At the end of the 130-day period the 4 lambs that had not received nicotinic acid were changed to a ration in which the unpurified casein was replaced by "Labco Vitamin-free Casein." These lambs were fed this ration for 90 days, while Nos. 270 and 300 were continued on the ration containing unpurified casein. During this period all of the lambs except No. 253 made excellent gains of between 114 and 164 g per day. Since No. 253 made no improvement after being placed on a standard fattening ration at the conclusion of the experiment it is believed that his failure to make as good growth as the others was due to causes other than the nature of the ration fed. There was no significant difference in the performance and rate of growth between the lambs fed the ration containing the unpurified casein and those fed the ration containing the purified casein, which is a typical pellagra-producing ration. The gains made by the lambs in both groups were comparable with the gains of lambs of similar weight on a well-balanced fattening ration.

Summary. Lambs developed normally on a ration that causes cessation of growth and produces pellagra-like symptoms in pigs and black tongue in dogs. From this it appears that nicotinic acid is either not a dietary essential for this species or that the requirements are much lower than for the pig, dog, or human.

10440

A New Fibrinogen Preparation.*

JOHN H. FERGUSON AND B. NIMS ERICKSON.

From the Departments of Pharmacology and of Pediatrics, University of Michigan, Ann Arbor.

In a recent presentation of a series of standardized procedures for the *in vitro* study of coagulation reactions,[1] it was noted that the essential thrombin components (prothrombin, calcium, cephalin) could all be obtained in stable form and readily converted into appropriate solutions. The fibrinogen, on the other hand, was always freshly prepared, consuming much valuable time and risking a poor preparation from denaturation and other causes. In searching for a method of obtaining a stable "stock" preparation of this labile

* One of us (B. N. E.) begs to acknowledge the receipt of a Sigma Xi research grant in aid of the accompanying investigation.

1 Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.

protein, it was recalled that most modern serological laboratories are equipped with a high-vacuum, low-temperature desiccating apparatus for the dehydration of sera and similar preparations. Through the courtesy of Dr. M. H. Soule of the Bacteriology Department of the University of Michigan, we were enabled to use such an apparatus in the investigation of the present problem.

Fibrinogen was "salted-out" from fresh citrated dog plasma by the usual methods. The final deposit from 50-60 cc of the original plasma was firmly packed at the bottom and adjacent side in *each* of a number of 50 cc centrifuge tubes, the angle centrifuge being utilized because of the advantage of the large sloping surface. With complete removal of the gross supernatant fluid by suction and drainage, the water content of the deposited protein was relatively small. Eight tubes could be handled at a time, their contents representing the fibrinogen from some 500 cc of citrated plasma or, approximately, a liter of blood. The tilted tubes were immersed in a mixture of "dry ice" and "methyl cellosolve" and the contents rapidly solidified without noticeable interference by the adherent precipitating salt. By distillation at high vacuum (20-40μ) into an intervening condenser tube surrounded by the freezing mixture (in a vacuum flask), the fibrinogen was reduced to a dry flaky powder in a few hours (overnight, by preference). On redissolving in distilled water or 0.9% NaCl, with enough 0.5% $NaHCO_3$ to render alkaline to phenol red, a gradual resolution of the bulk of the protein occurred. Filtration yielded a clear opalescent solution indistinguishable in properties from the original *fibrinogen* solution. It clotted excellently on the addition of an appropriate coagulant. Traces of *prothrombin* from the original preparation were also apt to be preserved as shown by slow coagulation with calcium and cephalin alone.

Among several modifications of the method which were tried the following may be mentioned:

1. Berkefeld-filtered citrated dog plasma was precipitated with $(NH_4)_2SO_4$ (one-quarter saturation) and the redissolved fibrinogen reprecipitated 3 times. The desiccated preparation was satisfactory for routine clotting tests where the trace of prothrombin present could be ignored. After several months, good resolution is still being obtained although there is a gradual increase in denaturation. From the amount of dilute alkali needed to restore the pH, it is surmised that there is sufficient acidity in the ammonium salt to favor such denaturation.

Analyses for total N and N.P.N. by the Van Slyke technic indicated that some 90% of the nitrogen present belonged to the precipi-

tating salt. Besides being too high a concentration of N.P.N. to permit accurate protein estimation by N-analyses, this amount of ammonium salt has a certain measure of inhibitory action on the clotting phenomena.

We have routinely used *Berkefeld-filtered* plasma[1] in order to remove all possible traces of platelet and similar material. Analytical comparisons with twice-centrifuged plasma showed a minor reduction (5-6%) in total N and no loss of phospholipids. While the ultrafiltration may be dispensed with for many purposes, the facts that good fibrinogen and prothrombin preparations may be made from the filtrate accord with these chemical analyses in favoring the view that the plasma is not greatly disturbed by such filtration when properly conducted.

2. By the use of *sodium* salts and greater care to keep the fibrinogen at ice-cold temperatures during the course of preparation (Florkin[2] recommends the cold room at 1-2°C) it should be possible to control denaturation and obtain a protein free from N.P.N. The fibrinogen prepared with sodium salts is in our experience always very gelatinous and tenacious and much less easily recovered (by centrifugalization) and redissolved than that prepared with $(NH_4)_2SO_4$.

We, therefore, recommend a "combined method" in which 3 ammonium sulphate precipitations are followed by one with NaCl. If desired, the final precipitate, after centrifuging, is resuspended and washed once or twice in half-saturated NaCl solution. The dehydrated preparation from this material appears to keep better, to redissolve more completely (although equally slowly), and to yield solutions with N.P.N. values down to 5-10%, or lower, thus admitting of accurate protein analysis.

3. A well-washed, N-free Alumina *Gel* (ortho Al $(OH)_3$, β-form[3]) was found to adsorb all the protein from redissolved weak fibrinogen solutions. It was, therefore, found practicable to prepare *prothrombin-free* fibrinogen only by adsorbing the original citrated plasma[1] and subsequently salting-out the fibrinogen. The desiccated preparation gave (filtered) solutions entirely free from all traces of prothrombin. The material subjected to such additional handling did not keep as well as the other preparations. Nevertheless, the undenatured (soluble) portion could be used for clotting tests even after several months.

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[2] Florkin, M., *J. Biol. Chem.*, 1930, **87**, 629.

[3] Method cited in C. Oppenheimer's *Die Fermente und ihre Wirkungen*, 1929, III, p. 480.

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).

WILLIAM ANTOPOL AND H. ROBINSON.

From the Laboratories of the Newark Beth Israel Hospital, Newark, New Jersey, and the Merck Institute of Therapeutic Research, Rahway, N. J.

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 $CH_3CONHC_6H_4SO_2NHC_5H_4N$. 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-

* By H. Mollitor and H. Robinson.

[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.