fertilization membrane; there is no effect on cilia formation and motility. Later, spherical or slightly elongated swimming larvae are obtained; they possess no spicules and never take a prismatic form. The mesenchyme is abundant, and made up of spindle shaped cells, which are often multinucleate. The gut is present and stains pink. If the eggs are treated at later stages of development with EB (10 μg/ml.) it is necessary to wait until the early gastrula stage before small spicules, forming a disorganized skeleton, can be observed in the treated larvae.

In conclusion, EB is a useful agent for the analysis of morphogenesis: it inhibits, in a reproducible way, cap formation in *Acetabularia*, yolk platelet breakdown in amphibian embryos, spicule formation in sea urchin larvae. The biochemical basis for these effects is now under study; preliminary results indicate that, in *Acetabularia*, EB inhibits cytoplasmic DNA synthesis[12] and induces the disappearance of the DNA peak which has been ascribed to mitochondria[13]. In amphibian oocytes, observations with the fluorescent microscope show that EB binds to the yolk platelets, presumably to their DNA (ref. 14). If the arrest of yolk utilization is a consequence of such a combination between EB and yolk DNA, it could mean that the latter contains the information needed for the synthesis or activation of the enzymes which break down the constituents of the yolk platelets.

I thank Drs M. Steinert, V. Heilporn, S. Limbosch, N. Six and V. Vacquier for the communication of unpublished results.

J. BRACHET

Laboratory of Animal Morphology,
Free University of Brussels, and
International Laboratory for Genetics and Biophysics,
Naples.

Received September 23, 1968.

[1] Brachet, J., *Chemical Embryology* (Interscience, New York, 1950).
[2] Lerman, L. S., *Proc. US Nat. Acad. Sci.*, **49**, 94 (1963).
[3] Ruttenberg, G. J. C. M., Smit, E. N., Borst, P., and Van Bruggen, E. F. J., *Biochim. Biophys. Acta*, **157**, 429 (1968).
[4] Radloff, R., Bauer, W., and Vinograd, J., *Proc. US Nat. Acad. Sci.*, **57**, 1514 (1967).
[5] Pikó, L., Blair, D. G., Tyler, A., and Vinograd, J., *Proc. US Nat. Acad. Sci.*, **59**, 838 (1968).
[6] Slonimski, P., Perrodin, G., and Craft, J. H., *Biochem. Biophys. Res. Commun.*, **30**, 232 (1968).
[7] Steinert, M., and Van Assel, S., *J. Cell Biol.*, **34**, 489 (1967).
[8] Brachet, J., Denis, H., and de Vitry, F., *Develop. Biol.*, **9**, 398 (1964).
[9] Brachet, J., *Arch. Zool. Ital.*, **51**, 395 (1966).
[10] Brachet, J., *Nature*, **214**, 1132 (1967).
[11] Gross, P., and Cousineau, G., *Exp. Cell Res.*, **33**, 368 (1964).
[12] Heilporn-Pohl, V., and Brachet, J., *Biochim. Biophys. Acta*, **95**, 302 (1965).
[13] Green, B., Heilporn, V., Limbosch, S., Bolouknère, M., and Brachet, J., *Proc. US Nat. Acad. Sci.*, **58**, 1351 (1967).
[14] Baltus, E., Hanocq-Quertier, J., and Brachet, J., *Proc. US Nat. Acad. Sci.* (in the press).

Occurrence of Bufotenin in the Urine of Schizophrenic Patients and Normal Persons

BECAUSE of conflicting statements about the occurrence of bufotenin in the urine of schizophrenics and normal subjects[1–13] we have now developed a method which allows semi-quantitative estimation of about 0·7 μg total bufotenin in 24 h urine samples. The method consists of purification and isolation by successive column chromatography, sublimation, paper chromatography and thin-layer chromatography.

24 h urine samples are hydrolysed for 0·5 h at *p*H 1·6, chromatographed through a Dowex column and eluted as described by Faurbye and Pind[14]. The eluate is evaporated in vacuum to near dryness, the rest extracted with chloroform at *p*H 10. The chloroform extract is sublimated at 10^{-3} to 10^{-4} torr, and one-way paper chromatography of the sublimate follows, with (20 per cent) potassium chloride solution. The bufotenin zone is found by co-chromatography of authentic bufotenin, and eluted. The eluate is subjected to thin-layer chromatography with chloroform–methanol–acetic acid–water (65 : 30 : 2 : 3) and stained with *p*-dimethylaminobenzaldehyde. The amount of bufotenin is determined by comparison with co-chromatographed authentic bufotenin.

In order to secure identity of positive spots the thin-layer chromatography is repeated on two way thin-layer chromatography, the first direction as described, and the second direction with methylacetate–isopropanol–ammonium hydroxide 25 per cent (45 : 35 : 20).

Table 1. RESULTS OF THE BUFOTENIN ANALYSIS

Initials	Diagnosis	Days without drugs	Total bufotenin per 24 h (μg)
R.L.	Schizophrenia	5 years	1·7
O.D.J.	Schizophrenia	89 days	0·7
P.J.	Schizophrenia	8 days	2·6
G.A.	Schizophrenia	9 days	1·7
G.J.	Schizophrenia	13 days	3·7
A.F.N.	Schizophrenia	19 days	3·2
E.H.	Schizophrenia	22 days	0
S.L.L.	Control		0
K.P.	Control		0
A.M.J.	Control		7·5
A.R.	Control		3·6
E.M.	Control		1·6
G.H.	Control		1·6

All the bufotenin spots were compact and well isolated from other spots. Addition of 0·7 μg authentic bufotenin to a bufotenin-free 24 h sample of urine always gave a weak but distinct spot.

Because bufotenin is easily destroyed by oxidation, nitrogen was induced where possible, and diphenylamine and EDTA were added to the solvents.

Recovery was determined by addition of 0·7–5 μg of bufotenin to bufotenin-free urine samples. Ten determinations gave a recovery from 32 to 62 per cent, a mean of 48 per cent. The numbers in Table 1 are corrected according to this.

The subjects were seven chronic schizophrenic patients with active psychotic symptoms. They were treated with neuroleptic drugs but were without medicine for at least 8 days before the investigation. The controls were six healthy persons.

As shown in Table 1 six of the seven schizophrenics excreted 0·7–3·7 μg total bufotenin in 24 h while one did not excrete bufotenin (that is, less than 0·7 μg). Four control persons excreted 1·6–7·5 μg and two did not excrete bufotenin. Bufotenin therefore seems to be a normal metabolic product.

ARILD FAURBYE
K. PIND

St Hans Hospital,
Roskilde,
Denmark.

Received August 5, 1968.

[1] Faurbye, A., *Comprehensive Psychiat.*, **9**, 155 (1968).
[2] Axelrod, J., *Science*, **134**, 343 (1961).
[3] Bumpus, F. M., and Page, J. H., *J. Biol. Chem.*, **212**, 111 (1955).
[4] Brune, G. G., Hohl, H. H., and Himwich, H. E., *J. Neuropsychiat.*, **5**, 14 (1963).
[5] Fischer, E., Lagravere, T. A. F., Vazquez, A. J., and Di Stefano, A. O., *J. Nerv. Ment. Dis.*, **133**, 441 (1961).
[6] Fischer, E., and Spatz, H., *Intern. J. Neuropsychiat.*, **3**, 226 (1967).
[7] Perry, T. L., Hansen, S., and MacDougal, L., *Nature*, **212**, 146 (1966).
[8] Siegel, M., *J. Psychiat. Res.*, **3**, 205 (1965).
[9] Runge, T. M., Lara, F. Y., Thurman, M. T., Keyes, J. W., and Hoerster, S. H., *J. Nerv. Ment. Dis.*, **142**, 470 (1966).
[10] Rodnight, R., *Intern. Rev. Neurobiol.*, **3**, 251 (1961).
[11] Perry, T. L., Shaw, K. N. F., Walker, D., and Redlich, D., *Pediatrics*, **30**, 576 (1962).
[12] Gross, H., and Franzen, F., *Biochem. Z.*, **340**, 403 (1964).
[13] Tanimukai, H., Ginther, R., Spaide, J., and Himwich, H. E., *Nature*, **216**, 490 (1967).
[14] Faurbye, A., and Pind, K., *Acta Psychiat. Scand.*, **42**, suppl. 191, 136 (1966).

©1968 Nature Publishing Group