body. Nowhere throughout the preparation is there any sign of the zona pellucida; this had evidently been dissolved by the fixative.

In regard to the duration of early cleavage stages, it is pertinent to cite the report of Lewis and Hartman[4] on the culture *in vitro* of the monkey egg fertilized *in vivo*. They state that in their experiment, in which fertilization was believed to occur soon after ovulation, the one- and two-cell stages lasted at least 36 hours. We observed two eggs in the two-cell stage 40½ and 45 hours, respectively, following contact with spermatozoa. Lewis and Hartman considered that the three- and four-cell stages in their egg extended to the 48th hour following fertilization. Our two eggs were seen in the three-cell stage 46 hours after exposure to spermatozoa. Hence, our findings, in this respect, are in general agreement with those reported for the monkey egg.

These experiments will be described in greater detail elsewhere, and photographs of the fresh and fixed specimens will be included.

JOHN ROCK
MIRIAM F. MENKIN

DEPARTMENT OF GYNECOLOGY,
HARVARD MEDICAL SCHOOL, BOSTON,
and
FERTILITY CLINIC LABORATORY,
FREE HOSPITAL FOR WOMEN,
BROOKLINE, MASS.

THE PROLONGATION OF PENICILLIN RETENTION IN THE BODY BY MEANS OF PARA-AMINOHIPPURIC ACID[1]

THE very great rapidity with which penicillin is cleared from the blood stream and appears in urine is a major disadvantage in therapy, and suggests that it might be eliminated by renal tubular secretion in addition to glomerular filtration. If such were the case, it might be possible to suppress the secretion of penicillin by the simultaneous administration of p-aminohippuric acid (PAHA) which is known to be secreted by the tubular epithelium[2] and which we have found to be remarkably non-toxic. Rammelkamp and Bradley have reported that the excretion of penicillin in urine was depressed by the injection of diodrast.[3]

The purpose of our investigations was to determine whether a mutual competition between penicillin and p-aminohippuric acid existed and, if so, to evaluate the significance of that relationship. The penicillin content of urine and plasma was determined by a modification of the method of Rammelkamp[4] and the total amounts recovered were checked by the Florey cup plate method. The PAHA content of urine and plasma was determined by making use of the principle set forth in the method of Bratton and Marshall for sulfonamides.[5] All urine and blood samples were collected aseptically and periodic renal clearance determinations of PAHA and penicillin were made during the course of the experiments. It was established that penicillin contained in urine was sufficiently stable to permit complete recovery in the presence and absence of PAHA at a pH range of 4.5 to 8.0 and that PAHA did not influence the assay of penicillin.

Two-hour experiments using normal, unanesthetized trained dogs were designed in which 10,000 Oxford units of penicillin were injected intravenously as a single dose. In the control tests no PAHA was infused, but in other experiments intravenous PAHA infusion was started shortly before and carried out continuously during the experiments. These experiments demonstrated that PAHA markedly prolonged the maintenance of an elevated plasma concentration of penicillin, being 0.2 unit at 2 hours compared to only a trace of penicillin in the plasma of the control animals at 1.5 hours. The recoveries of penicillin in the urine of one dog when no PAHA was administered were 61, 77 and 97.7 per cent. When PAHA was administered intravenously only 29.6 to 36.6 per cent. of the penicillin injected was recovered in 2 hours. In the case of another dog the control penicillin percentage recovery ranged from 64.9 to 102.4, whereas when PAHA was administered in addition to penicillin the recoveries of the latter were 30.2 to 52.6 per cent. When the former dog was given sodium bicarbonate by stomach tube to maintain the pH of urine at 7.8 to 8.0 the recoveries of penicillin were 107.8 per cent. for the control experiment and 36.1 per cent. when PAHA was infused. The normal renal clearance of penicillin at plasma concentrations of less than 1.3 units/cc approximated the minimal renal plasma flow. When the plasma level of PAHA was maintained at levels of above 25 mgm/100 cc the clearance of penicillin was depressed to and below the glomerular filtration rate for these dogs. This may be taken as evidence that penicillin and PAHA compete for the same tubular secretory mechanism.

Twelve-hour experiments, similar to those outlined above but during which the dogs were anesthetized, substantiated and extended the above findings. In the control experiments, with one exception, penicillin was no longer detectable in the plasma within 2.5

[1] From the Departments of Pharmacology and Bacteriology, the Medical-Research Division, Sharp and Dohme, Inc., Glenolden, Pa.

[2] (a) N. Finkelstein, L. M. Aliminosa and H. W. Smith, *Am. Jour. Physiol.*, 133: 276, 1941. (b) R. J. Bing, *Proc. Soc. Exp. Biol. Med.*, 53: 29, 1943.

[3] C. H. Rammelkamp and S. E. Bradley, *Proc. Soc. Exp. Biol. Med.*, 53: 30, 1943.

[4] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. Med.*, 51: 95, 1942.

[5] A. C. Bratton and E. K. Marshall, *Jour. Biol. Chem.*, 128: 537, 1939.

hours following the single intravenous injection of 10,000 Oxford units. When PAHA was infused to maintain a plasma concentration of 30 to 50 mgm/100 cc, the end point at which the plasma concentration of penicillin was less than the lower limit of our test (0.02 unit/cc) was extended to 3.5 hours. Penicillin ceased to be excreted in the urine in determinable quantities within 7.5 hours during the control experiments but was still being excreted in small amounts at the end of 9.5 to over 12 hours when PAHA was administered continuously over this period of time. In these experiments, also, PAHA decreased the renal clearance and the recovery of penicillin in the urine as in earlier experiments.

Forty-eight-hour experiments were performed during which both penicillin and PAHA were infused continuously. As a result of these tests we found that, if the plasma level of PAHA was maintained at 20 to 30 mgm/100 cc, we could maintain a plasma penicillin level of 0.08 to 0.1 unit/cc when the penicillin was infused at a rate of 15 units/minute. On the other hand, when PAHA was not administered the penicillin level in the plasma was 0.02 units/cc or less. Also, by raising or lowering the plasma concentration of PAHA we could concomitantly raise or lower the penicillin content in plasma even though the penicillin was infused at a constant rate during the whole of the experiments. As the plasma concentration of penicillin was caused to rise by the intravenous infusion of PAHA there was a progressive decrease in renal clearance and urinary excretion of the antibiotic substance.

There appeared to be no pathological findings attributable to the PAHA-penicillin therapy in any of the 48-hour experiments. Toxicological studies have shown PAHA to be remarkably non-toxic to mice, rabbits and dogs; so much so that the physical characteristics of the high concentrations (20 to 40 per cent.) of the sodium p-aminohippurate solutions found necessary for intravenous toxicity studies were probably the principal causes of deaths which occurred at an LD50 dose of 5,300 mgm/kg administered intravenously to mice.

The detailed accounts of these experiments will be published elsewhere. These results indicate that with the aid of PAHA one may attain and maintain materially higher concentrations of penicillin in plasma than is practicable without the use of excessive amounts of penicillin. The ease and economy with which this can be accomplished experimentally suggest that the combined intravenous administration of penicillin and PAHA may offer sufficient therapeutic advantages to make its clinical trial indicated.

KARL H. BEYER
ROLAND WOODWARD
LAWRENCE PETERS
W. F. VERWEY
P. A. MATTIS

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD OF OBTAINING RENAL VENOUS BLOOD IN UNANESTHETIZED PERSONS WITH OBSERVATIONS ON THE EXTRACTION OF OXYGEN AND SODIUM PARA-AMINO HIPPURATE[1]

THE present concepts of human renal physiology are based upon somewhat unstable tenets because of the difficulty in obtaining blood from persons immediately before and after its passage through the kidney. The widely used clearance techniques for determining renal blood flow are based upon the assumption that the test substance used is completely removed from the blood during one circulation through the kidney. Arterial blood may be obtained with relative ease from a peripheral vessel, because its composition is the same throughout the body, but obtaining venous blood as it leaves the kidney has presented the major problem. In animals explantation of the kidney has enabled investigators to obtain renal venous blood for extraction studies. To our knowledge human renal venous blood has only been obtained during operative procedures on the kidney.[2] The method described here has enabled us to obtain blood directly from the renal vein in the unanesthetized resting subject. A similar technique is being successfully utilized by Dr. S. E. Bradley at the Boston University School of Medicine.

The method is essentially a modification of the technique of right auricular catheterization, introduced by Forssmann in 1929,[3] and recently extensively utilized by Cournand and his colleagues.[4] A long (100 cm) No. 8 or 9 radioopaque ureteral catheter[5] with an angulated tip is passed into the venous system through the antecubital vein. A slow drip of physiologic saline

[1] From the Medical Service of the Grady Hospital and the Department of Medicine, Emory University School of Medicine, Atlanta, Georgia. The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine.

[2] S. Weiss, F. Parker, Jr., and G. P. Robb, *Ann. Int. Med.*, 6: 1599, 1933.

[3] W. Forssmann, *Klin. Wchschr.*, 8: 2085, 1929.

[4] A. Cournand, R. L. Riley, S. E. Bradley, E. S. Breed, R. P. Noble, H. D. Lauson, M. I. Gregersen and D. W. Richards, *Surgery*, 13: 964, 1943.

[5] Obtained from the United States Catheter and Instrument Co., Glens Falls, N. Y.