in a non-competitive way (unpublished data), db-Guo-3′:5′-P contracts the tissue in a dose-response relationship. Atropine blocks completely the contraction evoked by db-Guo-3′5′-P, as shown in the graph.

Separate experiments on stomach strips, obtained from rats previously vagotomized, as described by PATON and VANE[13] show that db-Guo-3′:5′-P has completely lost its contracting action. These results suggest that Guo-3′:5′-P is related to the mechanism of cholinergic transmission. In agreement with this hypothesis are the results of GEORGE et al.[14] on the acetylcholine (Ach) effect on isolated perfused rat heart: Ach increases Guo-3′:5′-P levels in this tissue and decreases those of Ado-3′:5′-P. MURAD et al.[15] have demonstrated that in homogenized dog hearts choline esters are inhibitors of Ado-3′:5′-P formation. Furthermore, FERRENDELLI et al.[16] have shown that oxotremorine treatment in mouse increases the Guo-3′:5′-P levels in cerebral cortex and cerebellum, an effect readily blocked by treatment with atropine.

On the basis of our results and these observations, it may be concluded that Guo-3′:5′-P is physiologically related to the autonomic nervous system. In order to clarify the mechanism of action of this naturally occurring nucleotide (Guo-3′:5′-P) and its relationship to the second sympathetic messenger (Ado-3′:5′-P) experiments on other smooth muscle activities are in progress.

Riassunto. Viene mettona in evidenza l'effetto contratturante del Guo-3′:5′-P sulla muscolatura liscia di stomaco di ratto in vitro. Tale effetto è antagonizzato dall'Atropina e scompare dopo vagotomia. Una possibile interazione fra il Guo-3′:5′-P e la trasmissione colinergica sembra così emergere da questi primi risultati.

L. PUGLISI, F. BERTI and R. PAOLETTI

Institute of Pharmacology and Pharmacognosy, University of Milano, Via A. del Sarto 21, I-20129 Milano (Italy), 8 March 1971.

[13] W. D. PATON and J. R. VANE, J. Physiol., Lond. *165*, 10 (1963).
[14] W. J. GEORGE, G. B. POLSON, A. G. O'TOOLE, Proc. natn. Acad. Sci. *66*, 398 (1970).
[15] F. MURAD, Y. M. CHI, T. W. RALL and E. W. SUTHERLAND, J. biol. Chem. *237*, 1233 (1962).
[16] J. A. FERRENDELLI, A. L. STEINER, D. B. McDOUGAL and D. M. KIPNIS, Biochem. Biophys. Res. Commun. *41*, 1061 (1970).

Antiviral Substances in Starfish

Starfish have been known to contain toxic substances in their tissues[1]. In 1960, HASHIMOTO and YASUMOTO[2] first reported the occurrence of saponin-like compounds in a Japanese species, *Asterina pectinifera*. Similar findings were reported with other Pacific and Atlantic species[3]. The saponins were described as hemolytic and cytotoxic[3a, b, 4]. Their actions to the nerve system were also discussed in comparison to a sea-cucumber saponin, holothurin A[5].

In the crude extract of a common Atlantic starfish, *Asterias forbesi*, the author found the presence of substances which are active in the antiviral test using influenza virus in chicken embryo. Further investigation confirmed the activity in many other species. Purification of the active components has been carried out with 3 species: *Acanthaster planci* (a South Pacific species), *Asterias forbesi* (a common Atlantic species) and *Asterias pectinifera* (a Japanese species). A standard procedure for the purification of the active components was established using the activity as guidance.

Ground air-dried starfish were extracted with chloroform. The chloroform extract was discarded. The residue was exhaustively extracted with a mixture of methanol and chloroform (1:1). The concentrated extract was dissolved in water and centrifuged. The supernatant was dialyzed to distilled water. The non-dialyzable fraction was lyophilized and chromatographed on a silica gel column. This operation afforded the active fraction as a mixture of several substances having very close Rf values on thin-layer chromatograms (TLC). The major components were further separated by repetition of dry-column chromatography and preparative TLC. The fractions which show single spots on TLC were passed through cation exchange resin (Na^+ form) and recrystallized from EtOH and Acetone-H_2O. The recovery of the substances from the chromatography was very poor, and only two each of the major components could be brought to purified form. The yields of the purified products were generally a few mg each from 100 g of the dried starfish. The isolated substances and their activities are listed in the Table.

The compounds are of highly glycosidic nature, and seem to coincide with the so-called asterosaponins reported by YASUMOTO and HASHIMOTO[2]. A relatively high yield of ASP-I from *Asterina pectinifera* enabled the author to obtain more information about the chemical nature of the compound.

Antiviral activities of purified fractions from starfish

Origin	Compounds	Melting points	Concentration (mg/ml)	Results
Asterias forbesi	AF-I	amorph	0.4	512
	AF-II	205–210°	0.5	16
	AF-III	218–223°	0.4	16
Acanthaster planci	AP-I	228–235°	0.4	16
	AP-II	215–219°	0.5	16
Asterina pectinifera	ASP-I	181–185°	0.3	128
	ASP-II	229–232°	0.3	16
				Control 4096

[a] Chick embryo technic was used. The inhibition of influenza virus multiplication by 1 ml of the test solution in an embryonated egg is expressed by the maximum dilution ratio of the allantoic fluid which causes hemaglutanation. The value 16 indicates the highest activity measurable by this assay.

[1] B. W. HALSTEAD, *Poisonous and Venomous Marine Animals of the World* (U.S. Govt. Printing Office, Washington, D.C. 1965), vol. 1, p. 537.
[2] Y. HASHIMOTO and T. YASUMOTO, Bull. jap. Soc. Sci. Fish. *26*, 1132 (1960).
[3] a. G. J. RIO, G. D. RUGGIERI, M. F. STEMPIEN JR. and R. F. NIGRELLI. Am. Zool. *3*, 554 (1963). – b. G. J. RIO, M. F. STEMPIEN JR., R. F. NIGRELLI and G. D. RUGGIERI, Toxicon *3*, 147 (1965). – c. T. YASUMOTO, M. TANAKA and Y. HASHIMOTO, Bull. jap. Soc. Sci. Fish. *32*, 673 (1966).
[4] G. D. RUGGIERI and R. F. NIGRELLI, Am. Zool. *4*, 431 (1964).
[5] S. L. FRIESS, R. C. DURANT and J. D. CHANLEY, Toxicon *6*, 81 (1969).

ASP-I (I) shows the similar mobility of TLC as holothurin A, and forms prisms from acetone, mp 181–185°, and crystalline powder from ethanol, mp 200–205° (decomp.). The IR-spectrum displays its glycosidic nature [intense absorptions at 3400 cm^{-1} (OH), and 1050 cm^{-1} C-O], and the presence of a sulphate group (1220 cm^{-1}). Elementary analysis gave C, 54.02%; H, 8.29% and S, 2.06%. Assuming the presence of 1 sulphate group in the molecule, the molecular weight, ca. 1500, was speculated. The substance is a sodium salt of a sulphate ester, and upon treatment with cold HCl, it liberated a free sulphate compound (II), needles mp 105–110° (decomp.), KBr_{max} 3400 (OH), 1200 cm^{-1} (sulphate), which showed the same mobility on partition type TLC as I. I was hydrolyzed with 2*N* HCl at 80° for 2 h to the aglycone and sugar fraction. Examination of the sugar fraction with various solvent systems shows that it consists mainly of one kind of sugar (III) which has a very similar Rf value to 3-*O*-methylglucose, and a trace of quinovose.

The aglycone (IV), needles (from MeOH and water), mp 202–210° shows strong absorptions for hydroxyl groups at 3400 cm^{-1} and lacks absorptions for carbonyl groups in the IR-spectrum. High resolution mass spectrum of IV gave the molecular ion at *m/e* 434.33852, from which the molecular formula $C_{27}H_{46}O_4$ (Calcd. 434.33961) was computed. The NMR-spectrum showed a methyl signal pattern similar to that of cholesterol (a composite of methyl signals around 9.10 and a signal for 18-methyl group at 9.35)[6]. In the mass spectrum, peaks arisen by dehydration of up to 4 molecules of water and several demethylated fragments were observed. On the basis of these data, ASP-I is considered to be a hydroxylated cholestene derivative conjugated with 5–6 sugar molecules and a sulphate group probably on the sugar part. Recently, MACKIE et al.[7] also have reported a cholestene derivative as a constituent of the avoidance substance excreted by starfish, *Marthasterias glacialis*. The aglycones of holothurins from sea-cucumbers are known to be triterpenoids having lanostane skeleton[8]. YASUMOTO and HASHIMOTO reported asterogenin I and II for the aglycones of Asterosaponins from *Asterias amurensis*. The same empirical formula, $C_{22}H_{34}O_3$, was postulated for both aglycones[9]. They also claimed the steroidal nature of the compounds from color reactions. The variation of the aglycones in the same phylum Echinodermata arouses considerable interst[10].

Zusammenfassung. Eine Gruppe von teilweise chemisch charakterisierten Verbindungen, welche die Vermehrung des Influenza-Virus in Huhnembryo-Test verhindern, wurde aus den Seesternen *Asterias forbesi*, *Asterias pectinifera* und *Acanthaster planci* isoliert.

Y. SHIMIZU

Department of Pharmacognosy, College of Pharmacy, University of Rhode Island, Kingston (Rhode Island 02881, USA), 7 May 1971.

[6] N. S. BHACCA and D. H. WILLIAMS, *Application of NMR Spectroscopy in Organic Chemistry* (Holden-Day, Inc., San Francisco 1964), p. 5.

[8] J. D. CHANLEY, T. MAZZETTI and H. SABOTKA, Tetrahedron *22*, 1857 (1966). – J. D. CHANLEY and C. ROSSI, Tetrahedron *25*, 1897 (1969); *25*, 1911 (1969).

[7] A. M. MACKIE and A. B. TURNER, Biochem. J. *117*, 543 (1970).

[9] T. YASUMOTO and Y. HASHIMOTO, Agric. biol. Chem. (Japan) *31*, 368 (1967).

[10] This research was supported by the N.S.F. Sea-Grant Program. The author thanks Dr. H. W. YOUNKEN JR. and Dr. L. R. WORTHEN for their encouragement and suggestions throughout this work. Thanks are also due to Dr. HASHIMOTO, Tokyo University, and Dr. YASUMOTO, Tohoku University, for the specimen of *Asterina pectinifera*, and Dr. B. M. BRANHAM, University of Hawaii, for *Acanthaster planci* sample. The antiviral test was carried out by Dr. O. LIU, Northeast Shellfish Sanitation Research Center, D. H. E. W., Narragansett, Rhode Island, to whome the author expresses his sincere gratitude.

Effect of Cerium on Drug Metabolizing Activity in Rat Liver

Earlier investigations have shown that after i.v. injection of light lanthanons, including cerium, there are changes in the liver endoplasmic reticulum, which manifest primarily as dilatations of the cisternae and dissociation of ribosomes[1].

The liver microsomes, which consist of the fragments of the endoplasmic reticulum in vitro, contain enzymes which detoxicate foreign compounds and metabolize drugs. We were interested in finding out if and to what extent the morphological changes induced by cerium injection are accompanied by an impairment of the drug metabolizing capacity of rat liver.

Material and methods. In the present study, male Sprague-Dawley rats weighing 180–270 g were used. They were obtained from Orion Oy, Finland. The animals were given normal laboratory food and water ad libitum. Following a single i.v. injection of 2 mg cerium/kg of body weight as chloride in physiological saline solution of pH 3.5–4.0, the rats were decapitated after 1, 2, 3, 6 and 16 days always at the same time of the day.

The 20% liver homogenates were prepared in 1.15% ice-cold KCl with a motor driven Potter-Elvehjem type homogenizer. The 15,000 × *g* supernatant was used as enzyme preparation. The microsome fraction for cytochrome P-450 determinations was obtained by centrifuging the supernatant at 105,000 × *g* for 1 h and resuspending the pellet in 0.1 *M* phosphate buffer, pH 7.4.

The incubation mixtures and other procedures for determining the activities of oxidative enzymes, which hydroxylate hexobarbital and demethylate N-methylaniline, were similar to those previously described[2]. Cytochrome P-450 content was determined according to the method described by OMURA and SATO[3]. The blood glucose was determined by the glucose-oxidase method and the free fatty acids were titrated according to TROUT et al.[4]. Protein concentrations were assayed by the biuret procedure. Student's *t*-test was used in calculating the significance of the results.

Results. The cerium treated animals behaved normally. No differences in food or water consumption or in weight increase could be seen, compared with controls. Macroscopically the livers of cerium treated animals showed a typical occurrence of fatty degeneration already on the first day after injection.

In vitro studies demonstrated that both hexobarbital and N-methylaniline metabolism in liver had already decreased significantly on the 1st day after cerium injection. On the 2nd day these metabolic rates declined further and the lowest levels were reached on the 3rd day after injection when the hexobarbital oxidation and