Zusammenfassung

Nach Verabreichung von Vincamin sinkt bei akuten Versuchen an Ratten der Blutzuckerspiegel. Die nach Dextroseverfütterung verursachte Hyperglykämie wird durch Vincamin verhindert, der Blutzuckeranstieg jedoch bleibt nach Adrenalin unbeeinflusst.

Folic Acid Degradation by the Peroxidase from *Cicer arietinum*

In contrast to most other members of the vitamin B-complex, folic acid content of seeds has been found to decrease during germination[1,2].

Investigations on *Cicer arietinum*, designed to explore the mechanisms by which folic acid may be inactivated in seeds, have demonstrated the presence of a peroxidase which converts folic acid to 2-amino-4-hydroxy-6-formyl-pteridine and *p*-amino-benzoyl-glutamic acid.

Methods. Seeds of *C. arietinum* were sown on sterile cotton soaked in water and were allowed to germinate in the dark for 96 h.

Enzyme extracts. 15 g of seedling were extracted with 100 ml of 0.1 *M* phosphate buffer pH 5.5. After 1 h the extract was centrifuged for 10 min at 10,000 × *g* and the supernatant thus obtained was employed.

Heated extract. 20% crude extract prepared from seeds germinated for 96 h was heated at 90°C for 20 min, the coagulated proteins were removed by centrifugation and the supernatant was the source of active extract.

Enzyme assay. Activity was measured by estimating the diazotisable amine liberated from folic acid by the BRATTON and MARSHALL test[3] as described previously[4].

Results. Crude enzyme extracts prepared from *C. arietinum* have been found actively to liberate the aromatic amine from folic acid. The activity developed progressively during germination. In 96 h of germination in the dark, the seedling extracts could break down 50% of the added folic acid (300 µg) in 60 min. Dry seeds were inactive.

Attempts to study the cofactor requirements showed that extensive dialysis against water was not adequate to remove the cofactors. Resort to versene dialysis, followed by removal of versene by further dialysis against water, proved effective. Such preparations could be activated by the addition of heated extract. These observations indicate that metal ions may be required for the optimum activity of the enzyme system. In presence of heated extract, activity could be observed only in presence of air. The system was inactive under anaerobic conditions. Heated extracts prepared as described in the section on methods could be inactivated by further heating and reactivated by the addition of H_2O_2. As previous observations have demonstrated that folic acid may be inactivated by the peroxidatic action of methemoglobin[5], experiments were carried out to determine if a similar mechanism was involved in the present system. The results showed that in presence of enzyme extracts containing 100 µg of protein, folic acid could be degraded only if the inactivated heated extract was enriched with H_2O_2. The system was not active in absence of air. In presence of higher concentrations of enzyme (700 µg), folic acid degradation could be demonstrated with H_2O_2 alone without the addition of the heated extract. It was also observed that systems in which the heated extract could be dispensed with, were active both under aerobic and anaerobic conditions. In presence of oxygen, the extent of

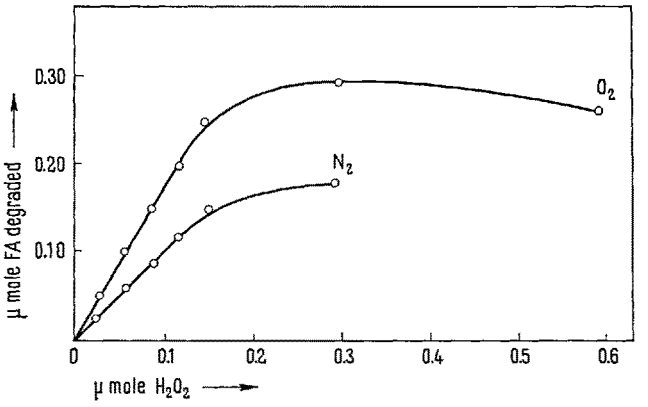

Fig. 1. Effect of varying concentrations of H_2O_2 on folic acid degradation by enzyme extract.
Reaction mixture consisted of FA 300 µg, PO_9^-. Buffer 0.025 M. pH 5.5, V. W. E. (700 µg protein) and varying amounts of H_2O_2. Total volume = 3.0 ml. Incubation time = 60 min. Temp. = 37°C.

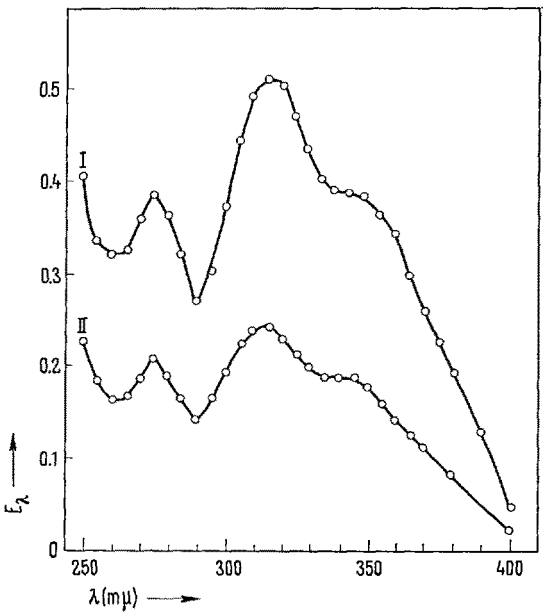

Fig. 2. Identification of carbonyl derivative as 2-amino-4-hydroxy-6-formyl pteridine.
Curve I. Acetic acid eluate containing enzymic degradation products of folic acid. *Curve II.* 2-amino-4-hydroxy-6-formyl pteridine in acetic acid.

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folic acid cleaved far exceeded the concentration of added H_2O_2 on molar basis. Under anaerobic conditions, the folic acid degraded was related to the amount of H_2O_2 added in the ratio of 1 : 1 within the range of 1 to 5 µg of H_2O_2 (Fig. 1). These observations suggest that, in absence of air, the cleavage of folic acid is purely due to the activity of the peroxidase. Addition of small quantities of catalase completely inhibited the system, indicating that H_2O_2 *per se* is essential for the enzyme system degrading folic acid.

A study of the products of reaction formed from folic acid by the peroxidase from *C. arietinum* showed that liberation of the aromatic amine was accompanied by a simultaneous increase in the carbonyl derivative. This was extracted by methods described previously[4] and identified as 2-amino-4-hydroxy-6-formyl-pteridine by the characteristic absorption spectrum (Fig. 2). The aromatic amine was identified as *p*-aminobenzoylglutamic acid by the ether extraction procedure described by STOKSTAD *et al.*[6]. It is thus clear that the peroxidase of *C. arietinum* brings about a cleavage of folic acid at C_9–N_{10} linkage.

Discussion. It is well known that peroxidases can function also as oxidases[7,8] and in many instances the requirements for trace amounts of H_2O_2 in the oxidase reaction have been clearly demonstrated. The results described here strongly suggest that folic acid is oxidised under aerobic conditions in presence of small quantities of H_2O_2 in a manner so as to generate more H_2O_2. Such a mechanism could account for the greater degradation of folic acid observed under aerobic conditions than expected on stoichiometric basis from the quantity of H_2O_2 added. Since the oxidase reaction cannot operate in absence of air, the breakdown of folic acid is then dependent on added H_2O_2. As only the peroxidase is effective under anaerobiosis, the ratio of folic acid degraded to H_2O_2 added is 1, as experimentally found. A possible reason for the lack of activity under anaerobic conditions when heated extracts were employed, could be that small quantities of added H_2O_2 were removed from the field of action by other peroxidase substrates present in the heated extract. Such an interference would be absent in the system containing only dialysed enzyme but no heated extract. Although the dialysed enzyme extracts are impure in so far as they contain adhering cofactors, it is clear from the results of the anaerobic experiments that they do not contain competing substrates for the peroxidase. Thus even 1 µg of H_2O_2 could degrade an equivalent quantity of folic acid.

The results reported are of interest in relation to the decrease in total folic acid content found to occur during the growth of seedlings of *C. arietinum*[9].

Since these seeds contain low catalase activity, it is conceivable that the peroxidatic breakdown operates *in vivo* and may play a role in the destruction of total folic acid observed during the germination of these and many other seeds.

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Résumé

La peroxidase des plantules de *Cicer arietinum* catalyse la transformation de l'acide folique dans 2-amino-4-hydroxy-6-formyl-pteridine et l'acide *p*-amino-benzoyl-glutamique. C'est peut-être le mécanisme responsable de la diminution de l'acide folique observée pendant la germination.

Wirkung von Δ^4-Cholesten-3,6-dion auf die Auslösung und Entwicklung von Tumoren

Aus zahlreichen tierexperimentellen Untersuchungen ist die Bedeutung von Steroidhormonen für die Auslösung und Förderung des Tumorwachstums unter bestimmten Bedingungen erwiesen[1]. Die Angaben über die Wirkung des Cholesterins und einiger Derivate sind nach bisherigen Versuchsergebnissen nicht übereinstimmend[2–6]. Besonderes Interesse hat das Auftreten ungesättigter Steroide mit UV-Absorption im Stoffwechsel Krebskranker erregt[7]. Bei dem von FIESER für Cancerogenese-Versuche synthetisierten, aber in seiner Wirkung umstrittenen Δ^4-Cholesten-3,6-dion (Cholesten-dion) handelt es sich um ein stark elektronenaktives ungesättigtes Sterin mit ausgeprägter UV-Absorption. Die zusätzliche Sauerstoff-Funktion in 6-Stellung ist zudem in bemerkenswerter Parallele bei speziellen Stoffwechsel-Reaktionen von Steroidhormonen zu finden.

Bei experimentellen, über viele Jahre an einer grossen Zahl von Mäusen durchgeführten Untersuchungen von HIEGER[8, 2, 3] sind nach subkutaner Injektion von Cholesterin lokal sarkomatöse Geschwülste aufgetreten, deren Frequenz bei verschiedenen Versuchen bemerkenswerte Schwankungen zwischen 0 und 14% aufweist. HIEGER nimmt an, dass diese unterschiedlichen Ergebnisse von individuellen Besonderheiten der Reaktionsfähigkeit der Tiere abhängig sind. Er vertritt jedoch die Meinung, dass dem Cholesterin eine schwache cancerogene Wirkung zukommt, wenngleich bisher nicht endgültig geklärt ist, ob das Cholesterin selbst oder endogen entstehende Oxydationsprodukte wirksam sind. BISCHOFF[5,6] hat nach subkutaner Verabreichung verschiedener von FIESER *et al.*[4] synthetisierter Oxydationsprodukte des Cholesterins bei einem bestimmten Mäusestamm (Marsh-Buffalo) in einem hohen Prozentsatz die Entwicklung lokaler sarkomatöser Geschwülste beobachtet, zum Beispiel nach Applikation von Cholesten-dion bei 33 Marsh-Buffalo-Mäusen in 34% Fibrosarkome. Diese Ergebnisse sind von HIEGER bei der Überprüfung an einem anderen Mäusestamm nicht bestätigt worden[3].

In eigenen Versuchen mit Cholesten-dion bei weissen Mäusen des Inzuchtstammes «Riems», einem Unterstamm des «Agnes-Bluhm»-Stammes, sowie bei schwarzen Mäusen des C57-black-Stammes ist nicht allein die lokale Wirkung nach subkutaner Injektion der Substanz in Sesamöl, sondern die allgemeine Reaktion der Versuchstiere durch die histologische Untersuchung vieler Organe geprüft worden. Da auf Grund der Erfahrungen von HIEGER nur mit einer schwachen cancerogenen Wirkung zu rechnen war, ist 2 Gruppen von Mäusen zusätzlich Cortisonazetat in Anlehnung an Versuche von SCHNITZER[9] verabreicht worden. Pro Versuchsgruppe sind 30 Mäuse verwendet worden,

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