killed 18 hr after administration of the labeled drug. Analysis of the various brain areas showed that with the exception of the cerebellum, residual [^{3}H]reserpine concentrations were markedly lowered in all brain areas (Table 2), indicating that mutual reserpine-tetrabenazine sites are widespread in the brain.

The other way of establishing the specificity of reserpine binding was by taking advantage of the observation that specific reserpine binding sites are readily saturated and that pretreatment with unlabeled reserpine well in advance of the labeled drug greatly reduces the persistent binding of the latter.[1] Accordingly, rats were given unlabeled reserpine (0·5 mg/kg, i.m.) 6 hr before [^{3}H]reserpine and were killed 18 hr after administration of the labeled compound. As was the case with tetrabenazine pretreatment, concentrations of [^{3}H]reserpine were markedly lowered in all areas except the cerebellum (Table 2).

These findings suggest that specific reserpine binding sites are widespread in the brain and that they are not well correlated with the anatomical distribution of any single brain monoamine although the degree of reserpine binding in peripheral organs seems to be correlated with the degree of adrenergic innervation.[1] The present results suggest further that specific and persistent reserpine binding in cortex, which has a low content of any of the endogenous monoamines, may reveal the presence of monoaminergic systems not associated with large amine storage pools. Other evidence that there may exist such systems has been presented by Snyder and Coyle[8] who showed that the cerebral cortex, a norepinephrine-poor area, takes up norepinephrine *in vitro* almost as well as the norepinephrine-rich hypothalamus. If such hidden systems should exist, it would follow that the subcellular site of reserpine binding in brain may not necessarily be limited only to amine storage granules within monoaminergic neurones or, alternatively, that in some brain regions such granules have a low monoamine content.

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REFERENCES

1. H. S. ALPERS and P. A. SHORE, *Biochem. Pharmac.* **18,** 1363 (1969).
2. S. NORN and P. A. SHORE, *Biochem. Pharmac.* **20,** 1291 (1971).
3. S. NORN and P. A. SHORE, *Biochem. Pharmac.* **20,** 2134 (1971).
4. J. GLOWINSKI and L. L. IVERSEN, *J. Neurochem.* **13,** 655 (1966).
5. R. P. MAICKEL and F. P. MILLER, *Adv. Pharmac.* **6A,** 71 (1968).
6. G. P. QUINN, P. A. SHORE and B. B. BRODIE, *J. Pharmac. exp. Ther.* **122,** 295 (1958).
7. L. MANARA and S. GARATTINI, *Eur. J. Pharmac.* **2,** 139 (1967).
8. S. H. SNYDER and J. T. COYLE, *J. Pharmac. exp. Ther.* **165,** 78 (1969).

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Inhibition of cholesterol side-chain cleavage by azacholesterols*

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INHIBITORS of cholesterol side-chain cleavage are of interest because of their possible diagnostic and therapeutic utility in diseases associated with hyperfunctioning adrenal glands.[1] One such inhibitor, aminoglutethimide (I), is known to block the conversion of cholesterol (III) to 20α-hydroxycholesterol

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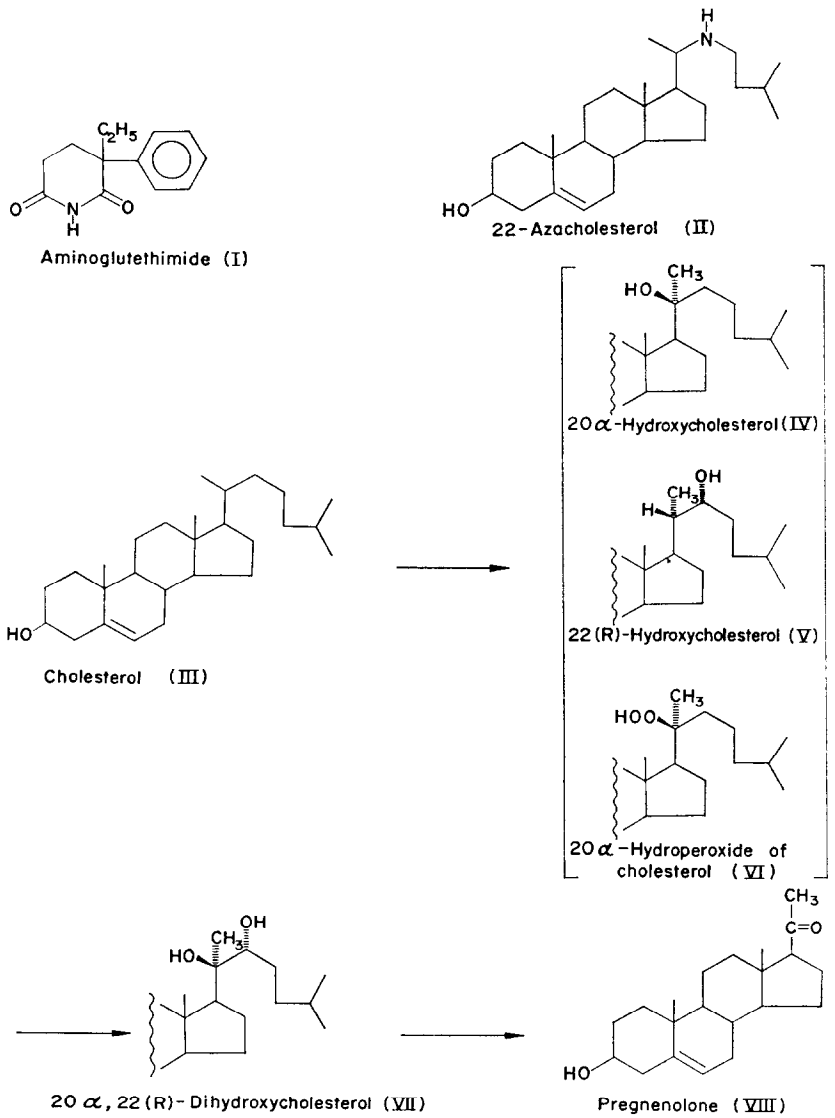

FIG. 1. Proposed intermediates in cholesterol side-chain cleavage.

(IV)[2,3] and thereby cause a reduction in the formation of all hormonal steroids, including cortisol and aldosterone.[4,5] Our previous studies[6] with azacholesterols suggested that some of these isosteres of cholesterol would be effective competitive inhibitors of the side-chain cleavage reaction. Investigation of several azacholesterols revealed that 22-azacholesterol (II) represents the most potent inhibitor of cholesterol side-chain cleavage reported to date.

Recent studies on the side-chain cleavage reaction in endocrine tissues showed that the sequence of events is more complicated than originally presumed. The initial proposals suggested that 20α-hydroxylation was the first and rate-limiting step in pregnenolone (VIII) and subsequent steroid biosynthesis.[7,8] More recent studies by Burstein and Gut,[9,10] however, revealed that 22 (R)-hydroxycholesterol (V) is the more important monohydroxycholesterol intermediate. Moreover, their kinetic data supported the assumption that a more significant pathway may be via the direct formation of 20α,22(R)-dihydroxycholesterol (VII). Additional support for V and VII as key intermediates was provided by the isolation of these sterols from bovine adrenal glands.[11] In addition, Van Lier and Smith[12]

presented evidence to suggest that the initial intermediate may be a 20α-hydroperoxide of cholesterol (VI). They found that VI could readily undergo enzymic conversion to the diol VII. Obviously many questions remain unanswered, but a selective inhibitor of any one of these enzymic reactions may prove a valuable tool to aid in the elucidation of the mechanism for the cleavage reaction.

Assays for cholesterol side-chain cleavage activity were performed according to the method of Doering.[13] An acetone powder preparation of the mitochondrial fraction of bovine adrenal cortex was used as the enzyme source. The powder was reconstituted in 0·01 M phosphate buffer (pH 7·4) to give a protein concentration of 5–8 mg/ml as determined by the method of Lowry *et al.*[14] Use of cholesterol-7α-^{3}H,26-^{14}C as the substrate allowed volatilization of one of the cleavage products as isocaproic acid-^{14}C. The enzymic activity was readily calculated from a change in the isotope ratios.

The inhibitory activity of the test compounds was determined at a final concentration of 0·1 mM or less. In all determinations, controls were run in the absence of inhibitor and the per cent inhibition was the average of at least two experiments. Table 1 compares the inhibitory activity of three aza-cholesterols and two diazacholesterols with that for aminoglutethimide. Pregnenolone was also included, since it is known to act as a feedback inhibitor of the cleavage reaction.[15]

TABLE 1. INHIBITORY ACTIVITY OF AZA ANALOGS OF CHOLESTEROL ON THE CONVERSION OF CHOLESTEROL* TO PREGNENOLONE

Compound	Concn (μM)	Per cent inhibition
20-Azacholesterol	100	79·1
	10	60·3
	1	4·2
22-Azacholesterol	100	100
	10	87·7
	1	26·3
	0·1	3·9
25-Azacholesterol	100	35·5
	10	0
20,25-Diazacholesterol	100	36·4
22,25-Diazacholesterol	100	69·7
Aminoglutethimide	100	40·2
Pregnenolone	100	29·4

* In all experiments a substrate concentration of $3·3 \times 10^{-9}$M was used. The incubations were carried out at 37·5° with 0·5 mg of adrenal protein from bovine adrenal cortex.

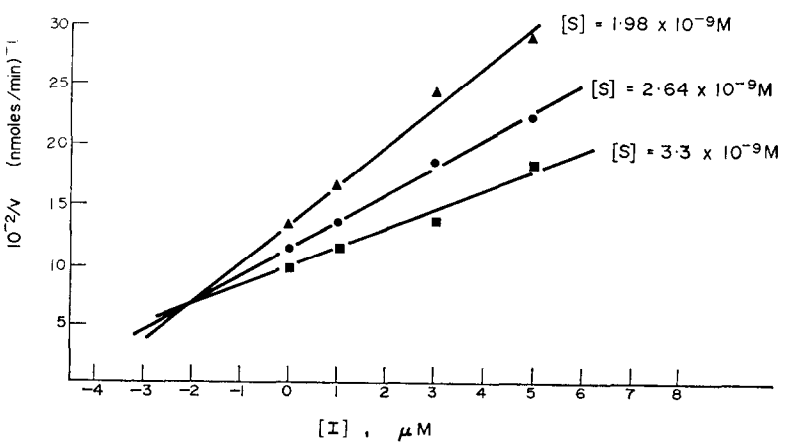

FIG. 2. Competitive inhibition of cholesterol side-chain cleavage by 22-azacholesterol (K_i = 2·2 μM).

22-Azacholesterol was the most potent inhibitor studied (K_i = 2·2 μM, Fig. 2) and has several times the activity of aminoglutethimide. The nature of the inhibition was determined by the method of Dixon[16] and was shown to be competitive with substrate (Fig. 2). Interestingly, 22-azacholesterol was slightly more inhibitory than 20-azacholesterol and agrees with the current view that 22 (R)-hydroxylation rather than 20α-hydroxylation is the more significant step in the cleavage reaction.

Previous studies with the azacholesterols showed that 25-azacholesterol and the two diaza analogs are potent inhibitors of cholesterol biosynthesis and cause a reduction in serum cholesterol levels of hypercholesterolemic rats at very low doses.[6,17,18] By contrast, 20- and 22-azacholesterol failed to cause any hypocholesterolemic effect at high doses. Thus the ability of 20- and 22-azacholesterol to inhibit the cleavage reaction appears to be somewhat specific and this specificity is decreased by replacement of C-25 with an N atom.

20,25-Diazacholesterol (Ornitrol) is currently employed as a chemosterilant in the control of pest pigeon populations.[19] Ingestion of this compound by pigeons causes inhibition of egg production and temporary sterility. Although the exact mode of action of this agent in pigeons is unknown, the above studies suggest that inhibition of cholesterol side-chain cleavage and an accompanying decrease in steroid hormone production may play a role in the antifertility action of the drug.

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REFERENCES

1. T. E. Temple and G. W. Liddle, *A. Rev. Pharmac.* **10,** 199 (1970).
2. F. W. Kahnt and R. N. Neher, *Helv. chim. Acta* **49,** 725 (1966).
3. M. P. Cohen, *Proc. Soc. exp. Biol. Med.* **127,** 1086 (1968).
4. R. Cash, A. J. Brough, M. P. Cohen and P. S. Satch, *J. clin. Endocr.* **27,** 1239 (1967).
5. L. M. Fishman, G. W. Liddle, D. P. Island, N. Fleischer and O. Kuchel, *J. clin. Endocr.* **27,** 481 (1967).
6. R. E. Counsell, P. D. Klimstra, L. N. Nysted and R. E. Ranney, *J. med. Chem.* **8,** 45 (1965).
7. T. T. Tchen, in *Functions of the Adrenal Cortex* (Ed. K. W. McKerns), Vol. 1, p. 3. Appleton-Century-Crofts, New York (1968).
8. G. J. Boyd and E. R. Simpson, in *Functions of the Adrenal Cortex* (Ed. K. W. McKerns), Vol. 1, p. 49, Appleton-Century-Crofts, New York (1968).
9. S. Burstein and M. Gut, *Steroids* **14,** 207 (1969).
10. S. Burstein, H. L. Kimball and M. Gut, *Steroids* **15,** 809 (1970).
11. R. Dixon, T. Furutachi and S. Lieberman, *Biochem. biophys. Res. Commun.* **40,** 161 (1970).
12. J. E. Van Lier and L. L. Smith, *Biochim. biophys. Acta* **210,** 153 (1970).
13. C. H. Doering in *Methods in Enzymology* (Ed. R. B. Clayton), Vol. XV, p. 591, Academic Press, New York (1969).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
15. S. B. Koritz and P. E. Hall, *Biochemistry, N.Y.* **3,** 1298 (1964).
16. M. Dixon, *Biochem. J.* **55,** 170 (1953).
17. R. E. Ranney and D. L. Cook, *Archs int. Pharmacodyn. Thér.* **154,** 51 (1965).
18. R. E. Counsell, P. D. Klimstra, R. E. Ranney and D. L. Cook, *J. mednl pharm. Chem.* **5,** 720 (1962).
19. J. E. Wooford and W. H. Elder, *J. Wildl. Mgmt* **31,** 507 (1967).