is little stimulation of lactate or glycerol output such as is seen on addition of adrenaline or when respiration is enhanced by addition of octanoate.

Caffeine-induced contractures can be prevented by various local anaesthetics, again by mechanisms thought to involve removal of Ca^{2+} from the scene of activity (Feinstein, 1963, 1964; Hauser & Dawson, 1968), and it is noteworthy that their addition (at 1 mM) to isolated diaphragm suppresses the enhancement of respiration induced by the methylxanthines and restores the appearance of the tissue to normal. Surprisingly, however, the rate of amino acid incorporation into protein is not restored to normal, but depressed further. Those compounds most successful in suppressing enhancement of respiration by methylxanthines (butacaine, dibucaine and tetracaine) were most potent in inhibiting protein synthesis in the tissue. Conversely procaine, cocaine and lignocaine had little influence on either parameter. The mechanism of this inhibition is again not clear, but is not explicable solely in terms of inhibition of amino acid uptake by the tissue, though this also occurs. Bloxam (1967a,b) noted the toxicity of various anaesthetics to liver, including interference with amino acid transport; these results in effect extend such observations to muscle.

The initial motivation of these experiments was the question whether the action of insulin on protein metabolism in muscle is influenced by the intracellular concentration of 3':5'-cyclic AMP. None of the treatments influenced the relative extent of stimulation of incorporation by insulin. Coupled with lack of direct evidence for any influence of the hormone on the 3':5'-cyclic AMP content of muscle, it appears unlikely that the effect of insulin on protein synthesis in this tissue involves the cyclic nucleotide.

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The Formation of *N*-Hydroxymethylcarbazole as a Metabolite of *N*-Methylcarbazole

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The biological demethylation of methylamines has been postulated as occurring via one of two intermediate pathways. One route suggested is that the

N-oxide is first formed, which rearranges to yield the demethylated product plus formaldehyde. The other suggestion is that the methyl group is first oxidized to yield a carbinol.

However, only with compounds having a carbonyl group adjacent to the nitrogen atom have the proposed hydroxymethyl derivatives been detected (for fuller discussion see McMahon, 1966).

We now report the formation of *N*-hydroxymethylcarbazole as a metabolite of *N*-methylcarbazole both *in vitro* and *in vivo*.

After incubation of *N*-methylcarbazole with a fortified hepatic microsomal preparation obtained from rabbit, one major and three minor metabolites were detected by chromatographic techniques. The principal compound had chromatographic properties and responses to spray reagents identical with those of authentic *N*-hydroxymethylcarbazole. Elution from chromatography plates showed that these compounds had identical u.v.-absorption spectra.

Treatment of urine, obtained from a rabbit after administration of *N*-methylcarbazole, with β -glucuronidase also produced an ether-soluble metabolite identical with the major product of the oxidation *in vitro*.

The minor metabolites observed in the microsomal oxidation of *N*-methylcarbazole were recognized as carbazole, 3-hydroxycarbazole and possibly 3-hydroxy-*N*-methylcarbazole, the first by comparison with authentic material or by comparison with the phenolic metabolite obtained from carbazole (Johns & Wright, 1964).

The demethylation of *N*-methylcarbazole was compared in various species by using the Nash assay for formaldehyde. The results showed a wide species variation in the order: mouse < rat < hamster < rabbit < cavy.

The detection of a hydroxymethyl compound as a metabolite from a methylamine supports the concept that this is an early step in dealkylation. In this case, like those observed by earlier workers, the methyl group was attached to a relatively non-basic nitrogen atom in the substrate. It may be that the basicity of a compound decides whether complete dealkylation or the formation of a hydroxymethyl derivative is the ultimate route of metabolism.

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