is an apparent increase in some cases in the ability of the tubule cells to excrete PAH upon exposure of the animal to an atmosphere of sufficiently reduced oxygen tension. This observation agrees with that of Alving *et al.*[1] in the case of chronic, intermittent exposure to anoxia.

[7] Eiler, J. J., Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 1943, **140**, 699.

[8] Heinbecker, P., Rolf, D., and White, H. L., *Am. J. Physiol.*, 1943, **139**, 543.

[9] Welsh, C. A., Rosenthal, A., Duncan, M. T., and Taylor, H. C., Jr., *Am. J. Physiol.*, 1942, **137**, 338.

An increase in the maximum tubular excretory ability has been effected by the administration of various hormone preparations,[7-9] indicating that hormonal influences are capable of causing alterations in the tubular transfer mechanism of the kidney. The possibility of such influences being operative here must be considered.

Conclusions. Upon exposure of animals to altitude, 1) glomerular filtration rate response varies widely with the individual, 2) TmPAH is increased or unaffected, and 3) TmG is decreased or unaffected.

16523

On Phenyletherase.*

CHARLES HUGGINS, ELWOOD V. JENSEN, AND ANNE STACK CLEVELAND.
From the Department of Surgery, University of Chicago, Chicago, Ill.

The enzymatic cleavage of the carbon-oxygen bond of phenyl ethers by rat tissues is reported in this paper. The methoxy-phenyl linkage occurs in a variety of biologically active compounds which include anti-fibrillatory compounds such as quinine, quinidine and α-fagarine[1] although here the methoxy radical apparently is not necessary[2] for the physiologic activity. This linkage also occurs in the anti-malarial compounds, atabrine and pentaquine; in the sedative papaverine; and in certain estrogen compounds such as dimethoxystilbestrol. The methoxy-phenyl linkage is present in the local anesthetic phenacaine. Barry, O'Rourke and Twomey[3] have shown that diphenyl ethers have potent antituberculous activity *in vitro*.

* This work was supported by grants from the American Cancer Society recommended by the Committee on Growth of the National Research Council and from Mr. Ben May, Mobile, Alabama.

[1] Moisset de Espanés, E., and Weksler, B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 195.

[2] Di Palma, J. R., and Lambert, J. J., *Science*, 1948, **107**, 66.

[3] Barry, V. C., O'Rourke, L., and Twomey, D., *Nature*, 1947, **160**, 800.

We have been unable to find earlier reports on enzymatic decomposition of ethers. The enzyme pectase catalyzes the cleavage of "methoxyl" groups of soluble pectin, a reaction studied extensively by Kertesz.[4,5] However, in pectin the methyl groups are attached to the carbohydrate as an ester and this bond is considerably weaker than an ether linkage. *p*-Nitrophenyl esters[6] are attacked at a much faster rate by esterase than are its ethers by the enzyme under consideration here.

It was found that the splitting of the methyl and ethyl ethers of *p*-nitrophenol (PNP) is carried out extensively by the intact rat and slightly by homogenates of liver and kidney. Following injection of these compounds in rats, appreciable or even large amounts of free and combined *p*-nitrophenol are excreted in the urine; it is well known that phenols are in large part excreted in the urine as conjugates of glucuronic and sulfuric acids. Since *p*-

[4] Kertesz, Z. I., *J. Biol. Chem.*, 1937, **121**, 589.

[5] McColloch, R. J., and Kertesz, Z. I., *J. Biol. Chem.*, 1945, **160**, 149.

[6] Huggins, C., and Lapides, J., *J. Biol. Chem.*, 1947, **170**, 467.

nitrophenyl ethers are nearly colorless and free *p*-nitrophenol is highly colored in alkaline solution, cleavage of the ethers is easily determined and in amounts as small as 0.02 micromoles.

Experimental. Under ethyl ether anesthesia the peritoneal cavity of a rat, 400-500 g in weight, was opened and exactly 0.5 millimoles of a *p*-nitrophenyl ether was deposited therein as a finely ground powder. In other experiments the compound was deposited subcutaneously. The weights of ethers used were: *p*-nitroanisole (the methyl ether) 76.5 mg; *p*-nitrophenetole (the ethyl ether) 83.5 mg; *p*-nitrodiphenyl ether, 130 mg. In 3 experiments 68.5 mg of *p*-nitrotoluene was used. The incision was sutured with silk. The urine was collected for 2 days using a cage and funnel device. The rats were not fed, but 15 cc of 0.9% sodium chloride was injected subcutaneously each day.

Both free and combined *p*-nitrophenol in the urine were determined. A standard curve was prepared relating varying amounts of recrystallized *p*-nitrophenol (from 0.05 to 3 micromoles in 10 cc of glycine buffer, pH 11.2) against the logarithm of the density of color. An Evelyn photoelectric colorimeter with 400 mμ filter was used throughout.

Free *p*-nitrophenol was determined as follows: 1 cc of urine was acidified with 2N hydrochloric acid and extracted 3 times with about 10 cc of ethyl ether; the extract was transferred to a separatory funnel. The ether solution was twice washed with approximately 20 cc of water and then once with 10 cc of M/15 phosphate buffer pH 8. The ether solution was transferred to a colorimeter tube and 10 cc of glycine buffer pH 11.2 added. The tube was stoppered and shaken to deliver *p*-nitrophenol to the aqueous phase and depth of color determined colorimetrically.

The conjugated excretion products of *p*-nitrophenol were hydrolyzed before extraction. One ml of urine was added to 5 ml of Folin-Ciocalteu[7] reagent and placed in a steam bath for 90 minutes and centrifuged; the total free PNP was then determined as above on the supernatant.

Results. When 5 millimoles of the methyl and ethyl ethers of PNP were deposited intraperitoneally the rat suffered paralysis of the lower extremities with a profound fall of body temperature. However, the subcutaneous implantation of these and smaller amounts of the ethers was not followed by any evident toxic symptoms. Intraperitoneal injection of 0.5 mM amounts was not toxic.

A. Recovery of compound from urine. In normal rat urine, especially after acid hydrolysis, there appear to be traces of colored phenols and also small amounts of what are presumably carboxylic acids; these are extracted along with PNP by ether from acidic aqueous solution. Carboxylic acids, being more strongly acidic than PNP, are removed from the ether extract by shaking once with buffer of pH 8. This treatment removes only a trace or no PNP, although repeated extractions by a solution of pH 8 will remove a slight amount of PNP from the ether. Phenols which do not contain a nitro group are less acidic than PNP and remain in the ether phase when the PNP is finally extracted by buffer of pH 11. There was a 94-97% recovery of 0.25 and 0.5 micromoles of *p*-nitrophenol added to 1 ml samples of rat urine. The extraction of Folin-Ciocalteu reagent by the technic described yielded no color. The ethers of PNP were not broken down by steaming for 1½ hours in 2N hydrochloric acid or in Folin reagent. The time required for hydrolysis of conjugated PNP in urine by Folin-Ciocalteu reagent in the steam bath was determined at 15 minute intervals; maximum hydrolysis occurred after steaming for 1-1½ hours.

B. Cleavage by Intact Rat. The methyl and ethyl ethers of PNP were split at nearly the same rate (Table I) after intraperitoneal injection. The maximum excretion was between 6 and 24 hours after administration of the ethers and much greater amounts were split during the first than the second day. *p*-Nitrophenol was liberated mostly in the conjugated form; the ratio of conjugated to free PNP was in the order of 25 to 1. The methyl and ethyl ethers of PNP possess characteristic

[7] Folin, O., and Ciocalteu, V., *J. Biol. Chem.*, 1927, **73**, 627.

TABLE I.
p-Nitrophenol in Urine After Phenyl Ether Administration.
500 micromoles of the phenyl ether inserted intraperitoneally or subcutaneously.

Hours	0-6	%*	0-24	%*	24-48	%*	Total recovery	%*
			micromoles					
			(a) *p*-Nitroanisole.					
Free	0.9	0.18	1.59	0.32	0.32	0.06	1.91	0.38
Conj.	22.5	4.50	210.8	42.2	87.35	17.47	298.15	59.63
Free	5.69	1.14	13.47	2.70	0.85	0.17	14.32	2.86
Conj.	88.00	17.60	284.33	56.87	11.24	2.25	295.60	59.12
Free	1.54	0.31	3.20	0.64	0.70	0.14	3.90	0.78
Conj.	123.08	24.60	292.99	58.60	34.4	6.88	327.39	65.48
			(b) *p*-Nitrophenetole.					
Free	2.44	0.49	9.33	1.87	0.71	0.14	10.04	2.0
Conj.	86.10	17.22	220.3	44.06	4.80	6.96	255.10	51.02
Free	2.68	0.54	12.23	2.45	1.36	0.27	13.59	2.72
Conj.	83.41	16.68	206.15	41.23	52.10	10.42	258.25	51.65
Free	0.54	0.11	10.39	2.08	2.31	0.46	12.70	2.54
Conj.	55.38	11.08	204.29	40.86	69.03	13.81	273.22	54.64
			(c) *p*-Nitrodiphenyl ether.					
Free	0.16	0.03	0.64	0.13	0.34	0.07	0.98	0.2
Conj.	3.66	0.73	14.36	2.87	7.10	1.42	21.46	4.29
Free	0	—	0.27	0.05	0.60	0.12	0.87	0.18
Conj.	1.89	0.38	6.28	1.26	10.96	2.19	17.24	3.45

* % of ether excreted in urine as *p*-nitrophenol.

odors which were detectable in the urine after injection; while the unattacked ether seems from its odor to be excreted in the urine, no effort was made to determine it quantitatively in these experiments.

The use of *p*-nitrodiphenyl ether showed that the etherase effect was also operative on aromatic ethers. The splitting of the aromatic ethers was much less than ethyl and methyl ethers of *p*-nitrophenol (Table I) which might be expected from the fact that the carbon-oxygen bond in aromatic ethers is stronger than that of aliphatic ether. There was no detectable splitting of *p*-nitrotoluene.

C. Nature of excretory product. The combined ether extracts of the urine from 4 experiments with the methyl ether of PNP were pooled and evaporated to dryness. The compound was recrystallized twice from tuolene yielding 109 mg of yellowish crystals, m.p. 112° (uncorrected). An intimate mixture of this substance with an authentic sample of *p*-nitrophenol (m.p. 112°) also melted at 112°. These results show that the excretory product obtained after administration of ethers of *p*-nitrophenol is actually *p*-nitrophenol.

D. Enzymatic cleavage by tissue homogenates. In a preliminary study of the mechanisms of splitting of phenyl ethers by living tissues, homogenates of rat liver, kidney and pancreas 140 mg per 1 ml were prepared. Incubation at 37° for 22 hours was carried out of the following system: tissue homogenate 1 ml; M/15 phosphate buffer pH 7.4, 3 ml; methyl or ethyl ethers of PNP 2.5 micromoles dissolved in 5 ml of water containing 1% methyl alcohol. After 22 hours 20 ml of Folin-Ciocalteu reagent was added and the mixture digested for 90 minutes with extraction of free PNP as described above. Liver and kidney homogenates split 0.05 to 0.074 micromoles of free PNP (2-3% of the ether) which is enough to yield a definite yellow color. Pancreas was inactive. Preliminary boiling of the tissue homogenates abolished their capacity of cleavage; in control tubes without tissue or without PNP ethers no color was liberated.

Summary. Injection of methyl and ethyl ethers of *p*-nitrophenol in rats is followed by

excretion of large amounts of *p*-nitrophenol and its "conjugates" in the urine. This must involve a breakage of the carbon-oxygen bond of the ether linkage. Homogenates of rat kidney and liver but not of pancreas cause a definite though slight cleavage of these phenyl ethers. Pancreas was inactive; boiling the liver and kidney extracts destroyed their activity against phenyl ethers. This evidence indicates that the breakdown of phenyl ethers by rat tissue is enzymatic and we propose for the enzyme the name phenyletherase.

16524

Nitrogen Retention after Intravenous and Oral Administration of Protein Hydrolysate and Native Protein Enterally.*

YOSHIO SAKO,† ARNOLD J. KREMEN, AND RICHARD L. VARCO.

From the Department of Surgery, University of Minnesota, Minneapolis.

Madden,[1] and Kozoll and Mok[2] have stated that of the two routes for supplying exogenous protein, the oral route is associated with greater nitrogen retention. Elman *et al.*[3] and Shohl[4] have indicated that a given protein digest is attended with the same nitrogen retention when supplied either parenterally or enterally. The addition of more experimental evidence on nitrogen retention under different modes of administration it was hoped might help resolve the conflicting published data.

Method. Mongrel dogs were used in this study. Dogs No. 4 and 5 were animals which had been placed on a low protein diet consisting essentially of apples, potatoes, carrots and sucrose. The diet was fortified with substantial amounts of vitamin B complex, cod liver oil, choline, and minerals. Caloric intake was limited to 40 calories per kilo body weight per day, and the studies were not begun until 30% of the original body weight had been lost. Dog No. 6 was placed on the same diet and on the same caloric intake and the study started prior to any significant weight loss. Animals were kept in metabolism cages and urine collected over a 24-hour period, and specimens were analyzed quantitatively for total nitrogen by the macro Kjeldahl method.[5] The amino acid nitrogen was determined by the method of Frame.[6] Samples of the diet, and protein hydrolysate, and calcium caseinate (Casec‡) were all analyzed for total nitrogen by the macro Kjeldahl method. At the beginning of each study period, the diet was colored with congo red and feces collected for total nitrogen determinations.

Protein hydrolysate (Amigen‡) was given in amounts equivalent to 2 g of protein per kilo of body weight. This was administered in a total volume of 500 cc of fluid over a 6 hour period. After 5 days of intravenous infusion, the same amount of protein hydrolysate was given orally over a 6 hour period by means of a Wangensteen tube passed through the nose into the stomach. Two g of protein per kilo body weight as calcium caseinate was then given orally over a 6 hour period. A control period of 9 days was interposed between each

* The researches presented here were supported by grants of the Graduate School of the University of Minnesota.

† Fellow of the United States Public Health Service.

1 Madden, S. C., Basset, S. H., Remington, J. H., Martin, F. J. D., Woods, R. R., and Skull, F. W., *Surg., Gyn., Obstet.*, 1946, **82**, 131.

2 Kozoll, D., and Mok, W. T., *Proc. Central Soc. Clin. Research*, 1947, **20**, 12.

3 Elman, R., Archar, L. A., Horwitz, A., and Wolif, H., *Arch. Surg.*, 1942, **44**, 1064.

4 Shohl, A. T., *J. Clin. Invest.*, 1943, **22**, 257.

5 Peters, J., and Van Slyke, D. D., *Quant. Clin. Chem.*, 1931, **2**, 548.

6 Frame, E., *et al.*, *J. Biol. Chem.*, 1943, **199**, 225.

‡ Casec (calcium caseinate) and Amigen (enzymatic digest of casein) were kindly supplied by Mead Johnson of Evansville, Ind.