method and appear to be the methods of choice for the isolation of purified cRNA.

Much work remains to be done on the characterization of cRNA. We hope that this report will help others to extend these studies and will stimulate further investigations of the cellular function of this RNA.

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References and Notes

1. R. C. Huang and J. Bonner, *Proc. Nat. Acad. Sci. U.S.A.* **54**, 960 (1965).
2. J. Bonner and J. Widholm, *ibid.* **57**, 1379 (1967).
3. M. Dahmus and D. McConnell, *Biochemistry* **8**, 1524 (1969).
4. H. W. Heyden and H. Zachau, *Biochim. Biophys. Acta* **232**, 651 (1971).
5. M. Artman and J. S. Roth, *J. Mol. Biol.* **60**, 291 (1971).
6. F. Szeszák and A. Pihl, *Fed. Eur. Biochem. Soc. Lett.* **20**, 177 (1972).
7. Y. Sivolap and J. Bonner, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 387 (1971).
8. D. Holmes, J. Mayfield, L. Murthy, J. Bonner, in preparation.
9. J. Mayfield and J. Bonner, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 7 (1972).
10. ———, *ibid.* **68**, 2652 (1971).
11. M. Dahmus, thesis, California Institute of Technology (1968).
12. T. Shih and J. Bonner, *Biochim. Biophys. Acta* **182**, 30 (1969).
13. M. J. Getz and G. F. Saunders, personal communication.
14. R. Jacobson and J. Bonner, *Arch. Biochem. Biophys.* **146**, 557 (1971).
15. R. Jacobson and J. Bonner, *Biochem. Biophys. Res. Commun.* **33**, 716 (1968).
16. Unpublished data; cRNA prepared from calf thymus by the method of Mayfield and Bonner (*10*) hybridizes to 2 percent of homologous DNA. The hybridization reaction was carried out as described by Gillespie and Spiegelman (*17*) and McConaughy *et al.* (*18*).
17. D. Gillespie and S. Spiegelman, *J. Mol. Biol.* **12**, 829 (1965).
18. B. L. McConaughy, C. D. Laird, B. J. McCarthy, *Biochemistry* **8**, 3289 (1969).
19. W. Marzluff, M. M. Smith, R. C. Huang, *Fed. Proc.* **31**, 1594 (1972).
20. R. C. Huang, *Biophys. J.* **9**, A86 (1969).
21. To isolated rat ascites chromatin (*3*) were added [^{3}H]tRNA (3×10^6 count/min) and [^{32}P]ribosomal subunits (5×10^7 count/min). Recovered in the CsCl skin (*3*) were 1.3×10^2 count/min from ^{3}H and 4×10^6 count/min from ^{32}P, and in the cRNA peak on DEAE Sephadex (*3*) were 96 and 8 count/min from ^{3}H and ^{32}P, respectively, values representing less than 1 percent of the cRNA when the input tRNA and rRNA is normalized to the mass of these species present in ascites cells.
22. A. Zamir, R. W. Holley, M. Marquisee, *J. Biol. Chem.* **240**, 1267 (1965).
23. R. C. Huang and P. C. Huang, *J. Mol. Biol.* **39**, 365 (1969); L. M. Shaw and R. C. Huang, *Biochemistry* **9**, 4530 (1970).
24. We acknowledge the continuing advice of our colleague S. Harris. We thank E. Davidson for critically reviewing the manuscript and to H. Tse for technical assistance. Supported in part by PHS grants GM-13762 and GM-86, and by a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft to G.S.

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Indole-3-Acetic Acid in Human Cerebrospinal Fluid: Identification and Quantification by Mass Fragmentography

Abstract. *Indole-3-acetic acid has been identified in human cerebrospinal fluid by the gas chromatographic–mass spectrometric technique called mass fragmentography. A specific and sensitive method for quantitative determination of indole-3-acetic acid down to 2 nanograms per milliliter of cerebrospinal fluid has been developed. Samples of cerebrospinal fluid from 24 patients with depression contained 6.1 ± 3.1 (range 2.6 to 15.8) nanograms of indole-3-acetic acid per milliliter.*

5-Hydroxytryptamine (5-HT) is catabolized in the central nervous system (CNS) by monoamine oxidase to 5-hydroxyindole-3-acetic acid (5-HIAA). Several investigators (*1*) have found low concentrations of apparent 5-HIAA in cerebrospinal fluid (CSF) of depressed patients compared to controls. The level of 5-HIAA in CSF has been used as an index of brain 5-HT metabolism (*2*).

The formation of 5-HT is a minor pathway in the biotransformation of tryptophan in animals and man. The

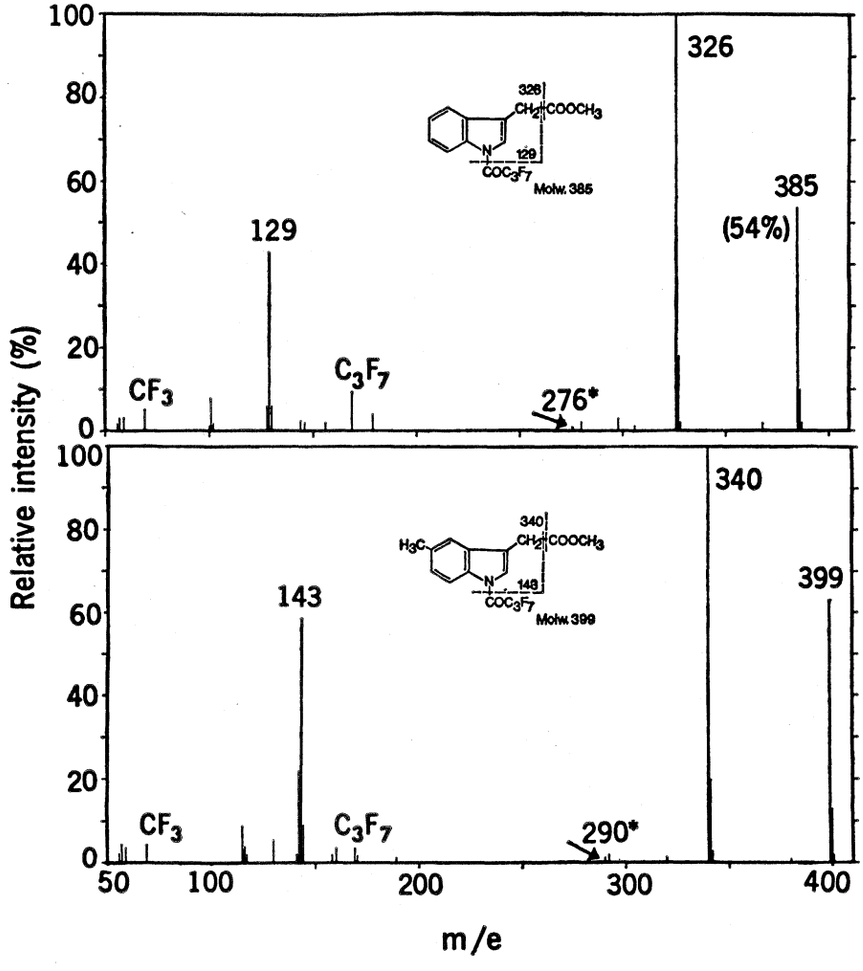

Fig. 1. Mass spectra and proposed fragmentation patterns for the heptafluorobutyryl methyl ester derivatives of reference IAA (top) and 5-MeIAA (bottom). Asterisks indicate metastable peaks. An LKB 9000 gas chromatograph–mass spectrometer with a silanized glass column (1 m by 3 mm, inside diameter), packed with 3 percent XE-60 on Gas Chrom P was used. Column, flash heater, and ion source temperatures were 130°, 210°, and 270°C, respectively. The ionizing potential was selected to 40 ev, as this was found to give the highest yield of *m/e* 326 from the IAA derivative (shown by mass fragmentography). The trap current was 60 μa; *Molw.*, molecular weight; *m/e*, mass-to-charge ratio.

catabolism by the liver enzyme tryptophan pyrrolase (or oxygenase) is a quantitatively more important pathway (*3*). It has been hypothesized that there is an increased pyrrolase activity in depression (*4*). An enhanced metabolism of tryptophan by way of the kynurenine pathway in the periphery should leave less tryptophan for the synthesis of 5-HT and tryptamine in the CNS.

Coppen *et al.* (*5*) measured the urinary excretion of tryptamine and its major metabolite indole-3-acetic acid (IAA) by fluorometry. Tryptamine was recently demonstrated in small amounts in rat brain by means of an enzymatic technique (*6*). However, it has not been possible to detect IAA in the CNS of animals or man because of the lack of analytical methods with sufficient sensitivity and specificity.

If IAA could be codetermined with 5-HIAA in human lumbar CSF, current theories about the biochemistry of depressive disorders could be further elucidated. Thus an increased tryptophan pyrrolase activity should decrease the 5-HT and tryptamine in brain with accompanying decrease in concentrations of 5-HIAA and IAA in CSF. Low amounts of 5-HT in brain (5-HIAA in CSF) may also be due to decreased activity of tryptophan-5-hydroxylase, which should be accompanied by increased brain tryptamine, possibly reflected by an increase in IAA in CSF. The hydroxylation is the rate-limiting step in the biosynthesis of 5-HT (*7*).

Due to the considerations above we report our effort to identify and quantitatively determine IAA in human lumbar CSF.

A method for the quantitative analysis of 5-HIAA in CSF by a gas chromatographic–mass spectrometric technique called mass fragmentography has been described (*8*). The 5-HIAA was extracted from CSF with ethyl acetate, and the methyl ester was prepared with diazomethane. Two heptafluorobutyryl groups were introduced on the phenolic hydroxyl and the indole nitrogen groups, respectively. The diheptafluorobutyryl methyl ester derivative of 5-HIAA was analyzed by mass fragmentography.

The above procedures for extraction and preparation of derivatives were used for the analysis of IAA. The mass spectrum of the heptafluorobutyryl methyl ester derivative of IAA (Fig. 1, top) prepared from milligram amounts of synthetic IAA (Merck, Germany) had a prominent molecular ion (*m/e* 385), and the base peak at *m/e* 326 formed by the loss of the carbomethoxy group. The metastable peak at *m/e* 276 is compatible with this transition.

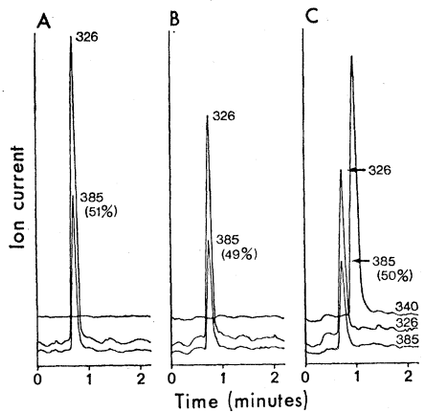

Fig. 2. Mass fragmentograms obtained from heptafluorobutyryl methyl ester derivatives prepared from (A) reference IAA, (B) material extracted fom CSF, (C) material extracted from CSF to which the internal standard (5-methylindole-3-acetic acid) had been added. The CSF sample contained 12 ng of IAA per milliliter. The mass spectrometer was set to detect *m/e* 340, 326, and 385 on channels 1 to 3 with relative sensitivities 1 : 4 : 4, respectively. The column temperature was 150°C. The other instrumentation conditions were as described in the legend to Fig. 1.

In analogy to the mass fragmentographic analysis of 5-HIAA, the mass spectrometer was set to detect compounds eluted from the gas chromatograph giving rise to the ions *m/e* 326 and 385. Derivatives were prepared (i) from synthetic IAA and (ii) from material extracted from human CSF. Mass fragmentograms (Fig. 2, A and B) were obtained. This shows that CSF contains a compound with the same retention time and relative proportion of the ions *m/e* 326 and 385 as synthetic IAA. The same results were obtained with another gas chromatographic column (5 percent OV-17 on Varaport 30). This shows that IAA is present in very small but measurable amounts in human CSF. The derivative from synthetic IAA gives, in addition to the above-mentioned ions, another prominent fragment at *m/e* 129 (Fig. 1, top). In view of the high background signal at this *m/e* from unknown compounds in CSF, it was impossible to use it to detect IAA in CSF.

For the quantitative determination of IAA in CSF, 5-methylindole-3-acetic acid (5-MeIAA, from Fluka, Switzerland) has been used as an internal standard. The mass spectrum of the heptafluorobutyryl methyl ester derivative of 5-MeIAA showed, in accordance with that of IAA, a molecular ion at *m/e* 399 with the base peak at *m/e* 340 (Fig. 1, bottom).

To 2.0 ml of CSF, 240 ng of 5-MeIAA was added along with the compounds added for the analysis of 5-HIAA (*8*). The procedures for extraction and making derivatives have been described. For the mass fragmentographic analysis of IAA, the ions characteristic of IAA (326 and 385) and the base peak of 5-MeIAA (340) were monitored (Fig. 2C).

The ratio of the peak heights of IAA (*m/e* 326) and 5-MeIAA (*m/e* 340) was calculated, and the concentration of IAA in CSF was determined from a standard curve. This standard curve was prepared by treating solutions of known amounts of IAA (2 to 20 ng/ml) in artificial CSF [see (*8*)] in the same way as the CSF samples. The peak height ratio (*m/e*, 326 to 340) was plotted against the IAA concentration of the standard solutions. The curve is a straight line going through origin. When 16.0 ng of IAA was added to a 2.0 ml CSF sample containing 4.1 ng of endogenous IAA per milliliter, this amount could be quantitatively recovered.

This sensitive and specific method has been tested on CSF samples that were available from 24 somatically healthy, depressed patients taking part in a biochemical, clinical, and pharmacological investigation of endogenous depression. The lumbar punctures were performed during a period when the patients were given a placebo prior to antidepressant therapy. The patients had been fasting for at least 9 hours, and the CSF samples were collected in glass tubes. The samples were immediately frozen and stored at −15°C until analyzed. Each sample was analyzed in duplicate for its content of IAA. In the investigated patients the concentration of IAA in CSF was 6.1 ± 3.1 ng/ml (mean ± standard deviation), with the range being 2.6 to 15.8 (*9*). The standard deviations of the analyses were 0.5 and 1.0 ng/ml in the concentrations below and above 5 ng/ml, respectively.

Further experiments are needed to elucidate how these data compare to IAA levels in CSF of psychiatrically healthy volunteers and whether IAA

in CSF originates from oxidative deamination of tryptamine in the brain or from the systemic circulation. There are indications that tryptamine, but not 5-HT or 5-HIAA, enters the brain from the bloodstream (*10*). Lovenberg *et al.* (*11*) have shown that brain contains the enzymes responsible for the transformations tryptophan → tryptamine → IAA. However, it cannot be excluded that IAA also is formed by transamination of tryptophan with subsequent oxidative decarboxylation (*12*). The intermediate in this pathway is indole-3-pyruvic acid.

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References and Notes

1. G. W. Ashcroft and D. F. Sharman, *Nature* **186**, 1050 (1960); S. J. Dencker, U. Malm, B.-E. Roos, B. Werdinius, *J. Neurochem.* **13**, 1545 (1966); H. M. van Praag and J. Korf, *Psychopharmacologia* **19**, 148 (1971).
2. A. T. B. Moir, G. W. Ashcroft, T. B. B. Crawford, D. Eccleston, H. C. Guldberg, *Brain* **93**, 357 (1970).
3. G. Curzon and P. K. Bridges, *J. Neurol. Neurosurg. Psychiat.* **33**, 698 (1970).
4. G. Curzon, *Brit. J. Psychiat.* **115**, 1367 (1969).
5. A. Coppen, D. M. Shaw, A. Malleson, E. Eccleston, G. Gundy, *ibid.* **111**, 993 (1965).
6. J. M. Saavedra and J. Axelrod, *Science* **175**, 1365 (1972).
7. W. Lovenberg, E. Jéquier, A. Sjoerdsma, *Advan. Pharmacol.* **6A**, 21 (1968).
8. L. Bertilsson, A. J. Atkinson, Jr., J. R. Althaus, Å. Härfast, J.-E. Lindgren, B. Holmstedt, *Anal. Chem.*, in press.
9. M. Åsberg, L. Bertilsson, D. Tuck, B. Cronholm, F. Sjoqvist, unpublished results.
10. D. F. Sharman, *Brit. Med. Bull.* **21**, 62 (1965).
11. W. Lovenberg, H. Weissbach, S. Udenfriend, *J. Biol. Chem.* **237**, 89 (1962).
12. H. Weissbach, W. King, A. Sjoerdsma, S. Udenfriend, *ibid.* **234**, 81 (1959).
13. We thank E. Dufva, Å. Härfast, and L. Roswall for technical assistance. Supported by grants from the Tri-Centennial Fund of the Bank of Sweden to B. Holmstedt (68/53) and to B. Cronholm and F. Sjoqvist (68/90); by NIH grants GM 13 978 and MH 12007; by grants from the Wallenberg Foundation, Swedish Medical Research Council (B72-40Y-2375-05); and by funds from Karolinska Institutet.

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Yellow Fever Vaccination, Avian Leukosis Virus, and Cancer Risk in Man

Abstract. *Comparison was made between 2659 veterans who died of cancer, during 1950 to 1954 or 1959 to 1963, and matched controls, based on the frequency of yellow fever immunization during World War II. The vaccine was produced from chick embryos that almost certainly contained avian leukosis-sarcoma viruses. Among the veterans, no relation was found between vaccination and leukemia, lymphoma, or other cancer.*

The use of chick embryos in the production of yellow fever vaccine during World War II suggests the presence of viruses capable of inducing neoplasia in avian species. To determine whether the vaccine could also induce cancer in man, we studied cancer mortality among army veterans of World War II in relation to their immunization against yellow fever during military service.

Leukosis-sarcoma viruses are ubiquitous in the avian species—viruses even having been isolated from "leukosis-free" flocks. The cell type of the neoplasia varies according to the strain of virus, and tumors occur mainly in the blood-forming organs, soft tissues, and kidney (*1*). Experimentally, avian viruses have seldom induced neoplasia in mammals, except for the Rous sarcoma virus which, when injected into various species, has induced spindle-cell sarcoma (*2*).

There is little doubt that most, if not all, of the yellow fever vaccine used during World War II was contaminated with avian viruses, but the extent of the contamination cannot be determined today. Although at that time viruses were known to cause avian leukosis, their presence in chick embryos was unknown. Furthermore, a viral origin of human cancer was considered to be remote. Subsequently, with the immense new understanding of viral oncology, the effects of live vaccines used in the past have come into question.

A third or more of World War II army veterans received yellow fever vaccine, but only about 1 percent had died of cancer 20 years later. Hence, a retrospective design, comparing veterans who died of cancer with living veterans on the basis of yellow fever vaccination, was adopted in preference to a prospective design, that is, one in which cohorts of the vaccinated and the nonvaccinated veterans would be compared as to subsequent cancer mortality. Representative deaths from cancer were obtained by sampling notices of death prepared by the Veterans Administration on virtually all deceased war veterans for the years 1950 to 1954 and 1959 to 1963 (*3*). The underlying cause obtained from the death certificate was used to identify deaths from cancer. Death certificates are 80 to 90 percent accurate as to the fact of cancer and about 60 percent accurate at the three-digit level of diagnostic classification (*4*). For lymphomas, death certificates are quite accurate, but for cancer of the pancreas or lung, much less so. For convenience and simplicity, sampling was restricted to white males born between 1912 and 1928 and with World War II service beginning prior to 1945. Similarly restricted controls were obtained from a "2-percent file" representative of National Service Life Insurance numbers

Table 1. Comparison of men who died of cancer with controls on the basis of previous yellow fever vaccination and by type of cancer. ICD, "International Classification of Disease"; +, vaccinated; −, not vaccinated.

Yellow fever vaccination	Cases		Controls	
	No.	(%)	No.	(%)
All forms of cancer (ICD 140–205)				
+	1221	45.9	1174	44.2
−	1438	54.1	1485	55.8
Lymphoma (ICD 200–203)				
+	147	46.1	139	43.6
−	172	53.9	180	56.4
Leukemia (ICD 204)				
+	84	43.5	81	42.0
−	109	56.5	112	58.0
Other cancer (ICD 140–199, 205)				
+	990	46.1	954	44.4
−	1157	53.9	1193	55.6

Table 2. Comparison of men who died of lymphoma with controls on the basis of previous yellow fever vaccination, by date of entry on active duty and by date of death. +, Vaccinated; −, not vaccinated.

Date entered active duty	Yellow fever vaccination	Cases		Controls	
		No.	(%)	No.	(%)
Lymphoma deaths, 1950–1954 and 1959–1963					
Before July 1942	+	76	72.4	77	74.8
	−	29	27.6	26	25.2
July 1942 or later	+	71	33.2	62	28.7
	−	143	66.8	154	71.3
Lymphoma deaths, 1950–1954					
Total	+	45	48.9	43	46.7
	−	47	51.1	49	53.3
Lymphoma deaths, 1959–1963					
Total	+	102	44.9	96	42.3
	−	125	55.1	131	57.7