Palaeoxonodon is correct, it follows that the talonid cusp seen in *Amphitherium* is the hypoconid, and not, as commonly supposed, the hypoconulid.

The upper molars which are here referred to the taxon *P. ooliticus* could have evolved into the types seen in *Peramus* and *Pappotherium*, the former by merely increasing the height of the metacone and displacing it lingually, while reducing the sizes of the metacrista cusps and the stylocone, and the latter by the development of a protocone on the lingual face of the paracone. In light of the incipient talonid basin in the holotype of *P. ooliticus*, the absence of even a cingulum on the lingual face of the paracone in the corresponding upper molars lends weight to Clemens' (*11*) conclusion that the early development of the talonid proceeded independently from that of the protocone.

Finally, as *Amphitherium* was nearly contemporaneous with *Palaeoxonodon* and the Kirtlington dryolestid, it cannot be the common ancestor of the dryolestids, the peramurids, and the higher theria, although it may illustrate what such an ancestor was like. It would seem that the Eupantotheria diversified earlier than is currently believed, certainly before the Bathonian, and probably during the Early Jurassic.

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5. The bed is exposed in the Old Cement Works Quarry, Kirtlington, and is that designated 3p by W. S. McKerrow, R. T. Johnson, and M. E. Jakobson [*Palaeontology* **12**, 56 (1969)]. There is some evidence that the mammal teeth at Kirtlington represent a coprocoenosis—that is, an assemblage derived from the excreta of a predator [see J. S. Mellett, *Science* **185**, 349 (1974)]. Teeth of small theropods (juvenile *Megalosaurus*?) also occur in the deposit.
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12. Of the many people to whom thanks are due, space allows me to thank individually only M. Ware and W. A. Clemens for much help and encouragement, G. McTurk for the scanning electron micrographs, and F. Pinchin, D. Mugridge, and E. King for help with the fieldwork.

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Effect of Charcoal-Broiled Beef on Phenacetin Metabolism in Man

Abstract. *When charcoal-broiled beef was fed to human volunteers, who were then given phenacetin orally, the concentration of phenacetin in the plasma was lowered, but its half-life in the plasma was not changed. The data suggest that feeding charcoal-broiled beef enhances the metabolism of orally administered phenacetin in the intestine or during its first pass through the liver, or both.*

Variability in drug response is a major therapeutic problem, and it is important to know whether normal dietary constituents can stimulate or inhibit the metabolism of drugs and thereby alter their biological effect in man. Charcoal-broiled beef contains benzo[*a*]pyrene and other polycyclic hydrocarbons (*1*). The *O*-dealkylation of the analgesic, antipyretic drug phenacetin to its major metabolite, *N*-acetyl-*p*-aminophenol, is catalyzed in rats by an enzyme system inducible by the polycyclic hydrocarbons benzo[*a*]pyrene (*2, 3*) and 3-methylcholanthrene (*4, 5*). These observations led to studies that we recently reported which showed, in rats, a stimulatory effect of a diet containing charcoal-broiled beef on the metabolism of phenacetin by intestine in vitro (*6*). We therefore initiated a study in humans to determine the effect of a diet containing charcoal-broiled beef on the metabolism of phenacetin. We now report that feeding charcoal-broiled beef to man enhances the metabolism and lowers the plasma levels of orally administered phenacetin.

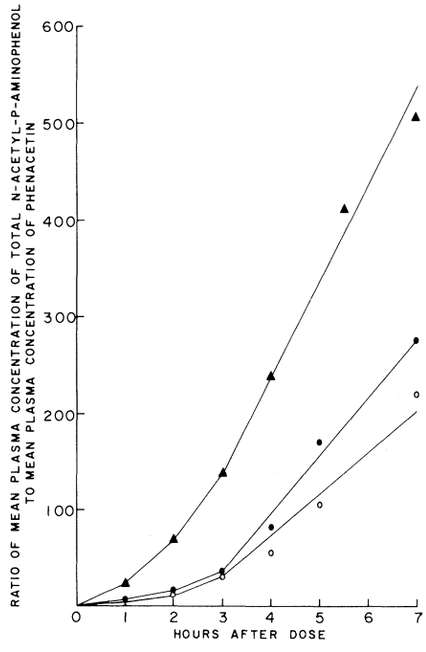

Fig. 1. Effect of a diet containing charcoal-broiled beef on the ratio of the plasma concentration of total (conjugated plus unconjugated) *N*-acetyl-*p*-aminophenol to the plasma concentration of phenacetin in subjects administered phenacetin. Subjects were given 900 mg of phenacetin orally after each dietary regimen. Each value represents the ratio of the mean plasma level of total *N*-acetyl-*p*-aminophenol to the mean plasma level of phenacetin for nine subjects. ▲, charcoal-broiled beef diet; ●, control hospital diet, second time; and ○, control hospital diet, first time.

Phenacetin metabolism was studied in nine healthy volunteers after they had been fed (i) a control hospital diet which was a balanced diet containing beef that had been cooked over burning charcoal but separated from the burning charcoal by aluminum foil, (ii) a charcoal-broiled beef diet which was a diet identical to the control hospital diet except that the beef had been exposed directly to the burning charcoal during cooking, and (iii) the control hospital diet for a second time. The phenacetin metabolism studies were carried out the morning after the subject had eaten (i) the control hospital diet for 7 days, (ii) the control hospital diet for an additional 3 days followed by the charcoal-broiled beef diet for 4 days, and (iii) the control hospital diet for the next 7 days.

The subjects were between 21 and 35 years old and weighed between 62 and 77 kg. Eight were male and one was female. The subjects were all nonsmokers and none were heavy drinkers of alcohol, coffee, or tea. No subject used any drug habitually, and no subject used any drug other than aspirin in moderation during the study.

Breakfasts were prepared by the subjects and eaten at home, and lunches and dinners were prepared by the diet kitchen of the Rockefeller University Hospital and eaten at the hospital. Breakfasts included only eggs, cereal, bread, rolls, muffins, pancakes, waffles, donuts, butter, syrup, jelly, fruit, fruit juice, carrots, milk, coffee and tea. Lunches consisted of a hamburger on a bun, tossed salad with French dressing, dessert (canned peaches, fresh apples, canned pears, fresh pears, fresh fruit cup, watermelon, canned fruit cocktail), and a beverage (coffee, tea, milk). Dinners consisted of steak, potato, vegetable (green beans, asparagus, carrots, peas, beets, waxed beans), bread and butter, dessert (ice cream, sherbert), fruit juice, and a second beverage (coffee, tea, milk). The hamburgers and steaks were lean beef and weighed, respectively, 8 and 6 ounces (1 ounce = 28 g) prior to cooking. They

Table 1. Effect of a diet containing charcoal-broiled beef on the plasma concentration of phenacetin in man. Subjects were given 900 mg of phenacetin orally after each dietary regimen. Each value represents the mean ± standard error for nine subjects. Results were analyzed statistically by Student's *t*-test. (a) Significantly different from control hospital diet (first time). (b) Significantly different from charcoal-broiled beef diet. (c) Significantly different from control hospital diet (second time). * $P < .05$. † $P < .01$. ‡ $P < .005$. § $P < .001$.

Diet	Phenacetin in plasma (ng/ml) at intervals after administration					
	1 hour	2 hours	3 hours	4 hours	5 hours	7 hours
Control hospital (1st time)	1328 ± 481	925 ± 166 (b§)	313 ± 60 (b‡)	149 ± 27 (b§)	66 ± 14 (b†)	17 ± 4 (b*)
Charcoal-broiled beef	319 ± 90 (c*)	163 ± 32 (a§, c‡)	74 ± 17 (a‡, c*)	34 ± 9 (a§, c*)	15 ± 4 (a†, c*)	7 ± 2 (a*)
Control hospital (2nd time)	1827 ± 661 (b*)	623 ± 128 (b‡)	271 ± 76 (b*)	99 ± 24 (b*)	40 ± 11 (b*)	14 ± 4

were placed either directly, or with a sheet of aluminum foil interposed, on a metal grill 2.5 inches (6.4 cm) from burning charcoal briquets and cooked for 12 minutes per side. The charcoal briquets were ignited with an electric fire starter.

For studies of phenacetin metabolism, the subjects were fasted overnight, except for water as they desired, and at approximately 8 a.m. were given 900 mg of finely powdered phenacetin by mouth with about 240 ml of water. No food or drink was permitted for the next 2 hours except for water as desired after 30 minutes. From each subject, a blood sample (20 ml) was drawn into a heparinized syringe just prior to the administration of phenacetin and at 1, 2, 3, 4, 5, and 7 hours after the dose. The blood samples were centrifuged immediately after being collected, and the plasma was removed and stored at −15°C.

Phenacetin and its metabolite, *N*-acetyl-*p*-aminophenol, were quantified in plasma by a gas chromatography–chemical ionization mass spectrometry procedure (*7*). In this method, deuterated phenacetin and deuterated *N*-acetyl-*p*-aminophenol were added to the plasma sample before extraction and served as internal standards. For determination of phenacetin and unconjugated *N*-acetyl-*p*-aminophenol, the plasma sample was extracted with a mixture of benzene and dichlorethane (7 : 3). The solvent was evaporated, and the residue was treated with diazomethane to convert *N*-acetyl-*p*-aminophenol to *p*-acetanisidine. The diazomethane was evaporated, and the residue was reconstituted in ethyl acetate and analyzed by gas chromatography–mass spectrometry with the mass spectrometer set to monitor isobutane-generated protonated molecular ions of phenacetin, *p*-acetanisidine, deuterated phenacetin, and deuterated *p*-acetanisidine in the effluent of the gas chromatograph. For determination of total *N*-acetyl-*p*-aminophenol, the plasma sample (0.1 ml), after the addition of deuterated *N*-acetyl-*p*-aminophenol, was treated with 16,000 units of β-glucuronidase and 64,000 units of sulfatase (Glusulase, Endo Laboratories) for 16 hours. The sample was then extracted with ethyl acetate, the solvent was evaporated, and the residue was treated with diazomethane and analyzed by gas chromatography–mass spectrometry for *p*-acetanisidine and deuterated *p*-acetanisidine.

Feeding subjects the charcoal-broiled beef diet, a diet identical to the control hospital diet except that the beef had been exposed directly to burning charcoal during cooking, resulted in a 59 to 82 percent decrease in the mean concentrations of phenacetin in the plasma from 1 to 7 hours after phenacetin administration (Table 1). Feeding subjects the control hospital diet for a second time resulted in a return of the mean phenacetin concentrations in the plasma to the levels observed after the subjects had been fed the control hospital diet for the first time (Table 1).

No significant changes in the mean half-life of phenacetin in the plasma were observed after the subjects had been fed the various diets. The mean (± the standard error) half-lives of phenacetin in the plasma were 59 ± 3, 69 ± 4, and 61 ± 4 minutes, respectively, after the subjects were fed the control hospital diet for the first time, the charcoal-broiled beef diet, and the control hospital diet for the second time.

The mean concentrations in the plasma of unconjugated or total (conjugated plus unconjugated) *N*-acetyl-*p*-aminophenol, phenacetin's major metabolite, were essentially the same after the subjects had been fed the control hospital diet for the first time, the charcoal-broiled beef diet, or the control hospital diet the second time before being given phenacetin. The ratios in the plasma of the mean concentration of the total *N*-acetyl-*p*-aminophenol to that of phenacetin were considerably higher after the subjects had been fed the charcoal-broiled beef diet than after they had been fed the control hospital diet either the first or second time (Fig. 1).

The foregoing data indicate that feeding charcoal-broiled beef to humans (for as long as 4 days) enhances the metabolism of phenacetin in the gastrointestinal tract or during the first passage of the drug through the liver. In support of intestine as a site of enhanced metabolism are studies showing that rat intestine contains an enzyme system capable of metabolizing phenacetin to *N*-acetyl-*p*-aminophenol (*2, 3, 5, 6*) and that the activity of this enzyme system is increased in intestine from rats that have been fed a diet containing charcoal-broiled beef as compared to intestine from rats fed a diet containing beef cooked on aluminum foil (*6*).

That foods can influence the metabolism of a drug is important because changes in an individual's diet or differences between the diets of different individuals can contribute, respectively, to intraindividual and interindividual variability in the bioavailability and, consequently, in the biological effect of the drug. Our data demonstrate variability in the metabolism and bioavailability of phenacetin in man resulting from alterations in diet. Charcoal-broiled beef in man's diet stimulates the metabolism and lowers the plasma concentration of orally administered phenacetin. Prior investigations have shown that Brussels sprouts or cabbage in the diet of rats stimulates the intestinal metabolism of phenacetin (*3*), 7-ethoxycoumarin (*3*), hexobarbital (*3*), and benzo[*a*]pyrene (*3, 8*). Additional studies are needed to determine the effects of vegetables and other foods on the metabolism and bioavailability of drugs in man.

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Plasma Alpha Amino-*n*-Butyric Acid to Leucine Ratio: An Empirical Biochemical Marker of Alcoholism

Abstract. *The plasma ratio of α-amino-*n*-butyric acid to leucine was elevated in ambulatory and hospitalized alcoholics as well as in baboons fed alcohol along with an adequate diet. There was a statistically significant positive correlation between this ratio and the degree of alcoholism assessed by three separate medical and psychological criteria in patients maintained on methadone.*

Alcoholism has been defined by many criteria (physiological, clinical, behavioral, psychological, attitudinal), but a universally accepted or practical definition is not available. Recently, we observed in alcoholics characteristic alterations in the plasma amino acids. The branched-chain amino acids (BCAA) were significantly depressed, whereas the concentration of α-amino-*n*-butyric acid was increased relative to the BCAA (*1*). The present study was undertaken to establish whether the ratio of plasma α-amino-*n*-buytric acid to leucine (A/L) could serve as a biochemical marker of long-term heavy drinking. Such a marker would enable the objective evaluation of different treatment modalities for alcoholism and would provide a means for the early detection and treatment of alcoholism. The study was conducted with 42 hospitalized alcoholics, 20 control subjects, 19 patients with nonalcoholic liver disease, and 25 participants in a methadone maintenance program. Alcohol was also fed to 13 baboons (as 50 percent of the total calories), and these were compared with 13 pair-fed control animals.

The 42 hospitalized alcoholics fulfilled the major criteria of alcoholism as defined by the National Council on Alcoholism (NCA) (*2*), and the majority had been drinking heavily until the time of admission. Blood was obtained after an overnight fast 1 to 60 days following admission. The blood was deproteinized with sulfosalicylic acid and analyzed for amino acids with a Beckman 119 amino acid analyzer according to the two-column method for physiological solutions (*3*). Twenty nonalcoholics (seven laboratory workers, seven patients, and six methadone maintenance patients) served as a common control group. Plasma ratios of A/L were determined for each subject, and the means for alcoholics and nonalcoholics were calculated. The hospitalized alcoholics had a greater than twofold increase in the ratio of A/L compared to the nonalcoholic controls (Fig. 1). A similar increase was observed in nine of the baboons fed alcohol (along with an adequate diet) (*4*) for 1 to 4 years. Furthermore, an increased ratio was present both in 14 hospitalized alcoholics and three baboons that had only minimal biochemical or morphological hepatic abnormalities due to alcohol, as well as in the remaining 28 patients and six baboons with more severe liver injury. However, no elevation of the A/L ratio was noted in 19 patients with nonalcoholic liver injury.

We observed an abnormally elevated ratio both in well-nourished alcoholics and in poorly nourished ones. Indeed,

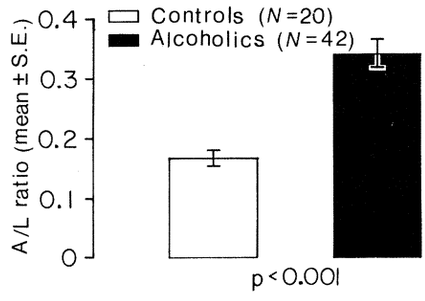

Fig. 1. Increased plasma A/L ratios in a representative population of hospitalized alcoholics. Alcoholism was defined by the fulfillment of major NCA criteria (*2*).

the ratio of A/L (mean ± standard error) among alcoholics with dietary protein deficiency for greater than 2 weeks (N = 21; A/L = 0.347 ± 0.039) was similar to that in alcoholics with deficiencies of shorter duration (N = 21; A/L = 0.350 ± 0.026), both ratios being high compared to controls (N = 20; A/L = 0.166 ± 0.011). Baboons fed alcohol along with a protein deficient diet (6 percent of calories as protein) developed an abnormal ratio (N = 4; A/L = 0.250 ± 0.054) when ethanol was given but not when it was omitted (N = 4; A/L = 0.090 ± 0.017). Furthermore, it was reported previously (*5*) that in extreme dietary protein deficiency (kwashiorkor), the calculated mean A/L ratio was within 2 standard deviations (S.D.) of the mean of our nonalcoholic controls.

The mechanism whereby long-term alcohol consumption results in plasma amino acid abnormalities is unknown. Extreme abnormalities of carbohydrate metabolism have been described in which the calculated ratio of A/L is increased: for example, in patients with massive obesity undergoing starvation (*6*) and in subjects consuming an experimental diet with very low carbohydrate and high fat content (*7*). The relationship of these findings to the abnormal ratios we observed in alcoholics is unknown since none of the subjects we studied displayed these severe disturbances. However, the possibility that other metabolic abnormalities, especially those of carbohydrate metabolism, increase the plasma A/L ratio and result in false positive tests warrants further study. The effects of a single large dose (1 g/kg) of ethanol on this amino acid ratio were investigated in four subjects. The mean ratio of A/L did not increase; actually it decreased progressively from 0.173 to 0.121 over an 8-hour period following ingestion. Only two of the 42 hospitalized alcoholics had measurable alcohol in the blood at the time of sampling and thus the presence of alcohol in the blood is not required for the positivity of this test. The effect of the time elapsed since long-term alcohol consumption on this ratio was assessed by comparing patients sampled within 1 week of cessation of drinking with patients sampled after this period. An elevated ratio was defined as being greater than 2 S.D. above the means for controls. In subjects sampled within 1 week of cessation of drinking 16 out of 18 had elevated ratios while in those sampled after this period 13 out of 24 had elevated ratios. Furthermore, all baboons receiving ethanol on a long-