stimuli may place an unusual stress on certain organs, such as the kidney, because of the interference with its normal blood supply. A continuing series of such reactions over prolonged periods may not only interfere with the organ's function, but could also adversely affect the vessel segment which is reactive to the repetitive stimulation. A clearer understanding of the stimuli which provoke this response, the specific area where the response occurs, and the cellular factors providing the energy for the response may afford us valuable etiologic information for diseases such as essential hypertension and specific diseases of vessels.

Conclusions. (1) Pharmacologic doses of intravenous epinephrine in dogs caused a significant decrease in the vascular phase and a slower ascent to peak concentration of the I^{131} radiorenogram. These changes are essentially the same as noted to occur in humans who suffered with acute apprehension during the test procedure. (2) These same doses of epinephrine caused an intense constriction of the renal arteries proximal to or at the first major bifurcation as visualized with angiography and cineradiography. In one-fourth of the dogs the renal artery constriction was noted to occur within one to one and one-half centimeters of their aortic origin. The renal arteries proximal to the constricted zone were dilated.

1. Isley, J., Baylin, G. J., DeMaria, W. J. A., Sharp, K. W., Sanders, A. P., *Am. J. Roentgenol., Rad. Ther. and Nuclear Med.,* 1963, v90, 141.

2. DeMaria, W., Krueger, R. P., Sanders, A. P., James, J. M., Politano, V. A., Baylin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 762.

3. Abram, H. J., Boijsen, E., Borgstrom, K. E., *Radiology,* 1962, v79, 911.

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Stimulation of Phosphogluconate Pathway in Rat Brain Mince by Ethanol. (28740)

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Certain cerebral effects of ethanol would be understandable on the basis of impaired intracellular energy metabolism. If this were the case, glucose metabolism might be expected to be deranged since free glucose is the predominant energy source in the central nervous system(1). Indeed, ethanol does depress glucose oxidation in stimulated cortical preparations(2,3) and it has generally been considered that the Embden-Meyerhof glycolytic pathway represents the predominant catabolic route in this tissue(4-6). However, the fact that enzymes of the phosphogluconote pathway are present in adult brain(7-10) suggests that this latter pathway may, under certain conditions, also assume importance. The difficulty in demonstrating operation of this pathway seemed to be due to failure to maintain adequate levels of NADP* in the cerebral preparations. Glutathione reductase is specific for NADPH and is present in brain(11) and it has been shown that GSSG stimulated oxidation of C-1 of glucose without affecting that of C-6(12). Moreover, cerebral tissues contain a potent system which, upon disruption of cell structures, liberates nicotinamide from its nucleotides. Therefore NAD and NADP were included in our preparations at physiological concentrations and nicotinamide was added to inhibit

* The following abbreviations are used: NADP and NADPH, oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide phosphate (formerly triphosphopyridine nucleotide); NAD and NADH, oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide (formerly diphosphopyridine nucleotide); GSSG, oxidized glutathione; ATP, adenosine triphosphate.

the nicotinamide nucleotide splitting enzymes(13). By utilizing these precautions we have demonstrated that ethanol shifted cerebral metabolism of glucose toward the phosphogluconate pathway.

Materials and methods. Male rats of the Sprague-Dawley strain weighing 200-250 g, maintained on Purina chow were sacrificed by decapitation. The brains were excised rapidly and immersed in ice-cold Krebs-Ringer phosphate solution which contained 25 mM nicotinamide. The cerebral hemispheres, washed free of blood, were dissected and forced through a cold garlic press fitted with a stainless steel 40 × 40 mesh screen. The mince was blotted and 150 mg portions were added to chilled Warburg vessels which contained (in a final volume of 2.5 ml): 0.15 M phosphate, pH 7.4, 25 mM nicotinamide, 5 mM ATP, 0.45 mM NADP, and 6 mg glucose which contained 200,000 counts/min of glucose-1-C^{14} or glucose-6-C^{14}. Activities of glucose solutions were determined both by direct plating and by oxidation to $C^{14}O_2$ by persulfate(14). Other materials and their final concentrations, when added, were 0.45 mM NAD, 2.5 mM GSSG, 350 mg% ethanol, 5 mM acetaldehyde. The sidearm of each vessel contained 0.2 ml of 1.0 g/ml trichloroacetic acid and the center well contained 0.2 ml of 10% carbonate-free NaOH on a small roll of filter paper.

To assess the influence of ethanol on cerebral carbohydrate metabolism, rats were injected intraperitoneally with 3 g ethanol per kg body weight. After 30 minutes they were sacrificed and brains were treated in the usual way except that both the cold Krebs-Ringer wash solution and the incubation medium itself contained 350 mg% ethanol. The blood alcohol level of these rats at time of sacrifice was 350-360 mg%. Although hypoactive, the animals were alert.

All experiments were conducted in quadruplicate. Oxygen consumption was followed manometrically at 37°C for 30 min in an air atmosphere. Trichloroacetic acid was then added to the main body of the flask from the sidearm and shaking was continued for an additional 30 minutes to expel CO_2 from the incubation medium. Contents of the center well were transferred quantitatively with several washings to rubber-capped centrifuge tubes. Carbonate was eluted from the filter paper by occasional shaking and standing overnight. The paper was washed well, washings being collected in the centrifuge tube, and carbonate was precipitated by addition of 0.25 ml 1M NH_4Cl and 0.5 ml 1M $BaCl_2$. After standing for an hour, the centrifuged precipitate was washed twice with 8 ml H_2O and finally with acetone, using a Vortex mixer for suspension of the $BaCO_3$ each time. The $BaCO_3$ was transferred as a slurry in several drops of acetone to aluminum planchets on a hot plate and the tube was rinsed several times with small volumes of acetone which also were added to the planchets. The acetone was evaporated and a minimum of 2000 counts were totalled for each sample in a micromil window-equipped flow counter. Since the amount of $BaCO_3$ per planchet never exceeded 4 mg, no correction was made for self-absorption.

Results of these experiments are shown in Table I. In all cases $C^{14}O_2$ arising from C-1 of glucose was greater than that from C-6 indicating operation of the phosphogluconate oxidative pathway in cerebral cortex. Furthermore, presence of 2.5 mM GSSG increased $C^{14}O_2$ production from C-1 in every case without affecting production of $C^{14}O_2$ from C-6. GSSG had no influence on total respiration. These results are in agreement with those of Hotta(12) who found that GSSG stimulated the phosphogluconate pathway by augmenting reoxidation of NADPH.

Of primary importance was the observation that ethanol treatment, while depressing oxidation of both carbons from glucose, decreased mainly C-6 oxidation thereby increasing the C-1:C-6 radioactivity ratios. A greater fraction of the glucose was metabolized *via* the phosphogluconate pathway during alcohol intoxication. This effect was manifest whether or not NAD was added to the medium. NAD addition did stimulate oxygen consumption and the oxidation of both C-1 and C-6 of glucose but did not influence the C-1:C-6 ratio. Thus, operation

TABLE I. Oxidation of Glucose-1-C^{14} and Glucose-6-C^{14} by Rat Brain Mince.

System	O_2 consumption (μl)		Counts/min in $C^{14}O_2$				$C^{14}O_2$ from C-1 / $C^{14}O_2$ from C-6	
			from C-1		from C-6			
	+GSSG		+GSSG		+GSSG		+GSSG	
ATP, NADP	82 ± 9*	86 ± 1	847 ±225	968 ± 13	210 ± 7	198 ± 20	4.03 ±1.09	4.89 ± .44
Idem + ethanol	76 ± 3	82 ± 6	728 ± 32	1015 ± 49	134 ± 5	130 ± 5	5.43 ± .04	7.81 ± .08
ATP, NADP, NAD	107 ± 2	112 ± 3	1240 ± 63	1520 ± 81	306 ± 11	298 ± 22	4.06 ± .35	5.10 ± .66
Idem + ethanol	111 ± 6	110 ± 6	824 ± 28	1091 ± 61	130 ± 16	146 ± 4	6.34 ± .61	7.49 ± .63
Idem + acetaldehyde	125 ± 4	127 ± 9	1113 ± 21	1410 ± 26	243 ± 22	270 ± 10	4.58 ± .52	5.22 ± .28

* Mean ± standard error.

of the phosphogluconate pathway was not due to any non-physiological imbalance between NADP and NAD. Addition of acetaldehyde *in vitro* in the absence of ethanol had little effect on the C-1:C-6 ratio and thus leaves unanswered the question of whether or not the above results were due to ethanol *per se,* its aldehyde oxidation product delivered to the brain from other tissues, or a combination of both. The somewhat higher oxygen consumption in presence of acetaldehyde, accompanied by lower radioactivity in both CO_2 fractions may be due to oxidation of the unlabeled aldehyde since this compound is capable of acting as a substrate in brain metabolism(15).

Discussion. Katz and Wood(16,17) have cautioned against indiscriminate use of $C^{14}O_2$ ratios from C-1 and C-6 in evaluating the relative contributions of various pathways for glucose catabolism operating in the same tissue. However, we did not attempt to determine the quantitative importance of the phosphogluconate pathway in comparison to glycolysis, for example, but rather only to detect a functional phosphogluconate route in brain and to observe any factors which might influence it. In fact, Hotta(12) demonstrated by the additional use of glucose-2-C^{14} and glucose-U-C^{14} that the $C^{14}O_2$ from C-1 in excess of that from C-6 does indeed represent the extent of phosphogluconate pathway activity in brain.

The observation of phosphogluconate pathway activity in cerebral cortex and the demonstration that ethanol *in vivo* results in glucose being metabolized preferentially *via* this route raise several important considerations in regard to acute and chronic toxic effects of ethanol. The phosphogluconate pathway is not generally considered to be closely associated with pyrophosphate bond synthesis as is the case with mitochondrial oxidations of the citric acid cycle. Since glucose is, for all practical purposes, virtually the exclusive energy source for the central nervous system, the consequences of this metabolic derangement may be profound. The similarity between symptoms of hypoxia and increasing levels of ethanol on the brain

is notable. First there is a depression of cortical control (probably mediated *via* a depression of the reticular activating system), then emergence of uninhibited sub-cortical manifestations (*e.g.*, rage reaction). This is followed by loss of equilibrium and consciousness with progressive depression of lower centers terminating in respiratory arrest and death. The similarity of these two syndromes suggests an involvement in oxidative metabolism as the common denominator.

The mechanism by which ethanol altered cerebral glucose metabolism (and by inference, energy metabolism) is not clear. Westerfeld(18) has concluded that although a minor mechanism is potentially available in brain for oxidation of ethanol to acetaldehyde, this is probably of little quantitative consequence. However, acetaldehyde is oxidized in this tissue and aldehyde dehydrogenase is also an NAD enzyme. Following alcohol ingestion, blood acetaldehyde readily passes into the brain(19) and though of low concentration, would result in a considerable quantity of this substrate being delivered over a period of hours. This might serve to depress the NAD/NADH ratio and thereby seriously hinder availability of NAD for glycolytic and citric acid cycle oxidations. This, in turn, would shift glucose oxidation toward the NADP-dependent phosphogluconate pathway. Moreover, aldehyde dehydrogenase is capable of coupling with lactic dehydrogenase *via* its coenzyme in the same way that alcohol dehydrogenase is known to do with the latter enzyme. Ridge(20) found that after ethanol administration lactate, pyruvate and NAD concentrations in brain fluctuated with acetaldehyde levels in this organ in the manner to be expected from the foregoing considerations and that acetaldehyde oxidation in rat brain is in fact coupled to pyruvate reduction. Hence, glucose metabolized by way of the glycolytic pathway would be directed toward lactic acid synthesis — again, a wasteful process from an energy standpoint. Finally, aldehyde dehydrogenase, unlike dehydrogenases of the citric acid cycle, is a cytoplasmic enzyme. If NADH produced by its action were to be reoxidized aerobically, the NADH would have to diffuse through cytoplasm and deliver its electrons to be enzymically "shuttled" (*e.g.*, glycerophosphate or hydroxybutyrate shuttles) into a mitochondrion in order for reoxidation to take place. This is a relatively slow process, so again acetaldehyde oxidation might be expected to maintain more NAD in reduced form than would otherwise occur, making less oxidized NAD available and increasing the importance of the NADP-dependent phosphogluconate pathway for glucose oxidation. These considerations concerning aldehyde dehydrogenase apply equally well to alcohol dehydrogenase so that ethanol oxidation by brain, even if minor, would tend to shift glucose metabolism toward the phosphogluconate pathway. Finally, Stotz *et al*(21) showed that acetaldehyde when added to cerebral homogenates is rapidly lost, to a considerable extent by condensation with pyruvate to form acetoin. Formation of acetoin can amount to 80% of that expected stoichiometrically. The subsequent fate of this compound is not clear, but by thus accelerating pyruvate removal, another avenue is open for acetaldehyde to divert pyruvate from citric acid cycle oxidation. In fact, acetaldehyde has been shown to inhibit specifically pyruvate oxidation by rat brain mitochondria(22).

Summary. Administration of a moderately intoxicating dose of ethanol to rats resulted in an increase in that portion of glucose metabolized *via* the phosphogluconate pathway in minced preparations of cerebral cortex.

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1. McIlwain, H., *Biochemistry and the Central Nervous System,* 2nd ed., Little, Brown & Co., Boston, 1959, p7.

2. Lindbohm, R., Wallgren, H., *Acta pharm. et toxicol.,* 1962, v19, 53.

3. Beer, C. T., Quastel, J. H., *Can. J. Biochem. Physiol.,* 1958, v36, 543.

4. Bloom, B., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 317.

5. DiPietro, D., Weinhouse, S., *Arch. Biochem Biophys.,* 1959, v80, 268.

6. Higgins, E. S., *Experientia,* 1962, v18, 461.

7. Yamada, K., Shimazono, N., *Biochim. biophys. Acta,* 1961, v54, 205.

8. Buell, M. V., Lowry, O. H., Roberts, N. R., Chang, M. W., Kapphahn, J. L., *J. Biol. Chem.,* 1958, v232, 979.

9. Dickens, F., Glock, G. E., *Biochem. J.,* 1951, v50, 81.

10. Glock, G. E., McLean, P., *ibid.,* 1954, v56, 171.

11. Rall, T. W., Lehninger, A. L., *J. Biol. Chem.,* 1952, v194, 119.

12. Hotta, S. S., *J. Neurochem.,* 1962, v9, 43.

13. McIlwain, H., Rodnight, R., *Practical Neurochemistry,* J. A. Churchill, Ltd., London, 1962, p214.

14. Abraham, S., Hassid, W. Z., in *Methods in Enzymology,* Vol. 4, S. P. Colowick, N. O. Kaplan, ed., Academic Press, New York, 1957, p549.

15. Hine, C. H., Shick, A. F., Margolis, L., Burbridge, N., Simon, A., *J. Pharm. Exp. Therap.,* 1952, v106, 253.

16. Katz, J., Wood, H. G., *J. Biol. Chem.,* 1963, v238, 517.

17. ———, *ibid.,* 1960, v235, 2165.

18. Westerfeld, W. W., *Tex. Rep. Biol. Med.,* 1955, v13, 559.

19. MacLeod, L. D., *Quart. J. Stud. Alc.,* 1950, v11, 385.

20. Ridge, J. W., *Biochem. J.,* 1963, v88, 95.

21. Stotz, E., Westerfeld, W. W., Berg, R. L., *J. Biol. Chem.,* 1944, v152, 41.

22. Kiessling, K. H., *Exp. Cell. Res.,* 1962, v26, 432.

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Hypocholesterolemic Action of 17α-Methyltestosterone (MeT) in Hypophysectomized Rats.* (28741)

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Administration of large doses of either naturally-occurring or synthetic androgens produces a significant lowering of the serum cholesterol concentration in several species of laboratory animals(1,2,3,4). The size of the dose required suggests that the effect of these substances is indirect and is not a result of their normal hormonal activity. The present study explores the possibility that the cholesterol-lowering effect of the androgens may be mediated via the pituitary gland.

Experimental. Normal and hypophysectomized male Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories. Two weeks after hypophysectomy the animals were divided into 4 groups of 8 animals each and were housed in individual cages. Water and the pertinent diets (Table I) were available *ad libitum* throughout the experimental period (4 weeks). During the first 2 weeks of the experiment all hypophysectomized animals received 5% sucrose in their drinking water. The unoperated control groups (Table I) consisted of 3 animals per group. They were included in the present study to ascertain that Sprague-Dawley rats exhibit the same response to methyltestosterone as the CFN Wistar rats reported on in detail previously(3).

The stock diet consisted of Rockland laboratory chow; cholesterol and methyltestosterone in the indicated amounts were added to this diet in ether solution(3). The rats were weighed and bled weekly. Blood was obtained under ether anesthesia from the capillary blood vessels around the eye. At the end of 4 weeks the animals were killed under anesthesia by exsanguination from the heart, and testes, adrenals and thyroid of the operated rats were examined to determine whether hypophysectomy had been complete.

Serum total cholesterol concentrations and cholesterol content of the "high density" (α) and "low density" (β) lipoprotein fractions

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