of the ligand-matrix bonds is pseudo first order (approximately constant OH^- concentration in a buffered solution), c) all bonds are similar and split with the same rate constant, k, d) the bonds are split in a consecutive order, i.e., given a bond numbering which is not further specified, bond 2 will be attacked by OH^- only if bond 1 is cleaved and so forth.

Retaining assumptions a) to c) let us assume a random nucleophilic attack of the hydroxyl ions. This is equivalent to the statement that the cleavage of the ligand-matrix bonds does not depend on numbering.

If a is again the total ligand concentration (μmole/ml wet gel) and s the number of bonds hydrolyzed, then we have, at any time: $a = c_n + c_{n-1} + c_{n-2} + \cdots + c_{n-s} + \cdots + c_0$ (1), c_0 = concentration of free ligand. The number of different forms of the ligand species with s bonds split is $\binom{n}{s} = n!/s!\,(n-s)!$ These $\binom{n}{s}$ forms are kinetically degenerated because of assumption c) this is, any one of the remaining $(n-s)$ bonds will be attacked with the same probability in the next step.

Writing down the differential equations, care must be taken of statistical factors. If k is the pseudo first order rate constant, which is proportional to the probability of cleavage of a given bond during a fixed time interval, then the probability that any one bond of the ligand species with n points of attachment will be cleaved is proportional to nk. The statistical factor for ligands with n-1 points of attachment is n-1 and so on.

We can write now:

$dc_n/dt = -nkc_n$
$dc_{n-1}/dt = nkc_n - (n-1)kc_{n-1}$
$dc_{n-2}/dt = (n-1)kc_{n-1} - (n-2)kc_{n-2}$, etc.

The solutions to these differential equations are easily found by the procedures of Bernoulli or Lagrange[4]:

$c_n = a\exp(-nkt)$
$c_{n-1} = na\,[\exp(-(n-1)kt) - \exp(-nkt)]$
$c_{n-2} = n(n-1)a/2\,[\exp(-(n-2)kt) - 2\exp(-(n-1)kt) + \exp(-nkt)]$

$$c_{n-s} = \binom{n}{s} a \sum_{r=0}^{s}{}' \binom{s}{r} (-1)^{s-r} \exp(-(n-r)kt) \qquad (2)$$

It follows then that the leakage-function is

$$c_0/a = 1 - \sum_{s=0}^{n-1} \sum_{r=0}^{s} \binom{n}{s}\binom{s}{r} (-1)^{s-r} \exp(-(n-r)kt) \qquad (3)$$

This equation can be rearranged to

$$c_0/a = 1 - \sum_{s=1}^{n} \binom{n}{s} (-1)^{s-1} \exp(-skt) \qquad (4)$$

k is a measurable quantity, so that half-lives, τ_n, can be computed from:

$$1/2 = \sum_{s=1}^{n} \binom{n}{s} (-1)^{s-1} \exp(-sk\tau_n) \qquad (5)$$

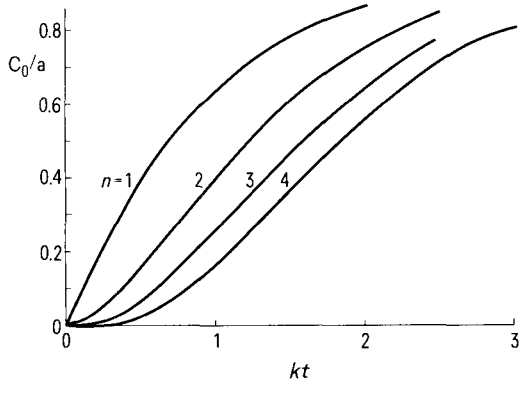

Fig. 1. Computed time course (cf. eqn. (4)) of the release of bioaffinity ligands attached to insoluble supports by the CNBr method.

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