The second blastocyst (blastocyst 2) developed approximately 24 h after the other. It was cultured in Ham's F 10 containing 25% human and 25% foetal calf serum. A blastocoelic cavity appeared after 159 h in culture. This cavity corresponded with that seen in the early stages of expansion in blastocyst 1, but the cell membranes traversing it were more pronounced and persisted for 12 h. Some of the trophoblastic cells had evidently failed to release their contents into the blastocoel. The thin zona pellucida and the shape of the inner cell mass were similar to those seen in blastocyst 1. Small extensions, somewhat resembling those seen in guinea-pig blastocysts[6], seemed to be passing through the zona pellucida (Fig. 2).

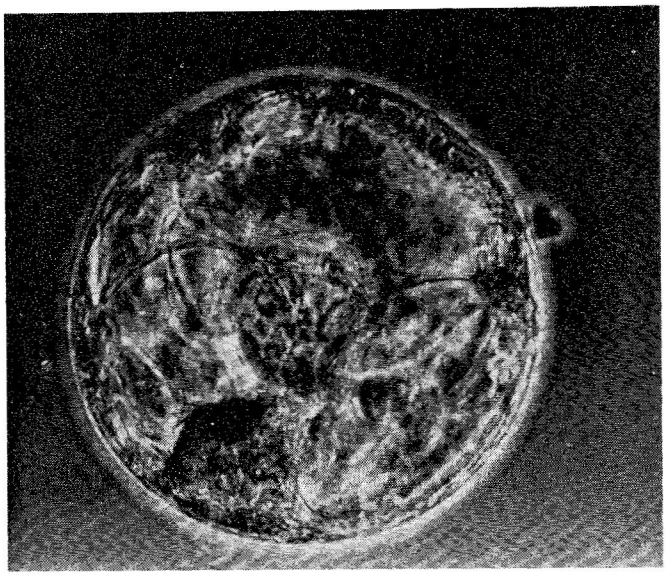

Fig. 2 Blastocyst 2 before it was fixed and stained. The thin zona pellucida and the underlying trophoblast can be seen; an extension is evidently emerging through the zona (at right). The inner cell mass (lower part of the embryo) is distinct. The cellular membranes persisting across the blastocoel can be seen.

The embryo was kept at room temperature for several hours before staining. It was found to have 110 nuclei. Bodies resembling sex chromatin were seen in a few nuclei.

We seem to have achieved this improved embryonic development through better handling of the cultures than previously. The preovulatory oocytes were kept at room temperature under reduced oxygen tension and 5% carbon dioxide until the spermatozoa were added. Small microdrops were used for culture, and enlarged when the embryos were eight celled. The embryos were left undisturbed for long periods after this stage.

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P. C. STEPTOE
R. G. EDWARDS
J. M. PURDY

Oldham General Hospital, Lancashire, and
Physiological Laboratory, University of Cambridge

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Excretion of *p*-Methoxyamphetamine administered to Humans

PSYCHOSIS can be induced by the administration of large doses of amphetamine[1] and some of its derivatives[2]. In man, a principal metabolite of amphetamine is *p*-hydroxyamphetamine. Biological O-methylation of this compound would yield a potent hallucinogen, *p*-methoxyamphetamine (PMA)[3]. In a previous report[4], we demonstrated that within the limit of detectability of PMA (about 80 μg/24 h urine) by a sensitive procedure employing gas chromatography of the acetyl derivative, PMA was not detected in the urine of four subjects to whom amphetamine sulphate was administered in large doses (mean of 629 mg) over an average period of 51 h. These subjects developed varying degrees of psychotic symptomatology.

Here we wish to demonstrate that PMA, when administered can be detected in human urine. Five normal subjects ingested 10–65 mg of PMA hydrochloride. Urine was collected in 12 h periods except when otherwise noted in Table 1. Aliquots (5 ml.) of the urine specimens were analysed in duplicate by gas chromatography as described previously[4,5]. In this procedure, which uses phenethylamine as an internal standard, the recovery of 10 μg of PMA-HCl added to 5 ml. urine samples was 108 ± 21 (s.d.).

Table 1 Excretion of PMA in Urine

Subject and trial	Weight (kg)	Dose PMA (mg)	Time of urine collection (h)	Volume (ml.)	Mg PMA excreted	PMA excreted (%)
1 A	65	10	0–12	660	580	2.64
			12–24	320	510	0.51
B		65	0–12	380	400	0.17
			12–24	510	230	0.08
			24–36	150	280	0.01
2	64	10	0–12	840	670	6.75
			12–24	1,020	1,280	6.15
			24–36	960	1,020	1.92
3	57	57	0–15	1,000	1,450	11.8
4	67	20	0–24	900	1,100	4.25
5	76	30	0–12	1,770	800	3.5
			12–24	1,280	1,190	1.4

The average excretion of PMA was 6.7% of the administered dose with a range of 0.3 to 15%. Variations in PMA excretion may, in part, be related to urinary *p*H [6].

Because PMA was detected in the urine of subjects who received as little as 10 mg PMA-HCl (a sub-hallucinogenic dose), yet was not detected in the urine of subjects receiving about 600 mg of amphetamine, it is unlikely that PMA is formed in amounts required for psychotogenesis when directly administered. The possibility of small amounts of the compound being formed at specifically sensitive central nervous system sites, of course, still exists.

JACK W. SCHWEITZER
ARNOLD J. FRIEDHOFF
B. M. ANGRIST
S. GERSHON

Department of Psychiatry,
New York University Medical Center, New York,
New York 10016

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Methadone increases Mouse Brain 5-Hydroxyindoleacetic Acid

METHADONE, a synthetic narcotic with many similarities to morphine, is currently being used extensively as a crucial part of treatment–rehabilitation programmes for heroin addiction. In the doses used for "methadone maintenance", this compound has minor tranquillizing effects, blocks the euphoric effects of heroin, eliminates drug craving, and prevents narcotic withdrawal symptoms. Although the mechanism of action of methadone is unknown, there are a number of recent reports which suggest that 5-hydroxytryptamine (serotonin) is involved in the perception of pain and in the mechanism of action of narcotic analgesics. For instance, Tenen first showed that *p*-chlorophenylalanine, which depletes tissues of serotonin by inhibiting synthesis, lowered the pain threshold of rats as measured by a flinch-jump technique[1]. He further found that *p*-chlorophenylalanine pretreatment lowered the analgesic effect of several major narcotics, including morphine and methadone[2]. Lints and Harvey made lesions in the medial forebrain bundle, septal area, and dorsomedial tegmentum of rats and concluded that the resulting increased sensitivity to electric shock and decreased telencephalic serotonin content were causally related[3]. Both effects were reversed by L-5-hydroxytryptophan but not by L-dihydroxyphenylalanine[4]. Rogers and Thornton showed that increased serotonin levels (but not dopamine or norepinephrine levels) were highly correlated with increased toxicity of principal narcotic analgesics following monoamine oxidase inhibitors[5]. Way *et al.* found that chronic morphinization of mice followed by pargyline administration resulted in a more rapid rise in brain serotonin compared with controls[6]. These investigators also showed that tolerance of and physical dependence on morphine in these animals was antagonized by *p*-chlorophenylalanine pretreatment and related the effects of morphine on serotonin metabolism to the development of tolerance and physical dependence.

Table 1 5-Hydroxyindoleacetic Acid Content of Mouse Brain after Administration of Methadone or Morphine

Drug regimen	Brain 5HIAA as percentage change from control*
(1) Morphine 10 mg/kg 3 times daily for 1 week, then 20 mg/kg 3 times daily for 2 weeks	+111 (4)
(2) Methadone 10 mg/kg 3 times daily for 3 weeks	+163 (5)
(3) Methadone 5 mg/kg	+45 (15)
10 mg/kg	+64 (4)
15 mg/kg	+76 (8)
(4) Controls+pargyline (75 mg/kg)	−54 (4)
(5) Methadone (10 mg/kg)+pargyline	−58 (4)†
(6) Methadone (10 mg/kg)+probenecid (200 mg/kg)	+171 (4)

* Absolute control values for thirteen determinations were 211±14 ng 5HIAA (free acid) per gram (wet weight) of brain. The number of determinations is in parentheses. Two brains were used for each determination.
† Controls were values for brain 5HIAA 2 h after methadone (10 mg/kg) only.

Haubrich and Blake found increased 5-hydroxyindoleacetic acid (5HIAA) in rat brain after acute and chronic doses of morphine[7]. These studies strongly support the idea that sensations of pain and certain effects of narcotic analgesics are mediated by serotoninergic systems. To compare methadone to morphine with regard to serotonin metabolism we performed the following experiments.

White mice (50–75 mg) were injected intraperitoneally with saline, d-l methadone (5–15 mg/kg), or morphine sulphate (10–20 mg/kg) at varying intervals. Acute animals were killed 2 h after a single dose; chronic animals were injected three times a day for 3 weeks. In some experiments pargyline (75 mg/kg intraperitoneally) was administered with methadone and these animals were killed 2 h later. In other experiments probenecid (200 mg/kg intraperitoneally) was given 15 min after methadone and these animals were also killed at 2 h. Two mouse brains (without cerebellum) were pooled for each determination of brain 5HIAA[8]. Methadone added to brain homogenates did not produce increases in the 5HIAA measurements. Prepared parenteral solutions of morphine, methadone, and probenecid (sodium probenecid) were used. Pargyline was dissolved in physiological saline before injection.

Values for mouse brain 5HIAA in the various experimental conditions are listed in Table 1. Morphine, when administered repeatedly according to a schedule designed to produce tolerance and dependence, increased brain 5HIAA. Methadone given by a similar schedule also produced a large increase in brain 5HIAA. Acute doses of methadone (5–15 mg/kg) also produced dose-related increases in brain 5HIAA. These increases were not as great as those produced by chronic administration of methadone. Thus the increase in brain 5HIAA produced by methadone is apparent after a single dose but becomes greater after repeated doses. The results of the experiments using pargyline indicate that the slope of the decay in 5HIAA after pargyline was the same both for untreated animals and for animals given a single dose (10 mg/kg) of methadone[9]. Similarly, probenecid, used in conjunction with a single acute dose of methadone (10 mg/kg), produced an increase in brain 5HIAA which was more than twice that produced by this dose of methadone alone. The pargyline and probenecid experiments show that methadone does not increase brain 5HIAA by affecting the transport system which removes acidic metabolites from brain[10]. So methadone, like morphine, apparently produces an increase in brain serotonin synthesis in the mouse. It is still unclear whether such an increase results from a primary effect on the synthesis of serotonin or from an increased requirement for this amine leading to an increase in synthesis. Further, it is important to bear in mind that the catecholamines have also been implicated in the action of the narcotic analgesics[11,12]. In this regard, important species