6 experiments was 1.4 ml/min/100 g of heart, which was about 18% of the oxygen utilization of the working heart.

Cooling the blood perfusing the arrested heart reduced myocardial oxygen consumption to about one-third of that observed at normal body temperature and to 7% of the oxygen consumption of the intact working heart. These observations indicate that the rapid post-arrest recovery of the cold arrested heart is most likely due to its very low oxygen requirement. The possibility cannot be excluded, however, that the beneficial effects of cold in the arrested heart are not related to the reduction in myocardial oxygen consumption, but to some specific effect of low temperature on the metabolic characteristics of the myocardium. Application of these data to surgical situations in which cardiac arrest is necessary, suggests that coronary blood flow can be interrupted about 3 times longer in the hypothermic than in the normothermic arrested heart.

Summary. Oxygen consumption of the potassium arrested heart averaged 2.1 ml/min/100 g of perfused heart at normal body temperature, and 0.66 ml/min/100 g of perfused heart at 21.5 to 25°C.

1. Melrose, D. B., Dreyer, B., Bentall, H. H., Baker, J. B. E., *Lancet,* 1955, v2, 21.

2. Lam, C. R., *J. Thorac. Surg.,* 1956, v32, 370.

3. Berne, R. M., Jones, R. D., Cross, F. S., *J. Applied Physiol.,* 1958, v12, 431.

4. Cross, F. S., Berne, R. M., Hirose, Y., Jones, R. D., Kay, E. B., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 210.

5. Shipley, R. E., Wilson, C., *ibid.,* 1951, v78, 724.

6. Van Slyke, D. D., Neill, J. M., *J. Biol. Chem.,* 1924, v61, 523.

7. Katz, L. N., Lindner, E., *Am. J. Physiol.,* 1938, v124, 155.

8. Driscol, T. E., Berne, R. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 505.

9. McKeever, W. P., Canney, P. C., Gregg, D. E., *Fed. Proc.,* 1957, v16, 86.

10. Berglund, E., Monroe, R. G., Schreiner, G. L., *Acta, Physiol. Scand.,* 1957, v41, 261.

Received June 26, 1958. P.S.E.B.M., 1958, v99.

Significance of Circulating Phenols in Anemia of Renal Disease.* (24256)

I. DUNN, I. M. WEINSTEIN, M. H. MAXWELL AND C. R. KLEEMAN

Wadsworth Hosp., Veterans Admin. Center, and Dept. of Medicine, University of California Medical Center, Los Angeles

Anemia is a frequent complication in patients with chronic renal disease. The degree of anemia is roughly proportional to the level of azotemia, but the exact mechanism whereby anemia is produced is still unsolved. The most widely supported theory is that anemia results from a suppression of erythropoiesis by "toxic" metabolic products retained in the blood(1-5). Recent reports also stress the importance of increased erythrocyte destruction associated with inadequate bone marrow response(1-5) The hemolytic process is thought to be extracorpuscular, but no definite mechanism has been set forth. Evidence has appeared to suggest that phenol and/or its derivatives are hematopoietic poisons(6, 7). Conceivably they could be implicated either as bone marrow "toxins" or hemolytic agents. Most earlier work suffered from lack of specific analytic reactions employed to detect blood phenols(6). Using one such nonspecific reaction, the xanthoprotein index, Bock and Thedering(7) reported that elevated xanthoprotein levels were seen in anemic patients with renal insufficiency, and suggested that these substances might be implicated in production of anemia of these patients. Schmidt, *et al.*(8) applied a more specific technic for detection of blood phenols

* This study was supported by research grant from Public Health Service.

Technical assistance of Miss Licia L. Petriello, Miss Donna L. Taylor and Miss Patricia Ludden is gratefully acknowledged.

TABLE I. Levels of Ether Soluble Phenols in Blood of Patients with Renal Insufficiency.

Diagnosis	Avg packed cell vol, %	Avg hemoglobin, g/%	Avg† creatinine, mg/%	Ether soluble phenols*: Free phenol-cresol	Conj. phenol-cresol	Free phenolic acids	Conj. phenolic acids
Chronic glomerulonephritis	26	8.3	11.3	*.34*	*.52*	*.70*	*.78*
Idem	39	13.8	3.5	.01	.06	*.15*	.15
"	35	12.5	3.2	.01	*.37*	*.16*	.09
Chronic pyelonephritis	32	11.2	8.4	.02	.18	*.17*	*.33*
Idem	35	11.6	8.4	.01	*.36*	*.29*	*.35*
"	39	13.2	6.5	.02	*.52*	.11	.20
"	34	11.6	11.6	.01	*.32*	*.41*	*.98*
Chronic glomerulonephritis	33	10.0	10.1	.00	*1.12*	*.20*	*.54*
Polycystic kidney	48	14.9	6.5	.00	*.45*	*.19*	*.43*
Chronic glomerulonephritis	49	16.6	2.6	*.24*	.11	*.23*	.17
Chronic pyelonephritis	19	8.3	15.9	.03	*1.10*	*.45*	*1.73*
Idem	19	8.3	27.6	.03	*1.19*	*1.19*	*1.97*
Chronic glomerulonephritis	25	8.5	1.6	.07	.04	.02	.04
Idem	26	8.6	28.8	.01	*.73*	*.13*	*.31*
"	30	9.8	12.2	.03	*.37*	*.77*	*2.12*
"	28	10.0	13.2	.02	*.93*	*.58*	*1.86*
Chronic pyelonephritis	24	8.4	10.5	.01	.13	*.23*	*.54*
Acute glomerulonephritis	34	11.6	2.2	.01	.02	.06	.02
Chronic glomerulonephritis	35	13.0	5.4	.03	*.58*	*.20*	.13
Severe nephrosclerosis	35	11.3	6.5	.03	*.64*	*.80*	*.47*

* Free and conjugated phenols expressed as mg % phenol; free and conjugated phenolic acids expressed as mg % p-hydroxyphenylacetic acid. Range of normal values (5 subjects): Free phenol-cresols, .01-.04; conjugated phenol-cresols, .01-.19; free phenolic acids, .02-.11; conjugated phenolic acids, .03-.20. Italicized figures indicate elevated findings.

† Normal range: .8-1.5 mg %.

from patients with a variety of diseases. They found no elevation of free circulating phenol and cresol in 5 patients with renal disease. The present study investigates the role of these compounds in anemia of chronic renal disease.

Methods. Routine hemograms and phenol determinations were performed on normal subjects, and on patients with documented renal disease. Serum creatinine determinations were performed on all renal patients by a technic employing the usual Jaffe reaction (9). Medications that interfered with the phenol reaction, such as aspirin, were withheld for 24-48 hours prior to blood letting. *Blood phenol determination*: A modification of the method of Schmidt, *et al.*,(7), was used. Heparinized blood samples were drawn and the plasma frozen at –20°C until used. Four ether-soluble fractions were determined: free phenols (phenol and cresol), free aromatic hydroxy acids (*e.g.*, p-hydroxyphenylacetic acid, p-hydroxybenzoic acid) and the conjugates of each of these groups. Blood filtrates were prepared in 1:5 dilution with tungstic acid and separation of the phenolic constituents accomplished by continuous extraction with ether at pH 10 and 1. Conjugates were released as free compounds after preliminary extractions by hydrolysis with sulfuric acid. The alkalinized extracts were then evaporated to remove the ether and treated with Folin-Ciocalteu reagent. Readings were made on a Beckman Model B spectrophotometer at wave length of 740 mμ.

Results. The results of these studies are

illustrated in Table I. A significant elevation of *free* phenol and cresol was found in only 2 instances in 20 patients with renal disease and azotemia; in one of these, no anemia was present. Conjugated phenols tended to increase with degree of anemia and azotemia, but the relationship was not consistent. This same relationship held between severity of anemia and level of serum creatinine.

Discussion. It would appear that uremic patients are able to conjugate potentially toxic free phenols despite severe derangement of kidney function. The site of this "detoxifying" process is unknown, but presumably it is mainly in the liver. It is unlikely that these conjugated phenols can be implicated *per se* in pathogenesis of anemia of renal insufficiency. Previous investigations have shown that larger amounts of free phenolic acids and conjugated phenols than those detected in our patients do not produce any significant deleterious effect on the blood(6,8).

It is still possible that minute amounts of circulating free phenol and cresol might accelerate naturally occurring plasma lysins, and evoke a hemolytic reaction. Such a mechanism has been postulated by Ponder (10) to explain the hemolytic effect of napthalene and other agents. However, it is difficult to accept this possibility in relation to free phenol and cresol in uremic patients inasmuch as the levels of these circulating compounds are not greater than in normal subjects.

Summary. Free and conjugated ether-soluble phenols in blood of uremic patients have been determined by a specific and reliable method. Free phenol and cresol are not elevated in the great majority of these patients. Reasons are given for discounting the role of elevated conjugated phenols and free phenolic acids in the pathogenesis of the anemia of chronic renal disease.

1. Chaplin, H., Mollison, P. L., *Clin. Sci.,* 1953, v12, 351.

2. Joske, R. A., McAlister, J. M., Prankerd, T. A. J., *ibid.,* 1956, v15, 511.

3. Emerson, C. P., Burrows, B. A., *J. Clin. Invest.,* 1949, v28, 779.

4. Loge, J. P., Lange, R. D., Moore, C. V., *Am. J. Med.,* 1958, v24, 4.

5. Desforges, J. F., Dawson, J. P., *A.M.A. Arch. Int. Med.,* 1958, v101, 326.

6. Von Oettingen, W. F., *Nat. Inst. Health Bull.* #190, 1949.

7. Bock, H. E., Thedering F., *Deut. Arch. Klin. Med.,* 1952, v199, 130.

8. Schmidt, E. G., McElvain, N. F., Bowen, J. J., *Am. J. Clin. Path.,* 1950, v20, 253.

9. Peters, J. H., *J. Biol. Chem.,* 1942, v146, 179.

10. Ponder, E., *Blood,* 1951, v6, 559.

Received June 27, 1958. P.S.E.B.M., 1958, v99.

Effect of a High Protein and High Nucleic Acid Diet on Occurrence of 2- Acetylaminofluorene-induced Cancer in Rats.* (24257)

W. F. DUNNING AND M. R. CURTIS

Cancer Research Laboratory, University of Miami, Coral Gables, Fla.

A previous report(1) showed an increased percentage of acetylaminofluorene-induced liver cancers in rats on a high protein diet. Fischer line 344 female rats on a synthetic diet containing 45% casein and 0.06% 2-acetylaminofluorene were compared with similar rats on a diet containing 26% casein. In the first group 11 of 12 rats died with liver cancers in an average of 303 days while only 6 out of 16 rats on the 26% casein diet developed liver cancers in an average of 283 days.

Harris(2) found no difference in incidence of induced liver cancers in Wistar rats on diets containing 0.04% acetylaminofluorene and a smaller difference in casein content, *i.e.* between 20 and 13% or with the addition of 3% liver extract. Morris, Wagner and Velat (3) reported a higher incidence of induced

* Supported by a Grant from the Am. Cancer Soc.