Mechanisms of Plasma Hemoglobin Clearance after Acute Hemolysis:

Studies in Open-Heart Surgical Patients

MURRAY N. ANDERSEN, M.D., CHRISTIAN V. MOURITZEN,* M.D., ELEMER GABRIELI, M.D.

From the Departments of Surgery and Medicine of the State University of New York at Buffalo and the Edward J. Meyer Memorial Hospital

THE relationship of acute hemolytic episodes to renal failure has been well documented in clinical conditions,[5, 18] and there is ample experimental evidence that the injection of free hemoglobin causes depression of renal function.[3, 8, 10, 15] The nephrotoxic effect of hemoglobin has also been the subject of speculation in studies demonstrating impaired renal function during and after extracorporeal circulation.[4, 20] However, studies of the effect of acute hemolysis on renal function in patients or experimental animals usually have failed to take into account the interaction of serum haptoglobin and plasma hemoglobin. Serum haptoglobin combines quantitatively with hemoglobin into a molecule which is nonfiltrable by the kidney and is removed from the circulation by the reticuloendothelial system.[12, 13, 17] In this combined form hemoglobin is presumably nontoxic, and it is the free,† or unbound, hemoglobin which should be considered the potentially toxic factor. It is therefore evident that in evaluating the effects of acute hemolysis on renal function, further information relating plasma hemoglobin to serum haptoglobin levels and more precise knowledge of the mechanisms of plasma hemoglobin clearance are needed.

Occurrence of varying and substantial degrees of hemolysis in patients undergoing extracorporeal circulation for open-heart surgery presented a unique opportunity to study basic mechanisms of plasma hemoglobin clearance in humans, as well as to gain further information relevant to the problem of renal failure. Studies were performed on a series of such patients in order to define the pattern of hemoglobin-haptoglobin relationships and excretion of hemoglobin in the urine, and to study the mechanisms of plasma hemoglobin clearance during episodes of acute and severe hemolysis.

Materials and Methods

Studies were performed in 47 patients having open-heart surgery with extracorporeal circulation. Twenty of these patients

Submitted for publication August 5, 1965.

These studies were supported by grants from the United States Public Health Service (H-3720 and HE 09268), the General Research Support Grant of the State University of New York at Buffalo, and the Heart Association of Erie County.

* Present address: Aarhus Kommune Hospital, Aarhus, Denmark.

† Throughout this paper *free hemoglobin* refers to hemoglobin in excess of the binding capacity of available haptoglobin; *plasma hemoglobin* indicates total plasma hemoglobin—both the portion combined with haptoglobin and that existing in a free state, if an excess is present.

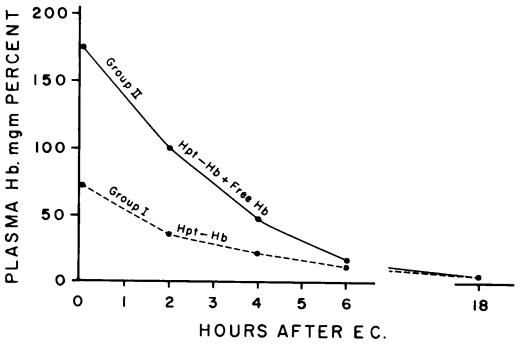

FIG. 1. Plasma hemoglobin concentrations in patients in whom the hemoglobin was completely bound to haptoglobin (Group I) and those in whom additional (free) hemoglobin was present (Group II).

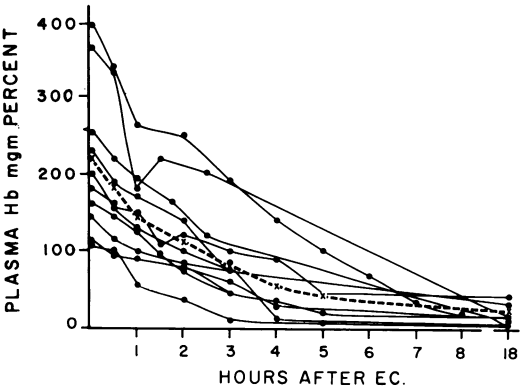

FIG. 2. Individual plasma hemoglobin concentrations in patients in whom hemoglobin levels exceeded the binding capacity of haptoglobin. Individual values and the mean are illustrated.

had replacement of one or more cardiac valves with ball-valve prostheses for acquired heart disease and 27 patients had repair of congenital cardiac lesions. None of the operative procedures for congenital cardiac lesions required more than 1½ hours of perfusion time. All operations for valve replacement required more than 1½ hours of extracorporeal circulation.

The variables measured serially before, during and after the period of extracorporeal circulation included serum haptoglobin, plasma hemoglobin, urine hemoglobin, total urine volume and rate of excretion of urine.

Prior to operation an indwelling catheter was placed in the bladder and urine was collected in separate samples to correspond with blood-sampling periods. A catheter was inserted through a saphenous vein into the inferior vena cava for periodic removal of blood samples for biochemical study.

Intravenous fluid was not given in specific amounts during the operative procedure and varied somewhat from patient to patient; however, hydration was considered satisfactory in all cases.

A Crafoord-Senning Pump Oxygenator was used for extracorporeal circulation with flow rates averaging 2.5 L./m.^{2}/min. All blood collected in the operative field during the period of extracorporeal circulation was aspirated and returned to the pump oxygenator.

Serum haptoglobin was measured by the technic of Lionetti *et al.*[14]

Plasma and urine hemoglobin levels were determined by a peroxidase method, as described by Gabrieli *et al.*[6]

"Free" plasma hemoglobin was calculated by subtracting haptoglobin-bound hemoglobin from the total plasma hemoglobin level. Haptoglobin-bound hemoglobin was assumed to be equal to the measured haptoglobin level since up to the level of available haptoglobin, hemoglobin combines with this substance quantitatively.[12, 13]

Results

Plasma Hemoglobin Clearance. Figure 1 illustrates the mean serial plasma hemoglobin concentrations in two series of patients undergoing open-heart surgery for congenital defects, grouped according to whether or not the plasma hemoglobin levels exceeded the binding capacity of the serum haptoglobin.

In 18 patients (Group I), the maximum plasma hemoglobin levels were less than the serum haptoglobin levels, averaging 72 mg.%. No free hemoglobin was present in the circulating plasma in these patients and hemoglobinuria did not occur in any case.

The maximum plasma hemoglobin clearance rate during the first 2 hours averaged 18 mg.%/hour with a progressive diminution thereafter.

In nine patients (Group II), plasma hemoglobin levels ranged from 120 to 220 mg.% with a mean of 175 mg.%. In each of these patients, plasma hemoglobin levels exceeded the haptoglobin concentration; free hemoglobin was therefore present in the plasma and hemoglobin appeared in the urine of all patients. In this group the maximum clearance rate during the first 2 hours was 38 mg.%/hour with subsequent decrease. This curve describes the combined clearance rate of haptoglobin-bound hemoglobin and free hemoglobin. The difference in slope between the two concentration curves in the two groups should represent the clearance rate of free hemoglobin which appears to be removed from the plasma at approximately the same or a slightly greater rate than hemoglobin bound to haptoglobin.

Figure 2 illustrates post-perfusion plasma hemoglobin concentration curves in 10 patients undergoing single or multiple valve replacement with ball-valve prostheses. Plasma hemoglobin levels in all cases exceeded haptoglobin levels (Table 1) and free hemoglobin was therefore present. In these patients the mean maximum plasma hemoglobin clearance rate during the first hour was 35 mg.%/hour, which closely approximates that in the group of patients with congenital heart disease. It may be noted from the individual curves that the clearance rate during the first few hours is roughly proportional to the initial plasma hemoglobin level. Since the higher levels represent increases in free hemoglobin, it is apparent that free plasma hemoglobin, when present in higher concentrations, was cleared at a more rapid rate than haptoglobin-bound hemoglobin. In this group of patients it was also noted that the plasma hemoglobin level on the morning following surgery remained somewhat elevated, reflecting a continuous degree of hemolysis produced by the ball-valve prostheses, as documented in other studies recently reported.[1]

TABLE 1. *Individual Values of Plasma Hemoglobin, Serum Haptoglobin (Representing Combined Hemoglobin), Free Hemoglobin, and the Percentage of Available Free Hemoglobin Excreted by the Kidneys*†

Patient	Plasma Hemoglobin mg. %*	Serum Haptoglobin mg. %**	Free Plasma Hemoglobin mg. %	Per Cent Free Hemoglobin Excreted
M. A.	360	91	269	1.8%
R. L.	160	75	85	7.5%
G. P.	110	20	90	12.0%
N. R.	252	122	130	0.3%
A. C.	180	160	20	2.3%
C. V.	390	87	303	2.4%
W. L.	200	45	155	8.4%
J. F.	230	100	130	11.0%
C. T.	110	100	10	2.5%
D. C.	146	112	34	42.0%

* Highest level obtained.
** Pre-perfusion level.
† Calculation of the last value is described in the text.

Renal Hemoglobin Excretion. Renal hemoglobin excretion data with serial measurements of serum haptoglobin and plasma hemoglobin were obtained in ten patients undergoing valve replacement.

Hemoglobin never appeared in the urine until the plasma hemoglobin concentration exceeded the haptoglobin level. The mean concentration of free plasma hemoglobin was 35 mg.% at the time of initial appearance of hemoglobinuria; and in several cases plasma hemoglobin had risen to 70 mg.% or more above the haptoglobin levels before hemoglobinuria appeared. However, this may in part be related to delay from kidneys to collection catheter. It was also found that the urine again became clear while substantial amounts of free hemoglobin remained in the plasma—the levels ranging from 35 to 85 mg.%. These relationships are illustrated in Figure 6 as discussed below.

To measure the effectiveness of renal ex-

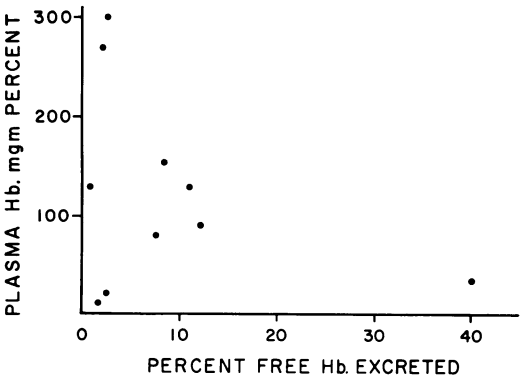

FIG. 3. The relationship of excretion of available free hemoglobin by the kidneys to plasma hemoglobin concentration.

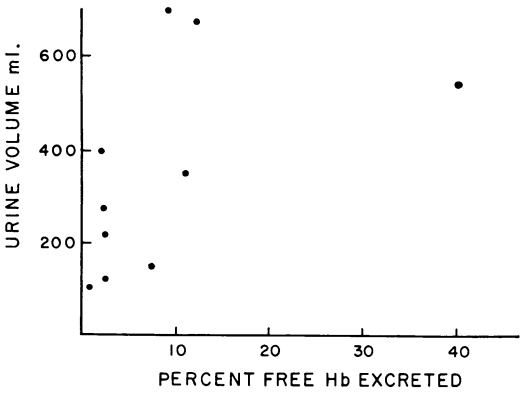

FIG. 4. The relationship of excretion of hemoglobin by the kidneys to urine volume.

cretion of free hemoglobin, the total amount of free hemoglobin present in the circulating blood was compared with the measured amounts of hemoglobin excreted in the urine. Total free plasma hemoglobin was calculated by multiplying free hemoglobin concentration (total plasma hemoglobin minus haptoglobin-bound hemoglobin) by the estimated blood volume. Total urinary excretion of hemoglobin was measured from the time of first appearance of hemoglobinuria until the urine became hemoglobin-free. The amount of hemoglobin excreted by the kidneys ranged from 0.3 to 42 per cent of the available free plasma hemoglobin pool (Table 1), and was above 12 per cent in only one patient. There was no apparent explanation for the one patient in whom a singularly high percentage of the available hemoglobin was excreted; however, the amount of free hemoglobin in this patient was smaller than average and the total amount excreted was still rather small.

Factors Influencing Renal Hemoglobin Excretion. An attempt was made to correlate the effectiveness of renal hemoglobin excretion with certain factors which were considered to have a possible bearing on this mechanism.

Figure 3 illustrates the per cent excretion of available free hemoglobin in relation to the total plasma hemoglobin concentration. It is evident that there is no correlation between these two factors.

In Figure 4 the per cent of available free hemoglobin which was excreted is compared to volume of urine measured from the beginning of extracorporeal circulation until 8 hours postoperatively, at which time the urine was hemoglobin-free in all instances. No direct correlation is observed.

Figure 5 illustrates the relationship between the rate of urine hemoglobin excretion and plasma hemoglobin concentration. Again no direct correlation is present.

The overall pattern of inter-relationships of serum haptoglobin, plasma hemoglobin and renal hemoglobin excretion, as it appears in this group of studies, is illustrated by serial data from a sample patient (Fig. 6). As documented in detail in the preceeding data, the following facts are evident.

1. Hemoglobin did not appear in the urine until after plasma hemoglobin levels had exceeded the binding capacity of haptoglobin by a significant amount, rather than at the first occurrence of free hemoglobin.

2. Urine became hemoglobin-free while the plasma hemoglobin level remained ap-

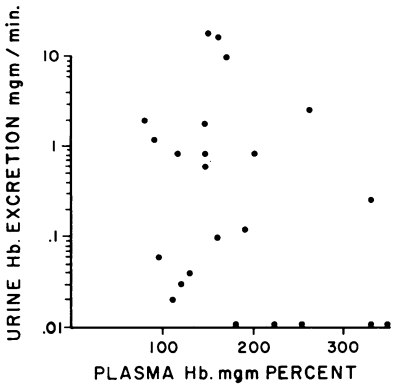

FIG. 5. The relationship of the rate of hemoglobin excretion by the kidneys to plasma hemoglobin concentration.

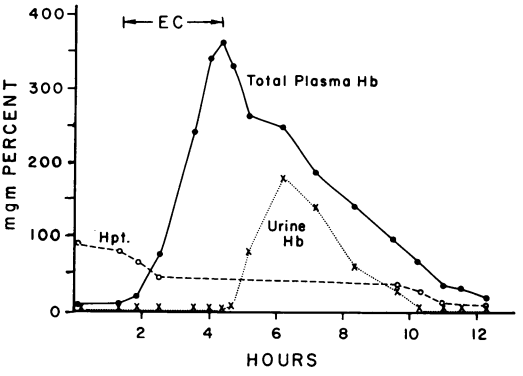

FIG. 6. Serial data from a sample patient illustrating the inter-relationship between plasma hemoglobin, serum haptoglobin and urinary excretion.

preciably above haptoglobin levels (i.e., free hemoglobin was still present in the plasma).

3. Clearance of haptoglobin-bound hemoglobin from the plasma was slower than free hemoglobin.

4. Correlation between urine hemoglobin concentration and plasma-hemoglobin concentration was not close, although there appears to be a certain similarity in the slope of decreasing concentration after maximal points have been reached.

Discussion

The primary defense of the body against the toxic effects of acute hemolysis appears to be the specific ability of haptoglobin to combine quantitatively with free hemoglobin into a firm molecule which is rapidly removed from the plasma, presumably by the reticuloendothelial system.[12, 13] If the degree of hemolysis is sufficient to saturate the available haptoglobin, free hemoglobin will accumulate in the plasma. Similarly, in a state of chronic hemolysis, if the rate of haptoglobin utilization by combination with hemoglobin exceeds the rate of production, the serum haptoglobin will fall to immeasurable levels and further hemolysis will result in the chronic presence of free hemoglobin in the plasma.

When the plasma hemoglobin level exceeds the binding capacity of available haptoglobin, free hemoglobin is filtered by the renal glomeruli and enters the tubular system with resulting hemoglobinuria.[12, 13, 19] It has commonly been assumed, therefore, that clearance of hemoglobin from the plasma is essentially accomplished by the combined mechanisms of haptoglobin-binding and renal excretion of the excess free hemoglobin.

The present data and recent experimental studies [2] suggest that some revision of the previous concept of plasma hemoglobin clearance may be indicated. Of primary importance is the fact that renal excretion of hemoglobin appears to be neither a useful nor efficient mechanism for elimination of free hemoglobin—a variable but small percentage of the available free plasma hemoglobin is excreted in the urine, but the major portion is not. The mechanism responsible for this seemingly random excretion is as yet uncertain, but it may well be related to variation in pore size of the glomeruli, or to variations in functional integrity of the filtration system during the abnormal state precipitated by acute hemoglobinemia.

The validity of this concept is supported by certain observations of others. It has

been noted previously that hemoglobin does not appear in the urine immediately after the haptoglobin pool is completely saturated and levels of 25 to 35 mg.% of free hemoglobin are reached in the plasma before urinary excretion occurs;[11] this figure is in agreement with the present data.

Further evidence is provided by studies in patients with chronic hemolysis secondary to ball-valve prostheses[1] and in certain hemolytic anemias.[11] In each of these conditions, the plasma may be devoid of haptoglobin and free hemoglobin is chronically present in the circulating plasma. However, unless the level of free hemoglobin rises above 20–25 mg.%, hemoglobin does not appear in the urine.

Free hemoglobin is removed from the plasma at a more rapid rate than the haptoglobin-bound fraction[17] and, since only a small proportion of the free hemoglobin is excreted by the kidneys, it is evident that the major portion is rapidly deposited in the body tissues. At present it is not possible to state with certainty the principal sites of deposition of free hemoglobin, nor the metabolic fate of such deposited hemoglobin in humans. However, recent experimental studies[2] have demonstrated that the principal area of deposition is the renal cortex, although small amounts of hemoglobin can be found in many of the body tissues after an episode of acute hemolysis. Furthermore, increasing levels of free plasma hemoglobin result in progressively higher concentrations of deposited hemoglobin in the renal cortex without a corresponding increase in other body tissues. The relationship of these data to renal toxicity remains to be determined; however, it seems probable that the known nephrotoxic effect of hemoglobin infusion is directly related to deposition within renal tissue, particularly the tubules.

Experimental evidence of renal toxicity secondary to hemoglobin infusion has commonly been obtained from dogs in which severe renal damage and renal failure have been observed chiefly in association with dehydration and shock.[10, 15] However, this evidence of renal damage may be more significant than is at first apparent; contrary to man and most other animals, the dog has a somewhat unique haptoglobin system in that substantial reserves of available haptoglobin appear to be present which are rapidly mobilized during infusions of hemoglobin.[2] For this reason proportionately higher concentrations of hemoglobin are necessary to result in the presence of free hemoglobin in the circulating blood of dogs than in man.

Recent reports have suggested that the incidence of renal failure has become somewhat higher with the advent of longer perfusions for prosthetic valve replacement and more complex intracardiac repair of congenital lesions.[4, 9] Since the rate of hemolysis in general increases progressively with increasing duration of perfusion, a certain potential correlation between degree of hemolysis and renal failure is evident. Although there is suggestive evidence that the incidence of renal damage is increased in cases with higher levels of plasma hemoglobin,[4, 9] this relationship is by no means exact. Since it is free, or unbound, plasma hemoglobin is toxic, and since a substantial variation in haptoglobin levels occurs in clinical cases, the relationship between plasma hemoglobin levels and renal failure after extracorporeal circulation may be more significant than previously recognized.

Presently available information has not yet indicated a definite program of management which might provide protection from the nephrotoxic effect of hemolysis. Studies have been performed in a group of open-heart surgical patients in whom the effect of mannitol on renal hemoglobin excretion was measured. It was found that although urine output increased substantially, neither the rate of hemoglobin clearance nor total excretion of available free plasma hemoglobin appeared to be increased significantly. Experimental studies have shown that haptoglobin production

may be stimulated to markedly increased levels by injection of certain substances such as elastase and papain,[16] and it is also known that haptoglobin levels are elevated in certain chronic inflammatory diseases. Whether this information could be employed to increase circulating haptoglobin, or whether supplementary administration of haptoglobin would be possible, is at present speculative. However, it is hoped that increased awareness of the mechanisms involved will lead to improved methods of control.

Summary

Mechanisms of plasma hemoglobin clearance after acute hemolytic episodes have been studied in a group of patients having open-heart surgery employing extracorporeal circulation. These studies were directed toward hemoglobin-haptoglobin relationships and renal hemoglobin excretion.

1. Clearance rates of hemoglobin bound to haptoglobin and free, or unbound, hemoglobin were determined. Free hemoglobin was removed from the plasma at a more rapid rate than that portion bound to haptoglobin.

2. Hemoglobin was not excreted in the urine until the plasma hemoglobin level rose significantly above the serum haptoglobin level, rather than at the first appearance of free hemoglobin.

3. Renal excretion of hemoglobin ceased before the plasma had been cleared of free hemoglobin and while appreciable levels were still present.

4. Only a small percentage of the free hemoglobin theoretically available for renal excretion was actually excreted in the urine, suggesting that the kidneys are not a primary factor in plasma hemoglobin clearance.

5. The major portion of free hemoglobin was removed from the plasma by mechanisms as yet not defined, but presumably by direct tissue deposition.

References

1. Andersen, M. N., Gabrieli, E. R. and Zizzi, J. A.: Chronic Hemolysis in Patients with Ball-valve Prostheses. J. Thor. Cardiov. Surg., **50**:501, 1965.
2. Andersen, M. N., Gabrieli, E. R. and Mouritzen, C. V.: Plasma Hemoglobin Clearance: Experimental Studies of Mechanisms Involved after Acute Hemolysis. Submitted for publication.
3. Atik, M., Manale, B. and Pearson, J.: Prevention of Acute Renal Failure. J.A.M.A., **183**: 455, 1963.
4. Doberneck, R. C., Reiser, M. P. and Lillehei, C. W.: Acute Renal Failure after Open-heart Surgery Utilizing Extracorporeal Circulation and Total Body Perfusion. J. Thor. Cardiov. Surg., **43**:441, 1962.
5. Doberneck, R. C., Reiser, M. P. and Yunis, E.: Acute Renal Failure after Hemolytic Transfusion Reaction. Surg. Gynec. Obstet., **119**: 1073, 1964.
6. Gabrieli, E. R. and Pyzikiewicz, T.: Assay of Level of Hemoglobin in Plasma. Amer. J. Clin. Path., **42**:4, 1964.
7. Giligan, D. R., Altschule, M. D. and Katersky, E. M.: Studies of Hemoglobinemia and Hemoglobinuria Produced in Man by Intravenous Injections of Hemoglobin Solution. J. Clin. Invest., **20**:177, 1947.
8. Goldberg, M.: Studies of the Acute Renal Effects of Hemolyzed Red Blood Cells in Dogs Including Estimations of Renal Blood Flow with Krypton. J. Clin. Invest., **41**:2112, 1962.
9. Grismer, J. T., Levy, M. H., Lillehei, R. C. and Lillehei, C. W.: Renal Function in Acquired Valvular Heart Disease and Effects of Extracorporeal Circulation. Surgery, **55**: 24, 1964.
10. Harrison, H. E., Bunting, H., Ordiray, N. X. and Alhink, W. S.: The Pathogenesis of the Renal Injury Production in the Dog by Hemoglobin or Methemoglobin. J. Exp. Med., **88**:339, 1947.
11. Herman, E. C.: Serum Haptoglobins in Hemolytic Disorders. J. Lab. Clin. Med., **57**:834, 1961.
12. Lathem, W.: The Renal Excretion of Hemoglobin: Regulatory Mechanisms and the Differential Excretion of Free and Protein-bound Hemoglobin. J. Clin. Invest., **38**:652, 1959.
13. Laurell, C. B. and Nyman, M.: Studies on the Serum Haptoglobin Level in Hemoglobinemia and Its Influence on Renal Excretion of Hemoglobin. Blood, **12**:493, 1959.
14. Lionetti, F. J., Valeri, C. R., Bond, J. C. and Fortier, N. L.: Measurements of Hemoglobin-binding Capacity of Human Serum or Plasma by Means of Dextran Gels. J. Lab. Clin. Med., **64**:519, 1964.
15. Maluf, N. S. R.: Factors Inducing Renal Shutdown from Lysed Erythrocytes. Ann. Surg., **130**:49, 1949.
16. Mambelloni, R. P. and Crosti, P.: Studies on Serum Haptoglobin in Experimental Connective Tissue Disorders. Proc. Soc. Exp. Biol. Med., **107**:499, 1961.
17. Murray, R. K., Connell, G. E. and Pert, J. H.: The Role of Haptoglobin in the Clearance and Distribution of Extracorpuscular Hemoglobin. Blood, **17**:45, 1961.

18. Reed, W. A. and Dunn, M.: Fatal Hemolysis Following Ball-Valve Replacement of the Aortic Valve. J. Thor. Cardiov. Surg., **48**: 436, 1964.
19. Whitten, C. F.: Studies on Serum Haptoglobin. New Engl. J. Med., **266**:529, 1962.
20. Yeh, T. J., Brackney, E. L., Hall, D. P. and Ellison, R. G.: Renal Complications of Open-heart Surgery: Predisposing Factors, Prevention, and Management. J. Thor. Cardiov. Surg., **47**:79, 1964.
21. Yuile, C. I.: Hemoglobinuria. Physiol. Rev., **22**:19, 1942.

The May issue includes papers presented at the Southern Surgical Association, December 7–9, 1965, Hot Springs, Virginia

Ectopic Non-cancerous Follicular Thyroid Tissue in the Neck: 18 Case Reports According to Etiologic Factors, *Calvin T. Klopp, George Washington University School of Medicine*

Thyroid Cancer Discovered Incidentally During Treatment for an Unrelated Head and Neck Cancer, *R. Lee Clark, University of Texas, M. D. Anderson Hospital and Tumor Institute*

Angiography in Tumor Diagnosis and Management: Review of 93 Cases, *Carlos M. Chavez, University of Mississippi Medical Center*

Radioactive Liver Scan in Differential Diagnosis of Upper Abdominal Disease, *William W. Shingleton, Duke University Medical School*

Hemodynamic Changes with Cirrhosis of the Liver: Control of Arteriovenous Shunts during Operation for Esophageal Varices, *George Johnson, Jr., University of North Carolina School of Medicine*

Recurrent Ulceration after Operation for Peptic Ulcer Disease, *James E. Thompson, Roosevelt Hospital, New York*

Relation of Endometriosis to Carcinoma of the Ovary: Report of Seven Cases, *Laman Gray, University of Louisville School of Medicine*

Malignant Tumors of Salivary Gland Origin, *Louis Rosenfeld, Vanderbilt University School of Medicine*

Trends in the Prognosis and Surgical Treatment of Cancer of the Stomach: 50-year Experience with 12,000 Cases at the Mayo Clinic, *William H. ReMine, Mayo Clinic, Rochester, Minnesota*

Type of Mastectomy in Cancer of the Breast: Analysis of 1,765 Cases, *Benjamin F. Byrd, Vanderbilt University School of Medicine*

Carotid Endarterectomy for Cerebrovascular Insufficiency (Stroke): Follow Up of 350 Cases, *Jesse E. Thompson, Baylor University Medical Center*

Low Molecular Weight Dextran in Vascular Surgery: Prevention of Early Thrombosis Following Arterial Reconstruction in 85 Cases, *John H. Foster, Vanderbilt University School of Medicine*

Factors Affecting Cerebral Blood Flow—Experimental Review: Sympathectomy, Hypothermia, CO_2 Inhalation and Pavarine, *Richard T. Shackelford, Johns Hopkins University Medical School*

"Washout" Acidosis Following Aortic Resection: Metabolic Study of Reactive Hyperemia, *Arlie R. Mansberger, Jr., University of Maryland School of Medicine*

Replantation of Amputated Extremities, *G. Rainey Williams, University of Oklahoma Medical Center*

Prevention of Experimental Atherosclerosis by Ileal Bypass: 20% Cholesterol Diet and I^{131} Induced Hypothyroidism in Dogs, *H. William Scott, Jr., Vanderbilt University Medical Center*