RESULTS AND DISCUSSION

SK&F 7690 inhibition of testosterone stimulation of hexobarbital oxidase activity; intact female rats

Testosterone (2·5 mg/kg) and, to a lesser extent, SK&F 7690 (100 mg/kg) stimulated hexobarbital oxidase activity (Table 1). When the agents were given together, stimulation was less than after testosterone alone. The results suggest that a "weak" enzyme inducer (SK&F 7690) may compete with a "strong" inducer (testosterone) for occupation of a common receptor site for enzyme induction. The antiandrogen SK&F 7690 exhibited antitestosterone activity at an enzymatic level in liver. These results are compatible with those of Saunders *et al.*[3] wherein SK&F 7690 competed with testosterone for *extra*hepatic receptors.

Since doses of SK&F 7690 higher than 100 mg/kg were incompletely absorbed from subcutaneous sites, it was difficult to describe accurately the effects of high doses. Such doses of SK&F 7690 stimulated hexobarbital oxidase and inhibited testosterone stimulation, but there was no relationship between dose and effect of SK&F 7690. Fifty mg SK&F 7690/kg stimulated enzyme activity slightly and when given with testosterone produced a small but insignificant reduction of testosterone stimulation.

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REFERENCES

1. B. B. Brodie, *J. pharm. Pharmac.* **8**, 1 (1956).
2. R. Kato, E. Chiesara and G. Frontino, *Biochem. Pharmac.* **11**, 221 (1962).
3. H. L. Saunders, K. H. Holden and J. F. Kerwin, *Steroids* **3**, 687 (1964).
4. J. R. Cooper and B. B. Brodie, *J. Pharmac. exp. Ther.* **114**, 409 (1955).

Biochemical Pharmacology, 1965, Vol. 14, pp. 1899–1900. Pergamon Press Ltd., Printed in Great Britain.

α-Aceto-γ-hydroxybutyramide—a new anti-metabolite

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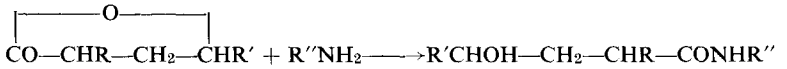
α-ACETO-γ-hydroxybutyramide has been examined in a microbiological system for anti-metabolic activity having shown signs of such action by interrupting pregnancy in mice. Some information on the mode of action of this compound has been obtained using *Escherichia coli* as a test system.

MATERIALS AND METHODS

Chemically, hydroxyamides can often be prepared by the reaction of the appropriate lactone with ammonia or amine—



α-Aceto-γ-hydroxybutyramide. 0·1 mole (12·8g) of α-aceto-γ-butyrolactone was warmed to 30° and a solution of concentrated ammonia (d = 0·880) (0·1 mole, ~ 5·5 ml) dissolved in water (10 ml) was added over a period of 1 hr with vigorous stirring, the temperature being maintained at 30–35°. The reaction product was concentrated by distillation under reduced pressure to obtain the crude solid. Recrystallization from water gave the pure product in 57 per cent yield as a white solid, m.p. 64–65° (Found : N, 9·7 per cent, $C_6H_{11}NO_3$ requires N,9·7 per cent). Compounds prepared similarly are in the appendix.

Effects on foetal growth. As a quick routine check on potential anti-metabolic activity, the materials under test were administered orally to pregnant mice on 3rd–6th days after mating. Doses up to 1·0 mg/g body wt/day were used.

Microbiological testing. The effects on growth of *Escherichia coli* were determined in a simple double strength aqueous medium of pH 7·2. Growth was investigated by turbidity measurements read 48 hr after innoculation, the growth being under static conditions at 37°.

RESULTS

Of the eight compounds tested, only one, α-aceto-γ-hydroxybutyramide, showed signs of interrupting pregnancy in mice. This was the only compound which was examined further.

It was found that 3·5 mg of amide/ml of medium completely prevented growth of *E. coli*. With test solutions containing sufficient inhibitor to prevent any growth (5 mg/ml) amino-acids, at the same concentration, were examined individually for ability to permit growth. L-Alanine, L-glutamine, L-glutamic acid and L-proline caused complete restoration of growth. Exactly half the amount of amide administered was all the amino-acid required to reverse the inhibition. The reversal of inhibition by proline was not competitive.

When administered to C3H mice which develop spontaneous mammary tumours, the α-aceto-γ-hydroxybutyramide at oral doses of 20 mg/day for four days caused a significant prolongation of life (R. Christie Brown—private communication). In addition, the amide was effective in suppressing the secondary reaction in immune response testing (J. A. C. Parke—private communication).

DISCUSSION

Microbiological examination of α-aceto-γ-hydroxybutyramide confirmed it to be an anti-metabolite in its action as a growth inhibitor of *E. coli*. Four amino-acids were found to cause reversal of the inhibition. These four compounds, alanine, glutamic acid, glutamine and proline, are all fairly closely associated metabolically in the medium. It seems likely, therefore, that one way in which this antagonist is working is by inhibiting the formation of glutamate.

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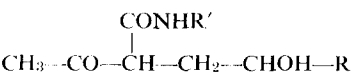
APPENDIX

The following new compounds were also synthesised but none of them showed any signs of anti-metabolic activity.



R	R′	m.p. (°C)	Found N %	Formula	Reqd. N %
CH_3	H	b.p. 150–4/1 mm	9·5	$C_7H_{13}NO_3$	8·8
C_2H_5	H	85	8·1	$C_8H_{15}NO_3$	8·1
$(CH_3)_2CHOCH_2$	H	b.p. 164–6/1 mm	6·4	$C_{10}H_{19}NO_4$	6·5
$C_6H_5OCH_2$	H	b.p. 220–2/1 mm	5·4	$C_{13}H_{17}NO_4$	5·6
H	C_2H_5	40–1	8·6	$C_8H_{15}NO_3$	8·1
H	$CH_3CH_2CH_2$	69–70	7·5	$C_9H_{17}NO_3$	7·5
H	$(CH_3)_2CH$	b.p. 146/1 mm	8·0	$C_9H_{17}NO_3$	7·5