munoglobulin G (IgG) is thought to depend on the simultaneous attachment of two IgG molecules (*11*), perhaps neuraminidase treatment allows more antibody to attach to the cell in such a manner that more complement can be bound. It is also possible, since Ray *et al.* (*12*) found that rabbit serum treated with cobra venom used as complement was fully lytic for neuraminidase-treated cells and that the removal of sialic acid uncovers a site essential for the completion of the lytic action of complement rather than the initiation step of complement fixation. Whatever the mechanism causing the increase in sensitivity of treated cells, it appears from our studies that many other CYNAP-like reactions are occurring for both HL-A and other lymphocyte antigens and that these reactions give positive cytotoxicity tests when neuraminidase-treated lymphocytes are used.

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8. The buffered calcium saline contained 0.145*M* NaCl, 0.003*M* $CaCl_2$, and 0.004*M* $NaHCO_3$ in H_2O.
9. One unit of enzyme activity is defined by Behring Diagnostics, Inc., as the amount of enzyme necessary to release 1 μg of *N*-acetylneuraminic acid from an acid α-1-glycoprotein substate in 15 minutes at 37°C and *p*H 5.5. The original sample of Behring neuraminidase used in the experiments (provided by Dr. B. Sanford) was reported free of detectable proteolytic activity by J. F. Codington [*J. Nat. Cancer Inst.* **45**, 673 (1970)].
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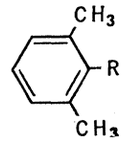
Oxidative N-Dealkylation: A Mannich Intermediate in the Formation of a New Metabolite of Lidocaine in Man

Abstract. *Evidence is presented for a new metabolite of lidocaine. Its structure,* N^1*-ethyl-2-methyl-*N^3*-(2,6-dimethylphenyl)-4-imidazolidinone, suggests reactive electrophilic intermediates for the oxidative removal of* N*-alkyl groups in general.*

The local anesthetic lidocaine **1** has been useful for the control of acute cardiac arrhythmias. However, despite its extensive clinical use, little is known about its metabolism in man.



1 R, $NHCOCH_2N(CH_2CH_3)_2$

2 R, $NHCOCH_2NHCH_2CH_3$

3 R, NH_2

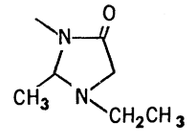
4 R, $NH-C\equiv\overset{+}{O}$

5 R, NH^+

6 R, +

7 R, $NHCOCH_2N$ 

8 R, $NHCOCH_2N(CH_2CH_3)(CH{=}CH_2)$

9 R, 

In 1966 Beckett *et al.* reported that after intravenous administration of **1** (three subjects), under conditions where the urine was maintained at constant acidic *p*H, approximately 10 percent of the administered dose could be accounted for as **1**; ω-ethylamino-2,6-dimethylacetanilide, **2**; and 2,6-dimethylaniline, **3** (*1*). To our knowledge these are the only metabolites of **1** that have been reported as occurring in man.

We now report our studies on this problem, in particular the finding of a new metabolite, and on the basis of the structure of this new metabolite we suggest the possibility of a reactive electrophilic intermediate for enzymatically mediated *N*-dealkylation reactions in general.

Three normal subjects were given 500 mg, by mouth, of the hydrochloride of **1**, containing 50 μc of randomly tritiated **1** as hydrochloride. Feces and urines of these subjects were collected, frozen, and stored over a period of 72 hours at which time 50 percent of the administered radioactivity had appeared in the urine. Since most of the radioactivity appeared in the urine within the first 8 hours after administration this fraction was selected for the initial isolation of metabolites.

A fraction composed of organic bases containing approximately 12 percent of the activity present in the urine was obtained by making the urine basic, exhaustively extracting it with ether, and the back extracting the ether fraction with aqueous acid. The ether was evaporated and the residue contained little activity, as did the residues after evaporation of ether extracts of urine obtained at various *p*H's. In addition, prior treatment of portions of the urine with a mixture of glucuronidase and sulfatase, followed by extraction with ether under a variety of conditions yielded insignificant activity in these organic extracts.

Since we expected to find **1** and **2** in the base-containing fraction, we sought conditions that would allow their separation and characterization by means of gas-liquid chromatography (GLC). Subsequently, a system (5 percent Carbowax on KOH-washed Chromosorb W, 1.8 m, 165°C) that gave excellent separation was found. In addition a high-resolution mass spectral study was made of **1** and **2** in order to elucidate the structures of any unknown metabolites. The study indicated that, as expected (*2*), both compounds fragmented primarily by homolytic cleavage of the carbon-carbon bond between the carbonyl and methylene groups to generate immonium ions at *m/e* 86 ($C_5H_{12}N$) and *m/e* 58 (C_3H_8N) as the base peak ions in the spectra of **1** and **2**, respectively. Exact measurements of the ions at *m/e* 148, 120, and 105 are consistent with structures **4**, **5**, and **6**, respectively. These structures represent

the other half of the molecule. Thus it appeared that metabolic transformations could conceivably be detected by changes in the atomic composition of these ions.

A gas-liquid chromatogram of the mixture of bases isolated from urine indicated five major components and a number of minor components. The presence of **1**, **2**, and **3** was confirmed by retention times on two different GLC systems, isolation of small quantities of each by GLC (flame ionization detector, stream splitter), scintillation counting of the fractions to establish the fact that they were radioactive, and high-resolution mass spectrometry.

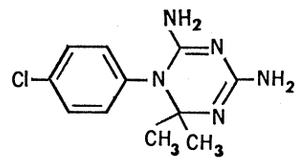
The largest peak in the GLC trace was unknown. Collection and scintillation counting of this material indicated that it was a metabolite of **1**, while its mass spectrum gave a molecular ion at *m/e* 232 ($C_{14}H_{20}N_2O$). The empirical formula for **1** is $C_{14}H_{22}N_2O$, suggesting that the unknown contained one additional unit of unsaturation. A surprising feature of the mass spectrum for this material was that the base peak ion occurred at *m/e* 85, $C_5H_{11}N$, and not at either *m/e* 86 or *m/e* 84. If the diethylaminomethylene portion of the molecule were intact, the compound should still give a base peak at *m/e* 86. However, if this portion of the molecule had undergone cyclization to a substituted pyrrolidine such as **7**, or some other isomeric substance, the base peak ion should occur at *m/e* 84. On the basis of the mass spectrum two substances were considered likely, the enamine **8** and the imidazolidinone **9**.

However, enamines are fairly reactive substances, hydrolyzing readily, and it seemed unlikely that such a substance could withstand the conditions of isolation. Compounds similar to **9** have been prepared by the condensation of an aldehyde with a suitable secondary amine (*3*). Hence acetaldehyde was condensed with **2**, and the reaction mixture was examined by GLC, which indicated the presence of two components distinct from the starting material, the major one having identical retention time to that of the unknown. Isolation of this material and determination of its mass spectrum showed that its mass spectrum was identical to that of the unknown, while analysis of its nuclear magnetic resonance (NMR) spectrum indicated that the compound was N^1-ethyl-2-methyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone **9** (*4*). Admixture of the synthetic material to the urinary metabolite and recrystallization to constant specific activity established the structure of the metabolite as **9**. The synthetic material arises presumably by way of a Mannich type of intermediate, as shown in Fig 1, where the intermediate undergoes cyclization rather than rearrangement to an enamine (*5*).

There is considerable evidence that enzymatic *N*-dealkylations occur by way of a carbinolamine intermediate, such as that shown above, which then either spontaneously or enzymatically rearranges to an amine and an aldehyde (*6*).

Since both these reactions involve a common intermediate, it would seem probable that the (*7*) production in vivo of **9** follows a similar pathway. In the case of lidocaine, a nucleophilic center, the amide nitrogen, is built into the molecule five atoms away from the reactive center and is thus ideally situated to "scavenge" the reactive intermediate and lead to a noncharged molecule. Normally an amide nitrogen is a very weak nucleophile, and in the case of lidocaine this problem is compounded by the steric hindrance provided by the ortho methyl groups of the aromatic ring. This implies that good nucleophiles such as the sulfhydryl group of cysteine, or the ring nitrogen of histidine, could effectively compete for reaction with this reactive intermediate provided that such groups are at the enzymatic site. If the formation of such a reactive electrophilic intermediate is a general phenomenon in *N*-dealkylation processes, then perhaps the biological responses, either efficacious or toxicological, of various amine drugs may, at least partially, be explained in terms of reaction of such an intermediate with nucleophiles at a critical enzymatic site.

At least one precedent does exist for a similar metabolic transformation. In the early 1950's, Carrington and Crowther found that the triazine **10** occurred as a natural metabolite of the antimalarial proguanil **11** in man and a number of other species (*8*).



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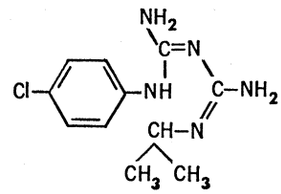

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In this particular case, **10** was found to be a much more potent antimalarial agent than the parent drug **11**. These investigators did not speculate on the mechanism of how such a metabolite might be formed, since knowledge of the microsomal metabolizing system of the liver was just beginning to emerge (*9*). A possible mechanism for formation of this compound is one directly analogous to that postulated for the formation of **9**.

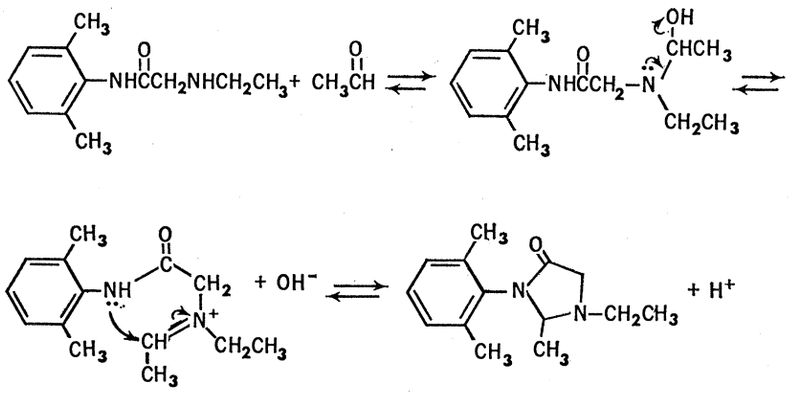

Fig. 1. Proposed mechanism for the formation of **9**.

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4. The NMR was determined in $CDCl_3$ with tetramethylsilane as an internal standard at 100 Mhz, and the results are as follows: methyl, doublet, J (spin-spin coupling constant) 5.5 hz, 0.87 ppm; methyl, triplet, J, 7.3 hz, 0.86 ppm; aryl methyls, nonequivalent singlets, 2.05 ppm and 2.56 ppm; side chain methylenes, nonequivalent, octets, J_{gem} 14.5 hz, J_{me} 5.5 hz, 2.49 ppm and 1.95 ppm; ring methylenes, nonequivalent, doublets, J_{gem} 14.0 hz, 2.87 ppm and 3.66 ppm; ring methine, quartet, J_{me} 5.5 hz, 4.12 ppm. In addition, the infrared spectrum showed a strong absorption at 1715 cm^{-1}, which is consistent with the carbonyl stretch expected of a five-membered ring lactam.
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Regional Blood-Flow Changes during 72-Hour Avoidance Schedules in the Monkey

Abstract. *Systemic and regional blood-flow measurements were made in five restrained monkeys before and during 72 hours of continuous work on an avoidance schedule. Systemic arterial pressures were elevated throughout the stress, initially owing to an increase in cardiac output, and after 72 hours owing to an increase in total peripheral resistance. Changes in the resistance in skeletal muscle blood vessels were closely related to these changes in total peripheral resistance.*

Psychological factors can be a potent influence in the acute elevation of systemic arterial pressure, and it has long been suspected that they play a major role in the pathogenesis of essential hypertension. Although much work has been done on the hemodynamic response to acute psychological stimuli (*1*), there is little information available about the changes in systemic and regional blood flow that occur during prolonged periods of emotional stress or anxiety.

The recent development of the radioactively labeled microsphere method (*2*) has made possible up to five separate measurements of the simultaneous regional blood flow and resistance in the awake, restrained monkey (*3*). This study reports the use of this method to assess the systemic and regional cardiovascular changes occurring during a 72-hour period of work on an avoidance schedule known to elevate urinary levels of 17-hydroxycorticosteroids, epinephrine, and norepinephrine (*4*).

Five male rhesus (*Macaca mulatta*) monkeys, weighing from 3.8 to 4.7 kg, were kept sitting in restraining chairs inside isolation booths for the duration of the experiment. In each animal polyvinyl catheters were surgically inserted into the abdominal aorta below the renal arteries and the inferior vena cava via the external iliac artery and vein, respectively; a third catheter was passed retrograde into the left ventricle through the left common carotid artery. The catheters, brought under the skin to the umbilical area and then to the outside of the isolation booth, were kept patent by a continuous infusion (at 1 ml/hr) of 0.9 percent NaCl solution containing 5 USP units of heparin per milliliter. Arterial, venous, and left ventricular end-diastolic pressures were measured with Statham P23Gb strain gauges placed at the mid-thoracic level and recorded on a Beckman type R Dynograph.

Each time the regional measurements were made the cardiac output was determined in duplicate by the indocyanine-green dye method with a Waters ×301 densitometer. Then, a batch (5000 to 10,000) of plastic microspheres, 50 μ in diameter, labeled with 500,000 to 2,000,000 count/min of different gamma-emitting nuclides (either ^{125}I, ^{141}Ce, ^{51}Cr, ^{85}Sr, or ^{95}Nb), was injected over a 15- to 20-second period through the left ventricular catheter. The spheres mix with blood in the left ventricle and travel with the blood until trapped in the arterioles in the end organs; they do not disturb the circulation since only about 0.1 percent of the total number of arterioles are so blocked. At the completion of the fifth injection the monkey was killed and the major organs plus the remaining tissues (so that total body counts could be obtained) were removed, weighed, and counted (*5*) in glass vials with a Nuclear-Chicago scintillation counter and a calibrated pulse height analyzer which divided the radioactivity into 100 channels of 10 kev. Since each microsphere label has a distinctive gamma-emission spectrum, the amount of radioactivity for each isotope in each organ was determined with appropriate correction factors for the known overlap of the isotope energies.

The fraction of cardiac output to each organ at the time of each of the determinations was the percentage of radioactivity in that organ compared with the sum of the radioactivity of that isotope found in the total body. The blood flow to each organ was the fraction of cardiac output delivered to that organ times the cardiac output determined by dye-dilution. Organ resistance was calculated as the mean pressure gradient $(\bar{P}_a - \bar{P}_v)$/flow to that organ (flow being measured in liters per minute). Details of the procedure, baseline values, and validation data have been described in detail (*2, 3*).

Four to 7 days after the surgical procedure each monkey was trained on a standard Sidman avoidance procedure with a 20-second response-shock interval (*6*). On this schedule the monkey learns to push a lever that resets a 20-second timer which, if allowed to complete its cycle, causes a shock to be delivered through an electrode taped to the monkey's tail. The shock intensity was adjusted to the minimal level which would maintain avoidance behavior. After each animal was trained (from 3 to 5 days) he was allowed from 7 to 10 days of rest prior to the experimental procedure. Pressures from each catheter, cardiac output, regional blood flows, and arterial samples for measurement of hematocrit and blood gases were measured before (baseline), and 20 minutes, 4, 24, and 72 hours after the avoidance schedule was begun. No punishing shocks were delivered for 5 minutes before or during the regional blood-flow measurements.