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:

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

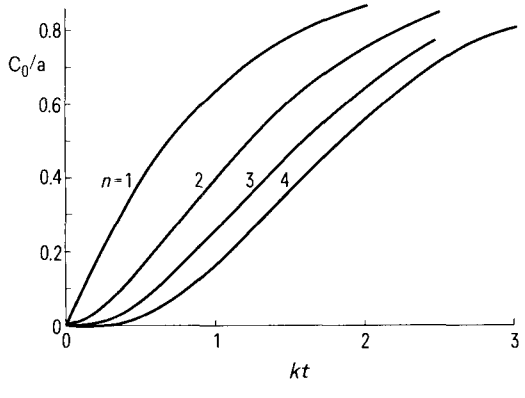

Fig. 1. Computed time course (cf. eqn. (4)) of the release of bioaffinity ligands attached to insoluble supports by the CNBr method.

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Calculation of half-lives, τ_n, using eqn. (5) and $k = 2.5 \times 10^{-5}$ (min^{-1}) determined experimentally by GRIBNAU and TESSER[2]

n	τ_n (days) Consecutive order model[2]	Random order model
1	19.16	19.16
2	46.67	34.17
3	74.67	43.89
4	101.94	51.11
5	129.72	56.67
6	157.50	61.67

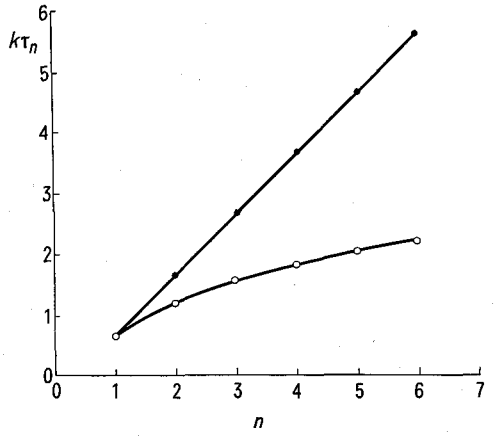

Fig. 2. Comparison of the $k\tau_n$ values computed from the consecutive order model[2] (●—●) and the random order model (○—○).

The time course of ligand release is computed from eqn. (4) (Figure 1). The shapes of these curves are qualitatively the same as in the GRIBNAU/TESSER model, although the analytical expression of the leakage-function is different (cf. eqn. (4)).

$k\tau_n$ values were computed from eqn. (5) using the iterative procedure of Newton and Raphson. They are compared with the values calculated by GRIBNAU and TESSER (Figure 2, Table). It is especially noteworthy that the increase of the $k\tau_n$ values with increasing n is not so steep as in the GRIBNAU/TESSER model. The interpretation of this somewhat surprizing result is that the stability gained by an additional point of attachment is partially offset by an increased probability of cleavage of a ligand-matrix bond. For $n = 1$, both models must yield the same $k\tau_1$. This condition is fulfilled by the randon model presented here (Figure 2).

Summary. A leakage function describing the hydrolytic release of ligand molecules covalently attached to insoluble supports by the CNBr method has been derived. Statistical factors were taken into account. The results of this random order model are compared with those of a consecutive order model proposed by GRIBNAU and TESSER.

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On the Role of Divalent Cations in the Reaction Mechanism of Malic Enzyme

NADP-linked malic enzyme (L-malate: NADP oxidoreductase (decarboxylating), EC 1.1.1.40) is known to require a divalent cation for activity[1]. Usually Mn^{2+} or Mg^{2+} are the better activators, but other cations, such as Co^{2+} and Ni^{2+}, are often able to replace Mn^{2+} or Mg^{2+}, at least to some extent[2].

We report here the results of some kinetic experiments on the activation of the NADP-linked malic enzyme partially purified from a marine Pseudomonad[3] by divalent cations.

The malic enzyme from the marine Pseudomonad was activated by several divalent cations. Mn^{2+}, Mg^{2+} and Co^{2+} were considerably more effective than Cd^{2+} and Ni^{2+}. When experiments with varying concentrations of divalent cation at fixed concentrations of the substrates L-malate (1 mM) and NADP (0.3 mM) were performed, the apparent V_{max} values obtained for the activation by Mn^{2+} and Mg^{2+} were similar, but the value for Co^{2+} was about half. The apparent K_a values were about 10^{-6} M, 2×10^{-6} M and 8×10^{-5} M for Co^{2+}, Mn^{2+} and Mg^{2+}, respectively.

The nature of the divalent cation used as activator affected the apparent kinetic constants for the substrates. Figure 1 shows the double reciprocal plots for the substrate L-malate obtained in the presence of 1 mM $MnCl_2$, $MgCl_2$ or $CoCl_2$. Substrate inhibition, previously reported for malic enzyme from other microorganisms[2], was clearly observed in the presence of Co^{2+} or Mn^{2+}, but not in the presence of Mg^{2+}. The apparent K_m values for L-malate obtained from the data of Figure 1 were 31,100 and 179 μM, in the presence of Co^{2+}, Mn^{2+} or Mg^{2+}, respectively. The apparent V_{max} value obtained in the presence of Co^{2+} was, however, considerably lower than those attained in the presence of Mn^{2+} or Mg^{2+} (Figure 1). The apparent K_m for NADP (not shown in the Figure) showed less variation with the nature of the divalent cation; under similar experimental conditions (1 mM L-malate) the values were 17, 24 and 22 μM, in the presence of Co^{2+}, Mn^{2+} or Mg^{2+}, respectively.

L-malate is known to be able to form complexes with divalent cations; the stability constant for the L-malate-Mn complex is greater than that for the L-malate-Mg complex[4]. The stability of the L-malate-Co complex might be expected to be of the same order or greater than that for L-malate-Mn, considering the usual order of effectiveness of the divalent cations to form complexes with organic ligands[4]. Two main roles for the divalent cation in the reaction mechanism of malic enzyme seem possible, both involving the ability of L-malate to form complexes with divalent cations. First, the L-malate-Me complex might be the true substrate of the reaction, as in the case of the $MeATP^{2-}$ complex for the kinases[5]; second, free cation might bind to the enzyme, and then act as a link between

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