The B analyses were made by J. Thoburn; the C, H and N by H. Beck and V. Stryker.

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## THE DEGRADATION OF CHOLESTEROL BY MAMMALIAN TISSUE EXTRACTS

*Sir:*

As part of a study concerned with the metabolic degradation of cholesterol and its conversion to bile acids, we have successfully prepared aqueous particle-free extracts of mammalian tissue which are capable of degrading the side-chain of cholesterol. The major product, obtained in yields up to 5%, has been isolated and identified as isocaproic acid. Active enzyme preparations have been obtained from beef adrenals, ovary, testis and rat liver. Extracts of the first three tissues were obtained by homogenization in 0.3 *M* sucrose followed by high speed centrifugation (85,000 *G*) at 0° for 30 minutes to remove the particulate fraction. The sedimented material was essentially inactive. The aqueous phase required only adenosine triphosphate and diphosphopyridine nucleotide for activity. This was demonstrated by precipitation of the active enzymes by half saturation with $(NH_4)_2SO_4$, followed by dialysis of the precipitate for 24 hours against cold water. The resulting solution, which was rendered inactive by stirring for a few minutes with charcoal, was restored to activity by the addition of the above cofactors. In most instances the enzyme preparations were incubated for three hours at 37° under oxygen or air at a *p*H of 8.3 (0.07 *M* tris-(hydroxymethyl)-aminomethane buffer). The substrate was an albumin stabilized emulsion[1] of cholesterol-26-$C^{14}$.[2]

The radioactive isocaproic acid was isolated by steam distillation of the acidified extracts followed by paper chromatography[3] (diethylamine–butanol; $R_f$ 0.67). After elution from the papers and subsequent addition of carrier isocaproic acid, the anilide (m.p. 110°) and the *p*-bromophenacyl ester (m.p. 76–77°) were prepared. The specific activity of these derivatives remained constant despite repeated recrystallizations. Although small amounts of radioactivity could be detected in other acids, the isocaproic acid invariably contained the greatest radioactivity.

Following incubation of the adrenal extracts with cholesterol-4-$C^{14}$ [4] several radioactive steroids have been isolated by standard chromatographic procedures.[5] Surprisingly, neither progesterone nor pregnenolone were found to be radioactive; negative results were obtained regardless of whether these two steroids were added as carrier prior to or following incubation.

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(4) R. B. Turner, THIS JOURNAL, **72**, 579 (1950); R. D. H. Heard and P. Ziegler, *ibid.*, **73**, 4036 (1951); W. G. Dauben and J. F. Eastham, *ibid.*, **73**, 4463 (1951).

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The active system in rat liver was found to be localized in the particulate fraction which could be extracted by stirring with water to obtain a soluble preparation. In this tissue the original cytoplasmic supernatant fluid was found to be inhibitory unless the proteins were denatured by boiling and subsequently removed by centrifugation.

It is hoped that a more extensive report of this study will be published shortly.

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## THE ACONITE ALKALOIDS. XXVI. OXONITINE AND OXOACONITINE

*Sir:*

Permanganate oxidation of aconitine,[1] $C_{34}H_{47}NO_{11}$, furnishes two neutral products, oxonitine[2,3,4] and oxoaconitine.[3,4] The nature and formulation of the former has long been in doubt.[2,4] However, the formula $C_{32}H_{41}NO_{12}$ based in part on an oxidative scission of the N-ethyl group is still accepted by some.[1,5] Because of the important relationship these substances bear to a correct interpretation of the structure of aconitine, we wish to present evidence showing that oxidation of aconitine to oxonitine as well as to oxoaconitine *proceeds without the loss of carbon atoms.*

It appears that the use of acetic acid or chloroform to recrystallize oxonitine has led to incorrect formulations due to retention of solvent. When recrystallized from ethanol, benzene or acetone, the data obtained clearly support a $C_{34}H_{45}NO_{12}$ formulation. Calcd. for $C_{34}H_{45}NO_{12}$: C, 61.90; H, 6.87. Found: (EtOH) C, 61.73, 61.66; H, 6.65, 6.86; (benzene) C, 61.83, 61.81; H, 6.74, 6.90; (acetone) C, 61.88, 62.10; H, 6.76, 6.73; (pyridine–acetone) C, 62.08; H, 6.64. Oxonitine from $CHCl_3$ or $CH_2Cl_2$ gave consistently low carbon values and showed the presence of several per cent. chlorine. Our oxonitine melted at 279–284° though occasionally 288–293° was noted apparently due to dimorphism. $[\alpha]^{27}D$ −49° (*c* 0.25 in chf.). Oxonitine in contrast to oxoaconitine did not form an oxime.

The formulation of oxoaconitine has now been revised to $C_{34}H_{43}NO_{12}$; m.p. 266–272.5°, $[\alpha]^{27}D$ −100° (*c* 0.3 in chf.). Calcd. for $C_{34}H_{43}NO_{12}$: C, 62.09; H, 6.59. Found: C, 61.90; H, 6.57. Oxime, m.p. 282–285.5°. Calcd. for $C_{34}H_{44}N_2O_{12}$; C, 60.70; H, 6.59, N, 4.17. Found: C, 60.59; H, 6.67; N, 4.37.

The formation of oxonitine and oxoaconitine from aconitine appears to involve oxidation of an N-ethyl group to N-acetyl. Their behavior toward

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