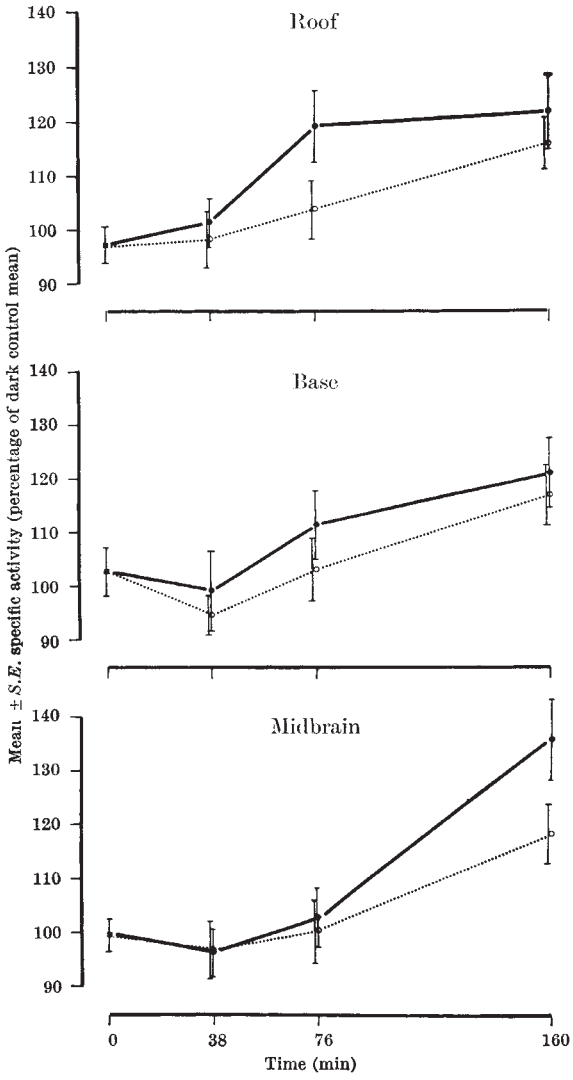

Fig. 1. Means and standard errors of standardized specific activities of RNA from the forebrain roofs, forebrain bases and midbrains of differently treated chicks. All chicks were injected with tritiated uracil and the time from injection to killing was kept constant. The experimental birds (———) were exposed to a flashing light and the light controls (. . .) to a continuous light.

controls. Fig. 1 gives the mean values and standard errors of the standardized specific activities (s.s.a.) for different regions of the brain after each treatment.

No change in the mean values of s.s.a. could be detected after 38 min of exposure in either the experimental or light control group. After 76 min exposure, a highly significant increase had occurred in the s.s.a. of the forebrain roofs of the experimental group (Table 1). No other differences between the dark controls (0 min exposure) and the experimental and light control groups exposed for 76 min are statistically significant. But after 160 min of exposure, the mean s.s.a. in all three brain regions in both experimental and light control groups are significantly greater than those in the dark controls (Table 1). The experimental group differs significantly from the light control in the forebrain roof after 76 min of exposure ($d = 1{\cdot}81$, $P < 0{\cdot}05$ 1-t) and in the midbrain after 160 min of exposure ($d = 1{\cdot}93$, $P < 0{\cdot}05$ 1-t). No significant differences were found in the liver at any time.

Table 1. VALUES OF d and PROBABILITY LEVELS FOR STATISTICAL COMPARISONS BETWEEN THE DARK CONTROL GROUP (0 MIN) AND (*a*) EXPERIMENTAL AND (*b*) LIGHT CONTROL GROUPS EXPOSED FOR 76 AND 160 MIN

Time of exposure (min)	Group	Roof	Base	Midbrain
76	Experimental	3·00†	1·13	0·48
	Light control	1·07	0·03	0·08
160	Experimental	3·23†	2·38*	4·70‡
	Light control	3·14†	2·05*	3·08†

* $P < 0{\cdot}05$. † $P < 0{\cdot}01$. ‡ $P < 0{\cdot}001$.

Clearly RNA synthesis in the brains of day-old chicks is very sensitive to visual stimulation. By varying the time of exposure to such stimulation while keeping the time from injection to killing constant, the effects of different treatments on different brain regions can be distinguished. Particularly striking is the rapid increase in RNA synthesis in the forebrain roof of experimental birds. This is the region where we previously detected an increase in protein synthesis after early hatching chicks had been exposed to an imprinting stimulus[1]. We should point out that because mere exposure to a continuously illuminated environment eventually affects RNA synthesis in all brain regions, the causes of the more rapidly occurring change in the forebrain roof of experimental birds are not clear. On the one hand, the difference between experimental and light control groups at 76 min might have arisen because the experimental birds merely received more visual stimulation. On the other hand, the difference might have been related in a more direct way to the imprinting process.

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Investigation of *p*-Methoxyamphetamine Excretion in Amphetamine Induced Psychosis

MANY differences exist between the symptoms of schizophrenia and those of drug-induced states caused by agents like LSD or mescaline[1]. Amphetamine psychosis, by contrast, can in some cases replicate virtually all of the manifestations of natural schizophrenic illness[2–5]. Such a correspondence justifies a study of the biochemical basis of this condition.

A provocative hypothesis about the genesis of amphetamine psychosis has been proposed[6]. Smythies *et al.* suggest that *p*-methoxyamphetamine (PMA) may be formed from amphetamine in patients who develop psychotic symptoms. In an animal test system utilizing a conditioned avoidance response and analysis of reaction time which is designed to correlate with human psychotogenicity[6,7], Smythies has found PMA to be highly potent as a disruptor of behaviour—second only, in this respect, to LSD of all the compounds tested[6]. Shulgin *et al.* have established that PMA is psychotogonic in humans and also that it has an order of potency of approximately five times that of mescaline[8]. The doses of amphetamine required to induce a psychosis in humans are such that pharmacologically active amounts of this metabolite

could be produced. We therefore examined the urine of subjects who were receiving amphetamine in psychotogenic doses to ascertain the presence or absence of PMA.

The subjects were four physically healthy non-schizophrenic volunteers to whom amphetamine was administered orally in doses of 5–50 mg/h for as long as was tolerated. A mean total cumulative dose of 629 mg was administered over an average period of 51 h. The method of administration and behavioural effects have been reported[5].

Urine was collected continuously in 6–12 h pooled specimens throughout amphetamine administration. The specimens representing hours before and immediately after amphetamine was last administered were examined. Urinary amphetamine was assayed by a method in which an internal standard, phenethylamine, is added to a 5 ml. aliquot of each urine sample, in duplicate. The amines, extracted with chloroform at *p*H 10, are acetylated directly with acetic anhydride. The residue on evaporation is dissolved in dioxane and a portion is injected into an F and M 'Biomedical 400' gas chromatograph fitted with a six foot U tube packed with 8 per cent ethylene glycol adipate on 'Chromosorb W'. For 8 inches at the two ends the packing was 3 per cent 'SE–30' on 'Diatoport S'. The column temperature was 215°.

The amount of amphetamine present can be calculated by comparing the height of its chromatographic peak with that of the internal standard[9]. PMA was assayed with the same method. Retention times for N-acetylamphetamine and N-acetyl-PMA were, respectively, 4 and 15 min. From peak height measurements of standard amounts (0·1–1·0 μg) of N-acetyl-PMA and, independently, from recovery studies of PMA added to urine, it is estimated that the limit of detectability of PMA in these urines is about 70–80 μg/l. of urine. In none of the nine studies was PMA detected even though large amounts of amphetamine were present in these urine samples (Table 1).

Table 1. SCHEDULE OF TESTING FOR PMA EXCRETION

Subject and trial	Cumulative dose (mg)	Duration of administration (h)	Urine volume (ml.)	Amphetamine excreted (mg)	Psychotic symptoms
1 A	325	28·75	95	10·0	Present
B	745	64·25	36·5	14·2	Present
C(+R)	950	54	735	123	Absent
2 A	390	45·75	485	18·3	Minimal
B	590	74·75	325	23·4	Minimal
3 A	955	71·5	245	21·4	Absent
B(+R)	465	26	405	9·20	Absent
4 A	595	46	79	9·05	Present
B(+R)	645	47	490	14·9	Absent

PMA was not detected in any of the trials. R indicates pretreatment with reserpine, which seemed to prevent the occurrence of psychotic symptoms[5].

Our study does not support the suggested formation of PMA from ingested amphetamine as a mechanism for the genesis of amphetamine psychosis, but the distinct possibility remains that amphetamine could undergo such transformation within the central nervous system and not peripherally, and then subsequently be further transformed before being excreted in the urine. Even the transient production of such a substance, should it occur at critical sites in the central nervous system, could cause significant behavioural effects. In partial support of this alternative, we have found that N-^{3}H-acetyl-PMA is almost entirely O-demethylated in rats *in vivo*.

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Inhibition of ^{3}H-Thymidine Incorporation into Rat Liver Nuclei by *E. coli* L-Asparaginase

THERE have been numerous reports dealing with the antitumour activity of *Escherichia coli* L-asparaginase in the therapy of human leukaemia. It has been postulated that the agent is without toxic action on normal tissues and that it is specific for asparagine dependent tumour cells, but neither of these assumptions has been proved[1,2]. When L-asparaginase was used in the treatment of human cancer, a satisfactory effect could be seen only with acute lymphoblastic leukaemia[3–5], but many investigators emphasized the toxicity of L-asparaginase. An alteration of liver function[2,5–7] has been demonstrated by the finding of hepatic lipoidosis; a marked increase in bromsulphthalein retention, and in serum levels of cholesterol, triglycerides and phospholipid; a decrease in human serum proteins and the demonstration of various clotting disorders in the course of therapy. Other authors[8] observed the total inhibition of the early wave of mitotic activity in regenerating rat liver approximately 30 h after partial hepatectomy. We decided to study the effect of L-asparaginase preparations of high specific activity on DNA synthesis in rat liver nuclei, and to determine nuclear DNA and RNA polymerase activity in rat liver under the influence of L-asparaginase.

The methods used in this study—with a few modifications—have been described[9–11]. Female Wistar rats with an average initial weight of 90 g have been used throughout this study. *E. coli* L-asparaginase (220 IU/mg protein, crystal suspension in 3·5 M $(NH_4)_2SO_4$) was a gift from Boehringer, Mannheim. The enzyme was injected intraperitoneally in an adjusted dilution of the crystal suspension which contained the administered doses in a solution volume of 0·5 ml. In all experiments control animals were injected with the same volume of an equimolar solution of $(NH_4)_2SO_4$. Labelling of nuclear DNA was achieved by means of a 1 h pulse of ^{3}H-thymidine (20 μCi/animal; 5 Ci/mmole) given intraperitoneally in 0·5 ml. 0·9 per cent NaCl. The nuclei were isolated using the procedures described elsewhere[11–13]. DNA as well as RNA polymerase assay followed the description in previous studies[9–11]. Nuclear DNA was estimated essentially as described by Burton[14]. To determine the incorporation of ^{3}H-thymidine into nuclear DNA, aliquot suspensions of liver nuclei were precipitated and washed with 2 per cent perchloric acid and radioactivity was counted in a toluene scintillator[15]. The results are presented in Tables 1–3.

Table 1. ^{3}H-THYMIDINE INCORPORATION INTO NUCLEAR DNA OF RAT LIVER FOLLOWING INTRAPERITONEAL INJECTION OF L-ASPARAGINASE

Group No.	Time of L-asparaginase injection before death (h)	Dose L-asparaginase (IU/kg)	Radioactivity (c.p.m./mg DNA)	Per cent inhibition
1	—	—	2,194 ± 1,313	—
2	1	780	478 ± 191	78 $P < 0.02$
3	4	780	912 ± 468	58 $P < 0.02$

Four animals were used in each group.