Ethnor Pty. Ltd.,
1 Khartoum Road,
North Ryde, N.S.W., 2113, Australia. A. G. BOLT

May 16, 1972

REFERENCES

FELDSTEIN, A. & WILLIAMSON, O. (1968). *Life Sci.*, **7**, II, 777–783.
HOGEBOOM, G. H. (1955), *Methods in Enzymology*, Vol. I, pp. 17–19, Editor: Colowick, S. P. & Kaplan, N.O. New York: Academic Press.
LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. Chem.*, **193**, 263–275.
SMITH, A. A. & WORTIS, S. B. (1960). *Biochem. biophys. Acta*, **40**, 569–570.
SOUTHGATE, J. & COLLINS, G. G. S. (1969). *Biochem. Pharmac.*, **18**, 2285–2287.
WURTMAN, R. J. & AXELROD, J. (1963). *Ibid.*, **12**, 1439–1440.

Metabolism of [^{14}C]griseofulvin in the mouse

The metabolism of griseofulvin has been studied in man, rats, rabbits and dogs. A single metabolite of griseofulvin, the 6-desmethyl derivative was demonstrated in rabbit dog and human urine. (Barnes & Boothfoyd, 1961; Kaplan, Riegelman & Lee, 1960; Harris & Riegelman, 1969; Riegelman, Epstein & Dayan, 1962). In rat, two major metabolites, the 4- and 6-desmethylated derivatives, were identified (Symchowicz & Wong, 1966a,b). McNall (1960) reported that in mice following a single oral dose of griseofulvin, the blood and serum drug levels reached a maximum 6–8 h later but he did not study the excretion pattern and metabolic fate of this drug.

Prolonged intake of large doses of griseofulvin in mice lead to the ultimate formation of multiple hepatomas in the enlarged liver (Barich, Schwartz & others, 1961: DeMatteis, Donnelly & Runge, 1966; DeMatteis & Rimington, 1963; Hurst & Page, 1963). We have therefore investigated the fate of griseofulvin in mice.

[^{14}C]griseofulvin (368·7 μCi/mM) was prepared biosynthetically (Symchowicz & Wong 1966b). The authentic 6-desmethylgriseofulvin (6-DM) and 4-desmethylgriseofulvin (4-DM) were supplied by Glaxo Group Ltd., England.

Charles River male mice, about 20 g, were given the drug (10 mg/kg, i.v.) in a solution of 75% *NN'*-dimethylformamide, or by mouth (25 mg/kg suspended in 0·5% Tween-80). The radioactivity in urine was measured directly by liquid scintillation spectrometry. The radioactivity in plasma, faeces, intestines and liver samples were combusted by Schoniger technique (Kelly, Peets & others, 1961; Woeller, 1961), before counting.

The urine was acidified to pH 1·0 with concentrated hydrochloric acid and extracted with ether. The metabolic pattern of ether extractable material in urine was then studied using ascending paper chromatography with benzene–cyclohexane–methanol–water (5 : 5 : 6 : 4, v/v). Glacial acetic acid (0·5%) was added to the organic phase after equilibrium. The radioactivity patterns on the paper strips were analysed by scanning with a Vanguard 4π automatic strip counter attached to an automatic integrator.

The plasma drug disappearance curve after an intravenous dose shows two components, one with a 10 fold fall from 6000 to 600 d/min over the first 2 h, with a half life of 30 min, the other with a fall from 600 to 200 d/min over the next 2 h with a half life of 75 min.

Over 96 h, about 74% of the dose was excreted in the urine while some 64% of the oral dose was excreted in the same period (Fig. 1). Almost 50% of the total radioactivity in the urine was excreted during the first 4 h after intravenous or 8 h after oral administration of the drug.

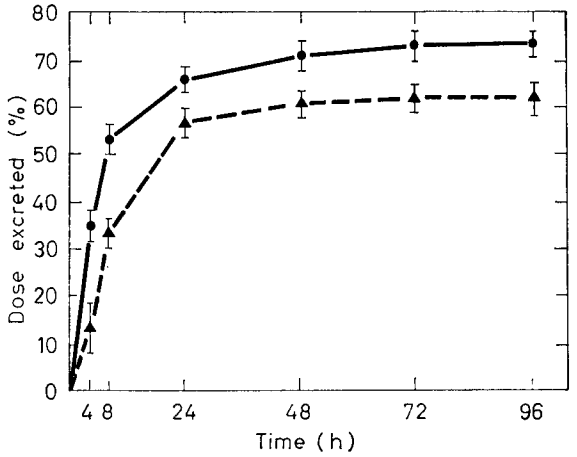

FIG. 1. Cumulative excretion pattern of radioactivity in urine after oral and i.v. administration of [^{14}C]griseofulvin. Each point represents mean value ± s.e. of ten mice. ●——● i.v. ▲- - -▲ oral.

Table 1. *Recovery of radioactivity in mice after ^{14}C-griseofulvin administration; p.o. (25 mg/kg) and i.v. (10 mg/kg).* Urine and faecal excretion represents 0–96 h periods. The liver and intestinal content was analysed 96 h post-drug.

	% of dose	
	Oral	i.v.
Urine	62·3 ± 3·1	73·8 ± 2·5
Faeces	18·3 ± 2·0	15·1 ± 1·1
Intestine	0·2 ± 0·0	1·4 ± 0·1
Liver	0·1 ± 0·0	1·3 ± 0·1
Total	80·9	91·6

Only negligible amounts of radioactivity could be detected in the intestine and liver 96 h after [^{14}C]griseofulvin administration, but 15% of the intravenous dose was recovered in faeces, indicating a biliary excretion of the drug (Table 1). The data also show that the drug was well absorbed from the gastrointestinal tract.

Four major radioactivity peaks were observed in the ether-extractable materials of urine after chromatography: 4-DM, 6-DM, an unidentified substance with R_F value of 0·12 and an unidentified peak remaining at the origin. No measurable amount of griseofulvin could be detected. Before hydrolysis with glusulase, the 4-DM peak was very small in the first 8 h, but it became clearly visible during the 24–48 h period. 6-DM was the predominant constituent at the early time period. After hydrolysis with glusulase, the 4-DM peak was present at all times and increased with time whereas 6-DM decreased with time. These results indicate that regardless of the route of administration, 6-DM is predominant in urine during the first 8 h and appears essentially in non-conjugated form while 4-DM is the major urinary metabolite present in the later time periods and appears in urine largely (90%) in conjugated form.

Our results indicate that the metabolism of griseofulvin in mice is different from that reported in rabbit, dog and man. In mice, both 4-DM and 6-DM were present as the major metabolites whereas in rabbit, dog and man, only 6-DM was found to be the major metabolite of griseofulvin (Kaplan & others, 1960; Barnes & Boothroyd, 1961; Riegelman & others, 1962; Harris & Riegelman, 1969). It has been reported previously

in our laboratory (Symchowicz & Wong, 1966a, b), that in rat urine both 4-DM and DM were present as the major metabolites. The rate of urinary drug excretion in rat was also similar to that of the mouse. Therefore, the mouse and the rat appear to have a similar metabolic pattern for griseofulvin. The unidentified metabolite with an R_F value of 0·12 observed in mouse urine was probably also present in rat urine but in a much smaller amount.

Department of Biochemistry,
Schering Corp.,
Bloomfield, New Jersey 04503 U.S.A.

C. LIN
R. CHANG
J. MAGAT
S. SYMCHOWICZ

July 14, 1972

REFERENCES

BARICH, L. L., SCHWARTZ, J., BARICH, D. J. and HOROWITZ, M. G. (1961). *Antibiot. Chemotherapy*, **11**, 566–571.

BARNES, M. J. & BOOTHROYD, B. (1961). *Biochem. J.*, **78**, 41–43.

DEMATTEIS, F., DONNELLY, A. J. & RUNGE, W. J. (1966). *Can. Res.*, **26**, 721–726.

DEMATTEIS, F. & RIMINGTON, C. (1963). *Brit. J. Dermatol.*, **75**, 91–104.

HARRIS, P. A. & RIEGELMAN, S. (1969). *J. pharm. Sci.*, **58**, 93–96.

HURST, E. W. & PAGE, G. E. (1963). *Br. J. Dermatol.*, **75**, 105–112.

KAPLAN, S. A., RIEGELMAN, S. & LEE, K. H. (1960). *J. pharm. Sci.*, **55**, 14–18.

KELLY, R. G., PEETS, E. A., GORDON, S. & BUYSKE, D. A. (1961). *Analyt. Biochem.*, **2**, 267–273.

MCNALL, E. G. (1960). *Arch. Dermatol.*, **81**, 657–661.

RIEGELMAN, S., EPSTEIN, W. L. & DAYAN, R. J. (1962) presented at the Scientific Section, Am. Pharm. Asso. Meet. Las Vegas, Nevada, March 1962.

SYMCHOWICZ, S. & WONG, K. K. (1966a). *Biochem. Pharmac.*, **15**, 1601–1606.

SYMCHOWICZ, S, & WONG, K. K. (1966b). *Ibid.*, **15**, 1595–1600.

WOELLER, F. H. (1961). *Anal. Biochem.*, **2**, 508–511.

Enhancement of audiogenic seizure by 6-hydroxydopamine

Recently investigations of the relation between changes in brain biogenic amines and changes in convulsive seizures were reported by Azzaro, Wenger & others (1972) and by Jobe, Picchioni & Chin (1972). The former group of workers showed that the ability of reserpine to lower electroshock seizure threshold is related to reduction in brain catecholamines and 5-hydroxytryptamine (5-HT). The latter group of workers concluded that endogeneous noradrenaline is a modulator of audiogenic convulsions. The present communication presents additional evidence to support the contention that catecholamines exert a modulation effect on sound-induced convulsions. The study involves the use of 6-hydroxydopamine, an agent which has the ability to cause selective degeneration of catecholamine-containing neurons (Bloom, 1971; Ungerstedt, 1971) and has been reported to lower minimal electroshock seizure threshold (Browning & Maynert, 1970).

Male rats, 280 to 320 g, from the University of Arizona colony of audiogenic seizure-susceptible rats, were used. Indwelling cannulae fashioned from 23 gauge stainless steel hypodermic needles were permanently implanted into the right lateral ventricle of the rats according to Grunden & Linburn (1969). One week after surgery, each rat in the test group was injected intracerebroventricularly with 6-hydroxydopamine, 200 μg in 20 μl of normal saline stabilized with 0·01 % ascorbic acid. Two such injections were given to each rat at an interval of 48 h. The control group of rats was similarly injected with the ascorbic acid-saline vehicle. A time-course study of audiogenic seizure response was conducted for 12-days. On the 13th day all the animals were killed and their brains compared for catecholamine content by means of he histochemical fluorescence technique of Falck, Hillarp & others (1962). The