

Summary. A method is described for dehydration (*in vacuo*) of salted-out fibrinogen for "stock" usage of the protein in coagulation studies. Modifications of the method of preparation are considered with a view to (a) lessening denaturation, (b) making the preparation available for biochemical analysis of its protein content, and (c) preparing it free from all traces of prothrombin.

10441 P

Urolithiasis and Renal Pathology After Oral Administration of 2(sulfanilylamino)pyridine (Sulfapyridine).

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In the course of an investigation of the pharmacology and toxicology of 2(sulfanilylamino)pyridine, which will be reported elsewhere,* we have observed the formation of uroliths in the urinary tract of rats, rabbits, and monkeys fed with this drug, which were found to consist of its acetyl derivative having the formula $\text{CH}_3\text{CONHC}_6\text{H}_4\text{SO}_2\text{NHC}_6\text{H}_4\text{N}$. The appearance of sulfanilamide crystals in the urine of patients has been previously reported by Stewart, Rourke and Allen.^{1†}

While concretions have been observed after the administration of a single large dose, the results were more striking after repeated feeding on successive days. The occurrence of urolith formation following the repeated daily administration of 2(sulfanilylamino)pyridine varied greatly in the different species and to some extent even in the same species. Thus uroliths were observed after feeding daily doses of 0.25 g per kilo to monkeys, 10 to 15 g per kilo to rab-

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¹ Stewart, J. D., Rourke, G. M., and Allen, J. C., *J. Am. Med. Assn.*, 1938, **110**, 1885.

† Lawrence mentions a human case of right lower quadrant pain and hematuria due to stone formation after sulfapyridine therapy.² Oakley in a short note mentions the presence of prontosil in the bladder and the straight and collecting renal tubules of mice fed with enormous doses of this drug.³

² Lawrence, E. A., *International Review of Recent Advances in Medicine*, 1939, **3**, 48.

³ Oakley, C. L., *Biochem. J.*, 1937, **31**, 729.

bits and 5 g per kilo to rats for 10 consecutive days, whereas the phenomenon was not observed in mice or dogs even after the feeding of such excess doses as 20 g per kilo. In a series of 25 monkeys fed doses of 0.25 to 14 g per kilo only one animal (receiving 10 g per kilo) failed to produce uroliths. Individual variation was found to be greater in a series of 30 rabbits and 120 rats, particularly with smaller doses (2 to 5 g per kilo) but the results were quite uniform when large doses (10 to 20 g per kilo) were employed. With small doses, urolith formation was more frequently observed on the right side, while the left ureter remained free of involvement. In contrast to the above results, daily feedings of sulfanilamide over prolonged periods of time produced a similar phenomenon only in 2 rats out of a series of over 300.

A few hours after the oral administration of a large dose of sulfapyridine, the urine in susceptible species is seen to contain needle-like crystals of the substance. This has already been found in rabbits by Stokinger.⁴ If the animal is sacrificed 24 hours later, aggregates of these crystals are observed, especially in the ureter, most frequently at the level of the bony pelvic brim. At times a calculus may be found in the ureter, where it enters the bladder wall, or in the bladder proper. The proximal ureter and renal pelvis is at first somewhat dilated and thinned out. Later these structures become indurated and hemorrhagic. Bloody urine and blood clots are found within the dilated channels. In some instances the initial obstruction occurs at the uretero-pelvic junction. In these cases an amorphous mass composed of compact crystals completely fills the pelvis and extends into the medulla of the kidney. The kidney proper becomes edematous and increases in size, varying from slightly larger than normal to twice the normal size.

The histology of the kidney varies with the degree of urinary stasis. The early picture is that of a calculus ureteritis and pyelitis; and with involvement of the kidney substance, a pyelonephritis ensues. In the more pronounced cases, there is marked retention of nitrogenous products in the blood.

There is in the experimental data a suggestion that the crystallized compound, when not excessive can either be redissolved or washed out. In a series of 5 monkeys, all of which received 4 g per kilo of sulfapyridine for 10 days, 4 of the animals which were sacrificed at the last day of feeding showed marked formation of uroliths while the fifth, sacrificed 79 days after discontinuation of the drug, exhibited a definite thickening and dilatation of the ureters and

⁴ Stokinger, H. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 61.

pelves, but no formation of concrement. This question is receiving further attention.

The uroliths consisting of the acetyl derivative of sulfapyridine permit penetration by X-rays. However, it has been observed that calcium can be deposited about these concrements which act as a nucleus in which case the shell may become X-ray opaque.

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Production of Mammary Carcinomas in Male Mice With a Single Implantation of Oestrone.

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Lacassagne¹ produced tumors in male mice of the R III strain, in which 70% of the females develop spontaneous mammary carcinomas, by the weekly injection of 300 I.U. of oestrone benzoate in oil. The tumors were found to develop sooner in males so treated than they occur spontaneously in females of the same strain. These findings have been repeated by Burrows,² by Bonser,³ by Cramer and Horning,⁴ and by Gardner, Smith, Allen and Strong.⁵ Lacassagne⁶ used oestrone, oestradiol, equiline, and equinine, all hormones being injected weekly in oily solution. Burrows painted his animals twice weekly with oestrone, .01% in benzene; Bonser used oestrone benzoate in olive oil as did Gardner, *et al.*, while Cramer and Horning used oestrone in chloroform applied to the skin twice weekly.

Recently Deanesly and Parkes⁷ have shown that prolonged effects may be obtained by the implantation of crystals of oestrone under the skin. A capon so treated with a 3 mg crystal of oestrone maintained the hen coloration of the breast feathers for over 3 months. It seemed that this method might be made use of to repeat the experiments of Lacassagne while decreasing the labor of weekly injection.

¹ Lacassagne, A., *Compt. rend. Acad. de sc.*, 1932, **195**, 630.

² Burrows, H., *Am. J. Cancer*, 1935, **24**, 613.

³ Bonser, G. M., *J. Path. and Bact.*, 1935, **41**, 217; 1936, **42**, 169.

⁴ Cramer, W., and Horning, E. S., *Lancet*, 1936, **1**, 247.

⁵ Gardner, W. U., Smith, G. M., Allen, E., and Strong, L. C., *Arch. Path.*, 1936, **21**, 265.

⁶ Lacassagne, A., *Bull. de l'assoc. fr. p. l'etude du cancer*, 1938, **27**, 1.

⁷ Deanesly, R., and Parkes, A. S., *Proc. Roy. Soc., B*, 1937, **124**, 279.