Pyridins stets viel mehr Radioaktivität als im C-2 lokalisiert war. Im Falle des Peganins liegen nach Fütterung von (XIII) ähnliche Verhältnisse vor wie bei [10]. Wir dürfen annehmen, dass Asparaginsäure oder eine nahe verwandte Verbindung (Malat, Oxalacetat) direkt an der Biosynthese des Pyrrolidinringes vom (I) beteiligt ist.

Experimentelles. DL-Asparaginsäure-3-C^{14} (Calbiochem) wurde mit inaktiver (XIII) verdünnt und an Jungpflanzen (Sprosslänge 8–10 cm) von *Adhatoda vasica* Nees über die Wurzeln appliziert. Die Versuche wurden in 2 Vegetationsperioden durchgeführt. Einzelheiten sind der Tabelle zu entnehmen. Die Isolierung des Peganins sowie der Abbau des Alkaloids haben wir nach [1] durchgeführt (Schema). Peganin wurde mit $KMnO_4$ zur 4-Oxo-3,4-dihydro-chinazolyl-3-essigsäure (XV) oxydiert. Dessen Methylester (XVI) haben wir mit KOH gespalten, wobei Anthranilsäure (II) und Glycin (XIV) entstehen. Glycin wurde wie folgt isoliert: Zur Entsalzung haben wir die fast anthranilsäurefreie Lösung über Dowex 50 H^+ gegeben und die Aminosäure mit 1*n* Ammoniak eluiert. Die Reinigung des Glycins erfolgte durch DC an alkalischen Kieselgel G «Merck»: 1. Laufmittel 70% Äthanol. 2. Laufmittel *n*-Butanol/Wasser/Eisessig (4:1:1). Zur Elution benutzten wir jeweils 70% Äthanol.

Glycin wurde quantitativ in Anlehnung an KRÜGER[11] bestimmt. Einen aliquoten Teil der Aminosäure haben wir mit Ninhydrin umgesetzt und den entstandenen Formaldehyd als Dimedon-Derivat kristallin erhalten. Die Umkristallisation erfolgte aus verdünntem Äthanol, Smp. 189°. Zur Radioaktivitätsmessung diente der Methandurchflusszähler der Firma Friesecke und Höpfner[12].

Summary. Aspartate-3-C^{14} (XIII) was administered to young plants of *Adhatoda vasica* Nees. After a feeding period of 1 or 2 days, the isolated alkaloid peganine (=vasicine) (I) was degraded. The distribution of the radioactivity in the anthranilic acid (II) and glycine (XIV) obtained has been determined (Figure). Most of the radioactivity (~80%) was localized in the glycine corresponding to the positions 1 and 2 of peganine. More radioactivity was recovered from position 2 of the alkaloid than from position 1. The labelling pattern is discussed. The results indicate either that aspartate or a closely related compound is an immediate precursor of peganine.

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[12] Anmerkung. Die mögliche Beteiligung der Asparaginsäure an der Biosynthese des Peganins ist erstmalig im Frühjahr 1966 während eines Vortrages mit Professor Dr. H. G. FLOSS, Purdue University, Lafayette (USA), früher Org. chem. Institut der T.H. München, diskutiert worden.

Influence of Cytostatic Agents on Fibrinolytic and Proteolytic Enzymes and on Phagocytosis of Guinea-Pig Leucocytes

Several authors[1,2] postulated that proteolytic enzymes from leucocytes are involved in phagocytosis. The purpose of this work is to investigate the effect of some cytostatic agents on fibrinolytic and proteolytic enzymes and on phagocytosis index of guinea-pig leucocytes.

25 guinea-pigs of both sexes (weight 300–400 g) were used. Peritoneal exudates were evoked by injection of 0.1% solution of glycogen. The exudates were collected 15 h after injection of glycogen. Phagocytosis index was calculated according to the DAVIES method[3]. Polymorphonuclear leucocytes from exudates were mixed with opsonized strain of Staphylococcus aureus 209 P. 100 cells were counted in each sample. Saline solutions of the drugs were injected i.p. 1 h before collection of exudate. The following doses/kg body weight were applied: endoxan 2.5 mg, trenimon 0.25 mg, 5-fluorouracil 125 mg, mitomycin C 2.5 mg and nitrogranulogen 0.25 mg. The control group comprised 9 animals and 0.9% NaCl solution was injected instead of cytostatics. The leucocyte count of all samples amounted to 10,000/mm^3. Homogenate of the leucocytes were dialysed against phosphate buffer at + 4 °C for 18 h. The activity of alkaline and acid leucocyte protease was determined by the caseinolytic method. The activities of both proteases were expressed as increase of optical density × 10^3. The fibrinolytic enzymes: plasminogen, plasminogen activator and spontaneous fibrinolytic activity were studied, using ASTRUP and LASSEN plate method. Details concerning preparation of leucocyte homogenate and proteolytic and fibrinolytic methods will be reported elsewhere[4]. The fibrinolytic activity was expressed as mm^2 of area of digested fibrin.

The Table shows that cytostatic agents rapidly induce a significant decrease of all investigated fibrinolytic and proteolytic enzymes. The activities of these enzymes in cytostatic treated leucocytes amounted on the average to about 20% of the values of control leucocytes. This decrease of enzymatic activities was statistically significant ($p < 0.01$) when 9 samples of control leucocytes were compared with 16 samples of leucocytes collected from animals treated with various cytostatic agents. A slight decrease of phagocytosis index in treated animals was observed but this is not statistically significant. No correlation between the degree of inhibition of phagocytosis index and the decrease of activities of fibrinolytic and proteolytic enzymes can be stated in particular animals. It is of interest to notice that in some leucocyte samples plasminogen disappeared while the phagocytosis index was only slightly depressed. Therefore it can be concluded that the plasminogen is not directly involved in phagocytosis. This observation is in good agreement with another finding of one of us[4] that plasminogen is not

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Phagocytosis index, activities of acid and alkaline protease, plasminogen, plasminogen activator and spontaneous fibrinolytic activity of the leucocytes in control and cytostatics treated guinea-pigs

Number of animals	Treatment	Phagocytosis index	Acid protease	Alkaline protease	Plasminogen	Plasminogen activator	Spontaneous fibrinolytic activity
9	Control	11.8 (4.3–13.4)	66.0 (35–129)	90.0 (59–102)	82.0 (35–124)	578.0 (397–877)	78.0 (30–96)
3	Mitomycin C	8.1 (5.3–10.4)	20.0 (5–28)	10.5 (4–16.5)	23.0 (16–28)	194.0 (82–273)	27.0 (6–46)
4	Nitrogranulogen	8.0 (6.5–9.8)	14.0 (3–27)	8.0 (2–16)	20.0 (0–30)	135.0 (93–222)	3.0 (0–12)
3	Endoxan	7.2 (5.5–8.8)	16.0 (10–21)	37.3 (22–50)	7.0 (5–9)	333.0 (325–343)	44.0 (16–69)
3	Trenimon	6.9 (5.8–8.7)	23.0 (19–26)	31.0 (23–35)	19.0 (8–35)	144.0 (71–226)	21.0 (9–44)
3	5-Fluorouracil	6.4 (5.5–6.8)	48.6 (40–54)	18.6 (9–25)	21.0 (0–42)	110.0 (70–159)	10.0 (7.5–12)

No. in brackets denotes minimal and maximal values.

particularly localized in lysosomes but it is almost equally distributed in the whole leucocyte. Acid and alkaline proteases are supposed to be most significant enzymes for phagocytosis process. If it is true, we can suggest that there is an excess of these enzymes in normal cell and their decrease to about 9% of initial values (e.g. alkaline protease after nitrogranulogen) may be without any significant influence on the phagocytosis index. Cytostatic agents added in vitro to the leucocytes suspension inhibited also fibrinolytic and proteolytic activities. The rapid disappearance of plasminogen from cytostatic treated leucocytes suggests that these cells may be the site of biosynthesis of this proenzyme[5].

Résumé. Nous avons trouvé que les agents cytostatiques (endoxane, trenimone, 5-fluorouracile, mitomycine C, nitrogranulogène) causent une inhibition considérable de l'activité des enzymes protéolytiques et fibrinolytiques des leucocytes du cobaye, sans affecter de manière significative la phagocytose. Le plasminogène des leucocytes n'est pas indispensable à la phagocytose et d'autres enzymes protéolytiques s'y trouvent probablement en excès.

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Department of Physiological Chemistry and Department of Pathological Anatomy of the Medical Academy, Bialystok (Poland), 24th April 1967.

[5] Aided in part by a grant from the Biochemical and Biophysical Committee, Second Department, Polish Academy of Sciences.

Lack of Effect of Constant Magnetic Fields on *Drosophila* Egg Hatching Time

Reports have appeared in the literature describing effects of constant magnetic fields in various biological systems[1,2]. Possible effects are, however, often obscured by the large spread in the biological parameter investigated, and by the small numbers of individuals treated.

The hatching time of *Drosophila melanogaster* wild type eggs should provide a sensitive test of such effects. Firstly, because it involves many of the crucial steps in the development of living organisms, such as synthesis of nucleic acids, enzymes and other vital molecules, as well as the more obscure processes involved in cell division and differentiation. Secondly, because it is a biological parameter of unusually small spread. Thus, in our experiments, its value at 25 °C was about 20 h, and the time required for the transition from 20% to 80% eggs hatched about 0.5 h, thus being only about 3% of the mean. Being familiar with this material[3], it seemed a good system for detection of a possible effect of magnetic fields.

Eggs were collected in 10 min periods, and placed in a constant magnetic field for approximately 18 h, until scoring was started. Reasonably homogeneous fields of about 1600 Gauss and about 5000 Gauss were used. In 2 experiments, using in all about 800 embryos, no difference in hatching time could de demonstrated between eggs in the magnetic field and simultaneously collected control eggs in the same temperature controlled environment.

Zusammenfassung. Die Wirkung homogener magnetischer Felder von 1600 und 5000 Gauss auf die Schlüpfzeit von *Drosophila*-Embryonen (Normalschlüpfzeit: 20 ± 0,5 h) wurde untersucht. Es konnte keine Wirkung festgestellt werden.

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