tion of the lipid or lipo-protein bodies[4]. The present author, in conformity with the views of GATENBY and MOUSSA[5], and ISSIDORIDES and SHANKLIN[6], considers these bodies to be some secretion products (endoerdonous[3]).

(3) *Pale-yellow substance:* This is seen as a pale-yellow diffuse substance in the cytoplasm and in the duplex bodies of the living spinal neurones of the frog, the toad, and rarely in those of *Natrix* and *Eryx*. This substance corresponds to a similar product described by THOMAS[3] and MALHOTRA[7] in the neurones of *Helix* and the frog respectively. The views regarding the elaboration of secretion products in the cores of the duplex lipid bodies have been advanced and bolstered for different animals by HIRSCH[8], BAKER[9], etc.

(4) *Hyaline spheres:* They originate from the nucleus and measure 0.2 μ to 5 μ and even more in diameter. After their discharge into the cytoplasm, they grow (Figure 2.) Although the chemical nature of the hyaline spheres remains undetermined, yet in the *Lissemys* nerve cells, a few spheres, which have in their vicinity an area of very small phospholipid granules, react feebly for the acidic lipids in the cortical sites. Sometimes, as seen in the *Eryx* and *Lissemys* neurones, extremely small phospholipid granules have also been seen attached to the outer borders of these spheres. All this tentatively suggests the participation of lipids as one of the 'building materials' of these bodies. Feeble acid phosphatase activity has been recorded in the cortical sites of a few hyaline spheres of the *Natrix* nerve cells. The hyaline spheres have been seen migrating into the inter-neuronal spaces in the case of the toad, *Lissemys*, *Eryx* and *Periophthalamus* nerve cells (Figure 3). Axonal transportation of these spheres has been demonstrated only in one or two *Lissemys* nerve cells. These bodies have been considered as some secretion products, both endoerdonous and exoerdonous[3]. PALAY[10] has also described the nuclear origin of the secretory products in certain fishes. Secretion in the form of vacuoles has been described by HAMMAR[11], MAZIARSKY[12,13], SMITH[14], GRZYCKI[15], etc.

Although it is not possible, with the prevalent histochemical techniques or with the methods alleged to be 'selective' for the neurosecretory substances, to detect the presence of the hormones – a fair indication of neurosecretion – the various intra-neuronal as well as inter-neuronal inclusions described above, cannot be dismissed as 'ordinary' cytoplasmic inclusions, particularly when they exhibit so many physiological characteristics: some significance, in the sense of their being 'neurosecretory' substances, seems essential.

The view that only those cells which show certain morphological specializations[16,17] can secrete the neurosecretory substances (NSS), seems untenable in the materials studied, although it may hold good for the other actively secretory centres, such as the mid-brain, hypophysis, etc. Also, the various stains – particularly the CH – which are alleged to be 'selective' for the NSS fail to warrant any substantial statement as to the histochemical background of the secretion products. Even the 'selectivity' of these stains is fraught with many pit-falls. Thus, in teleosts, skates, and amphibians, even some chrome haematoxyphobe substances have been labelled as the NSS[16].

In the author's opinion, it is more rational to pursue the problem of neurosecretion in the light of cellular physiological events rather than to attach unqualified importance to certain particular stains.

Résumé. Des recherches effectuées sur les neurones spinaux de quelques Poissons, Amphibiens et Reptiles ont amené l'auteur à considérer comme substances neurosécrétoires les 4 types d'inclusions telles que les sphères lipides, les sphères hyalines, la substance jaune-pâle et les corps pigmentés dits lipofuscins en se basant sur leurs caractères originels, leur foncton vraisemblable, leur rôle probable et leur destin final. Le chrome hématoxyphile ou l'aldéhyde fuchsine positive n'ont pas été observés.

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PRO EXPERIMENTIS

Determination of Mass Spectra of Non-Volatile Substances[1]

The main restriction to the wide application of mass spectrometry in organic chemistry has been the inability to volatilize in undecomposed form many organic substances. The use of heated inlet systems[2,3] (preferably all-glass) has greatly increased the use of this physical tool in the past few years as demonstrated especially by the very recent applications in the field of natural products[4–6]. Nevertheless, there exists a large number of interesting organic compounds which have resisted until now mass spectrometric investigation because of their non-volatility even in such heated inlet systems.

One way around this difficulty is the direct insertion of the substance near the electron beam. Such a system is available[3] in the Bendix Time-of-Flight mass spectrometer, but the latter suffers from the serious limitation

[1] This paper represents part XXII in the series *Mass Spectrometry in Structural and Stereochemical Problems*. For preceding article, see E. LUND, H. BUDZIKIEWICZ, J. M. WILSON, and C. DJERASSI, J. Amer. Chem. Soc., in press.
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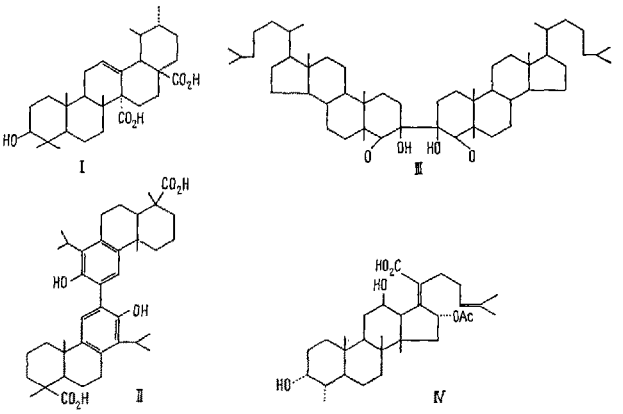
in that the mass range does not extend as far as the conventional magnetic instruments. REED et al.[7] have described a direct inlet system for the Metropolitan Vickers (A.E.I.) M.S.2 mass spectrometer, but the absence of a vacuum lock makes its use somewhat inconvenient. We should now like to describe a simple, all-glass inlet system which permits the direct insertion of the material near the ion source in a conventional Consolidated Electrodynamics Corp. mass spectrometer model 23-103C. As a result, we have been able to determine the molecular weights and mass spectral fragmentation patterns of organic substances, which have hitherto been outside the scope of practical mass spectrometry.

The glass system is reproduced in Figure 1 and is sealed directly to the inlet tube (F) of the CEC mass spectrometer, which is being continuously evacuated by the vacuum system of the mass spectrometer, stopcock A being closed. A small amount of the substance (less than 1 mg) is placed at the end of the glass sample holder (C), which is inserted through the joint (E). This system is now evacuated for less than 1 min by opening stopcock (B) which is then closed and stopcock (A) opened. Since the latter has a bore of 8 mm, while the sample holder (C) consists of a 3 mm glass rod, the sample can be moved very easily by means of the magnet holder (D) into the inlet tube (F). The position of the sample in the inlet tube (F) is governed by the volatility of the sample and is usually between 1–2 cm away from the repeller plate. For the majority of samples, spectra are obtained without any heating of the inlet tube (F), but the heating coil (G) is used to sublime the last traces of material from the inlet tube after the spectrum has been run and the sample holder (C) withdrawn.

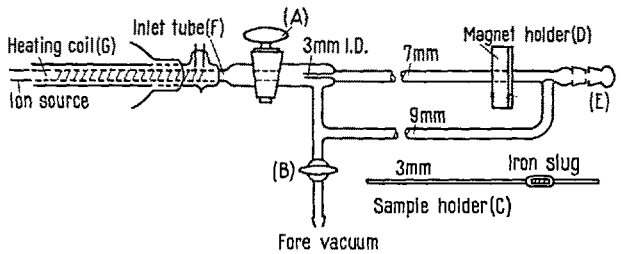
As an example of the remarkable utility of this simple system, we cite herewith examples of substances, the mass spectra of which could not be determined by any of the hitherto available procedures. Thus in our recent paper[8] on the relation of mass spectral fragmentation patterns and structure among pentacyclic triterpenoids, it was not possible to use directly free carboxylic acids and it was necessary to handle the corresponding esters. By means of the present system, we were able to obtain the mass spectrum, including the molecular ion peak at *m/e* 486 of even the triterpenoid dibasic acid quinovic acid (I) (m.p. 298° (dec.)). No difficulty was encountered in observing the molecular ion peak (*m/e* 630) of macrophyllic acid (II) (m.p. 238°)[9a] and the related podototarin[9b] dimethyl ether (m.p. 294°) or even that (*m/e* 802) of the steroid dimer cholestenone pinacol oxide (III)[10]. Dimeric indole alkaloids of molecular weight above 800 have been handled with ease and so have various porphyrins in the mass range above 700[11].

A particularly striking illustration of the use of this new inlet system is provided by the steroid antibiotics. The structure of fusidic acid (IV) has recently been established[12] and we found that its empirical formula ($C_{31}H_{48}O_6$) could be confirmed readily through the mass spectrum of its methyl ester, which exhibited a peak at *m/e* 530 corresponding to the molecular ion. Cephalosporin P_1 has been assumed[13] to possess the empirical formula $C_{32}H_{48}O_8$; in view of the additional acetyl function this would require that it contain one less methyl group than fusidic acid (IV). Recently, Dr. T. G. HALSALL of Oxford University supplied us with cephalosporin P_1 and several of its derivatives. In each instance, it was possible to obtain either a molecular ion peak or a *M*-60 peak (loss of acetic acid) which demonstrated that cephalosporin actually corresponded to the empirical formula $C_{33}H_{50}O_8$ and hence contained one additional methyl group[14]. Similarly, we find a *M*-60 peak at *m/e* 508 for helvolic acid (supplied by Drs. T. G. HALSALL and P. CRABBE), which makes untenable the earlier assigned structure[15] for this antibiotic since the mass spectrum requires the empirical formula $C_{33}H_{44}O_8$ rather than $C_{32}H_{42}O_8$[15].



The principal disadvantage of the presently described inlet system is its use of a lubricated stopcock (A). The hydrocarbon grease employed gives a fairly intense spectrum at lower mass numbers, but above *m/e* 150 the intensity of these peaks is negligible. Since the present inlet system will be used largely for non-volatile substances, notably natural products, of much higher mass range, this does not present any difficulties for molecular weight determinations, although it may interfere in the evaluation of mass spectral fragmentation patterns in the lower mass range. Work is currently in progress in our laboratory to develop a grease-less joint to substitute for stopcock (A) (Figure).

The above examples included substances[16] with molecular weights in excess of 800. We have found that while di-



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rect counting of the peaks from the photographic records is feasible to about 600, beyond this range, it is necessary to use the technique of measuring accurately the ion accelerating voltage[17].

Zusammenfassung. Ein einfaches Glas-Einlass-System wird beschrieben, das die direkte Einführung von organischen Substanzen in die Ionisationskammer eines Massenspektrometers gestattet. Es ist somit möglich Massenspektren von Substanzen zu bestimmen die man bisher nicht aufnehmen konnte. Beispiele werden angeführt.

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Dünnschichtchromatographischer Nachweis der Aminosäuren im Urin

Dünnschichtchromatographie ist ein vorzügliches Instrument zur qualitativen Analyse von Eiweisshydrolysaten und anderen relativ salzarmen Aminosäuregemischen[1–3]. Sie versagt aber oft, ähnlich wie die Papierchromatographie, bei der Untersuchung von Urin, Blutplasma, Preßsäften und ähnlichem Material. Die in solchen Fällen durch den Salzeffekt bedingte Störung lässt sich umgehen, wenn die Aminosäuren in Derivate umgewandelt werden, die sich vor der Chromatographie dank ihrer Löslichkeit in organischen Solventien von den Salzen abtrennen lassen. Besonders geeignet ist die Umwandlung der Aminosäuren in Dinitrophenyl-(= DNP-)-Aminosäuren mittels 2,4-Dinitrofluorbenzol (DNFB). Sie ist auch in verdünntesten Lösungen ohne langwierige Vorbereitungen rasch und praktisch quantitativ durchführbar. So haben zum Beispiel GERBER und GERBER[4] den β-Amino-isobuttersäure-Gehalt von Urin mittels Papierchromatographie des entsprechenden DNP-Derivates ermittelt; PERAINO und HARPER[5] beschreiben die Dinitrophenylierung von $HClO_4$-enteiweisstem Blutplasma und die papierchromatographische Bestimmung der erhaltenen ätherlöslichen DNP-Aminosäuren. Ähnlich, aber dünnschichtchromatographisch, haben sich einige Aminosäuren in Gäransätzen von Traubenmost nachweisen lassen[6].

Nr.	DNP-Aminosäure	Abkürzung für die zugehörige Aminosäure
1	DNP-Alanin	Ala
2	DNP-β-Alanin	β-Ala
3	DNP-α-Aminoadipinsäure	Aad
4	DNP-α-Aminobuttersäure	Abut
5	DNP-γ-Aminobuttersäure	γ-Abut
6	DNP-α-Aminocaprylsäure	Acy
7	DNP-β-Aminoisobuttersäure	β-AiB
8	DNP-Asparagin	Asp (NH_2)
9	DNP-Asparaginsäure	Asp
10	Di-DNP-Cystein	Cys
11	Di-DNP-Cystin	$(Cys)_2$
12	DNP-Glutamin	Glu (NH_2)
13	DNP-Glutaminsäure	Glu
14	DNP-Glycin	Gly
15	Di-DNP-Histidin	His
16	Di-DNP-Hydroxylysin	Hylys
17	DNP-Hydroxyprolin	Hypro
18	DNP-Isoleucin	Ileu
19	DNP-Leucin	Leu
20	Di-DNP-Lysin	Lys
21	DNP-Methionin	Met
22	DNP-Methiononsulfon	$MetO_2$
23	Di-DNP-Ornithin	Orn
24	DNP-Phenylalanin	Phe
25	DNP-Prolin	Pro
26	DNP-Sarkosin	Sar
27	DNP-Serin	Ser
28	DNP-Threonin	Thr
29	DNP-Tryptophan	Try
30	Di-DNP-Tyrosin	Tyr
31	DNP-Valin	Val
32	2,4-Dinitrophenol	DNP-OH
33	2,4-Dinitroanilin	DNP-NH_2
34	α-DNP-Arginin	Arg
35	DNP-Citrullin	Cit
36	DNP-Cysteinsäure	$CySO_3H$
37	DNP-Taurin	Tau

Die Verwendung der Dünnschichtchromatographie bietet in der Tat grosse Vorteile[7,8]. Es ist uns inzwischen gelungen,
(a) durch Modifikation der Fliessmittel und der Chromatographiertechnik den Trenneffekt bei den ätherlöslichen DNP-Aminosäuren noch weiter zu steigern, und
(b) durch Essigester/Butanolextraktion auch die säurelöslichen DNP-Aminosäuren von den Salzen abzutrennen und damit chromatographierbar zu machen. Somit liegt jetzt ein Verfahren vor, das es gestattet, die 37 in der Tabelle aufgeführten Verbindungen, die bei der Dinitrophenylierung von biologischem Material auftreten können, nebeneinander nachzuweisen.

Dinitrophenylierung der Urinaminosäuren. Die üblichen Dinitrophenylierungsverfahren[9] beziehen sich auf Lösungen mit relativ hoher Aminosäurekonzentration. Im vorliegenden Fall arbeiten wir in Anlehnung an eine Vorschrift von PERAINO und HARPER[5].

Die verwendete Menge DNFB (5facher Überschuss) haben wir anhand bekannter Daten[10] über die oberen Grenzen der Aminosäuremengen in 24-h-Urin festgelegt; die Zweckmässigkeit dieses Vorgehens ist durch Vergleichsversuche mit der 0,1-, 0,25-, 0,5- und 2fachen

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