rung nach gleichzeitiger Zugabe des Fällungsmittels und der Monosaccharide. Bei einer Initialgeschwindigkeit des Glucoseschwundes bzw. der Desoxy-D-glucose-6-phosphatbildung von 0,5 µMol/ml Zellen · Sekunde (30 °C)[5,8] ergaben die «Blindwerte» bei gleichzeitigem Zusatz von Trichloressigsäure bzw. Perchlorsäure und der Monosaccharide in beiden Fällen ohne Unterschied scheinbare Umsätze von weniger als 0,10 µMol, was einer Zeit bis zur Unterbrechung des Zellstoffwechsels von etwa 200 msec entsprechen würde. Auf Grund unserer Ergebnisse muss bei Hefezellen eine Fällung mit Perchlorsäure und nachfolgendem Aufschluss durch dreifaches Frieren und Tauen als die für die quantitative Bestimmung verschiedener Metabolite am besten geeignete Methode angesehen werden.

Summary. The exact assay of metabolites like NAD, ATP and other glycolytic substances in intact yeast cells greatly depends on the method of cell extraction. Reproducible and high levels can be obtained after prolonged incubation at high temperatures or by freezing and thawing of the denatureted cellular material in perchloric acid, thus eliminating possible errors due to different recoveries in the cell-free extracts.

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Physiologisch-Chemisches Institut der Medizinischen Akademie Magdeburg (DDR), 30. Januar 1967.

[8] H. W. AUGUSTIN und E. HOFMANN, Acta biol. med. germ. *11*, 628 (1963).

The Distribution of Quinone Pigments in Echinoderms

A characteristic feature of echinoderms is the occurrence of a variety of quinone pigments in their calcareous skeleta (spinochromes) and in parts of their viscera (echinochromes)[1]. Neither the biogenesis nor the function of these compounds is known at present, but some interesting aspects of their distribution have come to light in the course of our work.

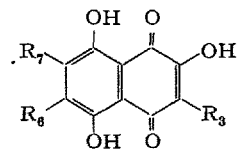
Of the 5 classes in the phylum *Echinodermata* (echinoids, holothurians, asteroids, ophiuroids, and crinoids) the echinoids (sea urchins) have received the closest attention. As a result of our work[2,3] and of research in other laboratories[1] it appears that this class of animals synthesizes predominantly (we have found a single benzoquinone[2]) polyhydroxynaphthoquinones, the hydroxy groups of which are not methylated and which frequently bear a single two-carbon side chain[1-3]. It is of biogenetic interest that no pigment with a one-carbon side chain, a prevalent feature of naphthoquinone pigments derived from higher plants and fungi, has ever been found among the naphthoquinone pigments of echinoderms.

All echinoids which had been examined up to very recently were representatives of the subclass *Euechinoidea*. We have now investigated 3 species of the more primitive subclass *Perischoechinoidea* and have found that these animals produce naphthoquinones of identical type. From the spines and tests (shells) of *Chondrocidaris gigantea* Agassiz and of *Prionocidaris* (*Stephanocidaris*) *hawaiiensis* Agassiz and Clark we have isolated and identified by direct comparison with authentic specimens spinochromes A, C, and D as the principal pigments. Similarly, we have demonstrated the presence of spinochromes A and C and of 2,7-dihydroxynaphthazarin in *Eucidaris metularia* Lamarck.

While none of the polyhydroxyquinones which have been isolated from echinoids possesses an alkylated hydroxy group, the holothurian (sea cucumber) *Polycheria rufescens* Brandt produces as its principal pigment namakochrome (I), which is the monomethyl ether of spinochrome E[4]. The structures of compound I and of all other naphthopurpurin derivatives described in this report are shown in the Table.

We have now examined for the first time representatives of 2 classes of echinoderms, the asteroids (sea stars) and the ophiuroids (brittlestars). From the spines of the asteroid *Acanthaster planci* Linn. we have isolated by previously described procedures[2] 2,6-dihydroxy-3,7-dimethoxynaphthazarin (II), m.p. 252–254 °C, and 2,7-dihydroxy-3,6-dimethoxynaphthazarin (III), m.p. 218–219 °C, in yields of 0.002 and 0.005%. The structures of II and III were rigorously established by comparison with authentic samples, by hydrolysis to spinochrome E, and by methylation to 2,3,6,7-tetramethoxynaphtha-

Naphthopurpurin derivatives from echinoderms



	R_3	R_6	R_7
I	OH	OH	OCH_3
II	OCH_3	OH	OCH_3
III	OCH_3	OCH_3	OH
IV	C_2H_5	H	H
V	$COCH_3$	H	H
VI	H	C_2H_5	H
VII	$COCH_3$	H	OH
VIII	$COCH_3$	H	OCH_3
IX	C_2H_5	H	OH
X	OH	OH	C_2H_5

[1] R. H. THOMSON, in *Comparative Biochemistry* (Ed. M. FLORKIN and H. S. MASON; Academic Press, New York 1962), vol. 3, p. 631.

[2] R. E. MOORE, H. SINGH and P. J. SCHEUER, J. org. Chem. *31*, 3645 (1966).

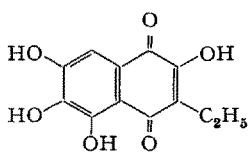
[3] C. W. J. CHANG, R. E. MOORE and P. J. SCHEUER, Tetrahedron Lett. 3557 (1964).

[4] M. YAMAGUCHI, T. MUKAI and T. TSUMAKI, Mem. Fac. Sci. Kyushu Univ. Ser. C. *4*, 193 (1961).

zarin[5]. We have thus shown that the asteroid *A. planci* and the holothurian *P. rufescens* elaborate closely related pigments: partially methylated derivatives of spinochrome E. It should be pointed out that, unlike the situation which exists in natural products of plant origin, methyl ethers of alcohols and phenols are only rarely encountered in animals, an observation which was first made by BERGMANN[6,7].

Examination of the spines of 2 species of ophiuroids, *Ophiocoma erinaceus* Müller and Troschel and *O. insularia* Lyman revealed a situation which is in sharp contrast with that found in the asteroid. These animals produce a complex array of pigments in striking parallel to the spectrum of compounds which we have encountered in 2 species of the echinoid genus *Echinothrix*[2]. From *O. erinaceus* and *O. insularia* we have isolated and identified 2-hydroxy-3-ethylnaphthazarin (IV), m.p. 185–186 °C, 2-hydroxy-3-acetylnaphthazarin (V), m.p. 163–164 °C (dec.), 6-ethyl-2-hydroxynaphthazarin (VI), m.p. 204–204.5 °C, spinochrome A (VII), and 2-hydroxy-3-acetyl-7-methoxynaphthazarin (VIII), m.p. 246–248 °C, in yields ranging from 10^{-6}–10^{-5}%. In addition, from *O. erinaceus* alone, we isolated and identified 2,7-dihydroxy-3-ethylnaphthazarin (IX), m.p. 190–192 °C, and echinochrome A (X). The structures of compounds IV–X were proven by comparison with authentic synthetic samples and/or by conversion to known methyl derivatives[5,8].

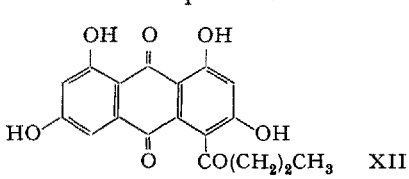
In addition to these 7 known compounds, we found in both species of *Ophiocoma* 2 new unmethylated (n.m.r. evidence) polyhydroxynaphthoquinone pigments. One of them, 2,6,7-trihydroxy-3-ethyljuglone (XI), m.p. 220–226 °C, was isolated in 10^{-4}% yield.



XI

Its structure was secured from the parent ion peak at m/e 250 in its mass spectrum, by conversion to the known 2,6,7-trimethoxy-3-ethyljuglone[5], and by comparison with the borohydride reduction product of 2,6,7-trihydroxy-3-acetyljuglone[9]. The second new compound, m.p. 258–262 °C (dec.), which we obtained in 10^{-5}% yield, is nitrogenous (mass spectral parent peak at m/e 251) and has not yet been characterized. It is interesting that we have also isolated this unusual nitrogenous pigment from the echinoid *Echinothrix diadema* Linn.

The remaining class of echinoderms, the crinoids (sea lilies), present an uncertain picture. DIMELOW[10] reported circumstantial evidence of the occurrence of naphthoquinones in the arms and pinnules of the crinoid *Antedon bifida* (Pennant). SUTHERLAND[11,12], on the other hand, isolated from the crinoid *Comatula pectinata* Linnaeus a series of anthraquinones, which are partial methyl ethers of rhodocomatulin (XII). SUTHERLAND's publications, it should be pointed out, do not state from what parts of the animals the pigments were isolated. We have now had the opportunity to examine the calcareous portions of a crinoid belonging to the genus *Antedon* from Eniwetok, from which we isolated small amounts of the naphthoquinone derivatives spinochrome A and 2,7-dihydroxynaphthazarin, which were present in the animal in addition to quantities of pigments which we did not recognize and which we have not so far investigated further.



XII

Our data therefore point to a close relationship between echinoids and ophiuroids on one hand, and between asteroids and holothurians on the other. The position of the crinoids is not entirely clear on the basis of existing data, but the fact that they alone seem to elaborate anthraquinones may place them apart from the other 4 classes. Similar relationships were established previously on the basis of other chemical parameters. BERGMANN[13] reported the occurrence of Δ^7-sterols in asteroids and holothurians, and of Δ^5-sterols in echinoids, ophiuroids, and crinoids. He further points out that the sterols of more primitive echinoids, such as *Cidaroida* and *Cassiduloida*, have not been examined. In another publication BERGMANN[14] reports the isolation of a possible Δ^7-sterol from a slate-pencil sea urchin (binomial not given). More recently HASHIMOTO and his group[15] have examined the distribution of steroidal glycosides among the echinoderms. Only holothurians and asteroids were found to elaborate this group of substances.

The distribution of all 3 chemical indicators, quinones, sterols, and saponins, points to the relationship indicated above. The importance of minor deviations, BERGMANN's possible Δ^7-sterol in an echinoid[14] and our single methyl ether (VIII) isolated from an ophiuroid cannot be assessed adequately on the basis of available data. It is worth noting in this connection that the genus *Ophiocoma* belongs phylogenetically to an advanced family (*Ophiocominae*) of ophiuroids[16]. Perhaps a better perspective of the relationship of ophiuroids to the other classes of the phylum will results from an examination of members of a more primitive family, e.g. *Asteronychidae*, and of fossils[16].

Our findings agree with the classical embryological theory that echinoids and ophiuroids have descended from a common ancestor, while asteroids and holothurians have evolved from another[17]. However, paleontological and anatomical considerations, based on fossil evidence, imply that asteroids and ophiuroids are derived from a common origin, and echinoids and holothurians from a different one[17,18]. According to both phylogenetic views crinoids have evolved from a third ancestry. Since

[5] R. E. MOORE, H. SINGH, C. W. J. CHANG, and P. J. SCHEUER, Tetrahedron, in press.
[6] W. BERGMANN and D. C. BURKE, J. org. Chem. *21*, 226 (1956).
[7] W. BERGMANN and M. F. STEMPIEN, J. org. Chem. *22*, 1575 (1957).
[8] R. E. MOORE, H. SINGH, C. W. J. CHANG and P. J. SCHEUER, J. org. Chem. *31*, 3638 (1966).
[9] We are indebted to Dr. M. D. SUTHERLAND, University of Queensland, for the sample of the 3-acetyl compound, the structure of which he had proven by deacetylation and subsequent conversion to 2,5,6,7-tetramethoxynaphthoquinone.
[10] E. J. DIMELOW, Nature *182*, 812 (1958).
[11] M. D. SUTHERLAND and J. W. WELLS, Chem Ind. 291 (1959).
[12] R. J. PARK, M. D. SUTHERLAND and I. VESSEY, Aust. J. Chem *18*, 182 (1965).
[13] W. BERGMANN, in *Comparative Biochemistry* (Ed. M. FLORKIN and H. S. MASON; Academic Press, New York 1962), vol. 3, p. 103.
[14] W. BERGMANN and I. I. DOMSKY, Ann. N.Y. Acad. Sci. *90*, 906 (1960).
[15] T. YASUMOTO, M. TANAKA and Y. HASHIMOTO, Bull. Jap. Sco. scient. Fish. *32*, 673 (1966).
[16] S. MURAKAMI, Trans. R. Soc. N.Z. (Zoology) *4*, 1 (1963).
[17] H. B. FELL, Rep. Smithson. Instn 457 (1962).
[18] D. NICHOLS, *Echinoderms*, revised edn (Hutchinson University Library, London 1966).

crinoids are the first echinoderms to appear in the fossil record, all echinoderms may ultimately have evolved from a now extinct crinoid. The presence of quinone pigments in crinoid limestone has been demonstrated[19], but it is interesting to note that the modern comatulid crinoids are apparently the only echinoderms which synthesize anthraquinones[11,12,20].

Zusammenfassung. Das Vorkommen chinonoider Pigmente bei allen Echinodermen deutet auf eine Beziehung zwischen Seeigeln und Schlangensternen, ebenso zwischen Seesternen und Seewalzen hin. Die Verteilung der Sterine und der Saponine weisen in dieselbe Richtung. Die klassische embryologische Theorie stützt diese Verwandtschaftsauffassung.

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Department of Chemistry, University of Hawaii, Honolulu (Hawaii 96822), 25th January 1967.

[19] M. BLUMER, Science *149*, 722 (1965) and earlier references cited therein.

[20] Supported by PHS Grant No. GM-10413 and by NSF Instrument Grant No. GP 5813. We thank Mr. D. M. DEVANEY for helpful discussions.

Circulating Plasma Kinin in Patients with Bronchial Asthma

Biologically active polypeptides, plasma kinins, have been considered to play a role in the pathogenesis of various clinical conditions, such as hereditary angioneurotic oedema[1], pancreatitis[2], or carcinoid syndrome[3]. According to COLLIER and his co-workers[4], bradykinin given i.v. to the guinea-pig increased the resistance of the lung to inflation. In the studies on asthmatic patients, HERXHEIMER[5] observed a decrease in the vital capacity and appearance of audible wheezing after the inhalation of bradykinin aerosol, while no changes were found in normal persons. However, these results are not direct evidence that bradykinin plays an actual role in asthmatic patients.

Recently, we developed a new method for determining the kinin content in peripheral blood[6]. With this method, blood kinin levels in asthmatic patients were determined and hitherto unreported data, which give further evidence for the participation of kinin in the etiology of bronchial asthma, were obtained.

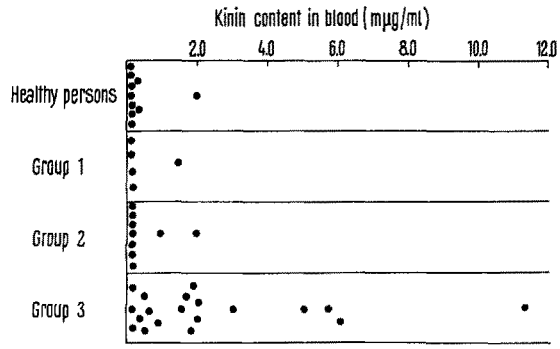
Patients. 10 healthy persons and 33 asthmatic patients were subjected to this study. The asthmatic patients were divided into 3 groups according to their clinical features, as follows: group 1 consisted of 5 patients in whom no complaints or clinical signs were recognized at the time of experiment. Group 2 consisted of 9 patients in whom wheezing and rhonchi were audible on auscultation, but dyspnea was not apparent. Group 3 consisted of 19 patients in whom wheezing was audible by the unaided ear. In this group, sibilant rales were heard on auscultation over all the lung field, and dyspnea was distinct, especially in the recumbent posture. Cyanosis was observed not infrequently.

Methods. A syringe and a needle were siliconized and kept in cold before use. 10 ml of venous blood were rapidly drawn from the antecubital vein into the syringe which contained ethylenediamine tetraacetic acid solution. The sample was immediately transferred to a centrifuge tube containing hydrochloric acid, and shaken sufficiently.

The mixed solution was adjusted to twice the volume with 20 ml of *n*-butanol. After the elimination of water in the butanol phase by adding 10 g of anhydrous sodium sulphate, the active substance was re-extracted twice with 4 ml and 2 ml of distilled water. The aqueous extract was concentrated around 0.6 ml under reduced pressure at below 40 °C. After neutralization, the final volume of the extract was adjusted to 2.0 ml with water. This solution was assayed on the isolated guinea-pig ileum suspended in a bath filled with Tyrode solution at 33–35 °C. Atropine sulphate (10^{-6}) and promethazine hydrochloride (10^{-7}) were added to Tyrode solution.

The active substance extracted by this method was thermostable and destroyed by the incubation with chymotrypsin. This substance also elicited the slow contraction on the isolated guinea-pig ileum and vasodilatation of a dog's femoral artery, and showed the same migration rate as that of synthetic kallidin of paper chromatography (butanol-acetic acid-water, 4:1:1 v/v). From these results, the extracted substance was tentatively identified as a kinin.

Results. The estimated values of blood kinin in healthy persons and asthmatic patients are shown in the Figure.



Kinin contents in peripheral venous blood in healthy persons and asthmatic patients.

[1] N. S. LANDERMANN, M. E. WEBSTER, E. L. BECKER and H. E. RATCLIFFE, J. Allergy *33*, 330 (1962).
[2] A. P. THAL, E. E. KOBOLD and M. J. HOLLENBERG, Am. J. Surg. *105*, 708 (1963).
[3] J. A. OATES, K. MELMON, A. SJOERDSMA, L. GILLESPIE and D. T. MASON, Lancet *1*, 514 (1964).
[4] H. O. J. COLLIER, J. A. HOLGATE, M. SCHACHTER and P. G. SHORLEY, Br. J. Pharmac. Chemother. *15*, 290 (1960).
[5] H. HERXHEIMER and E. STRESEMAN, J. Physiol. *158*, 38P (1961).
[6] K. ABE, N. WATANABE, N. KUMAGAI, T. MOURI, T. SEKI and K. YOSHINAGA, Tohoku J. exp. Med. *89*, 103 (1966).