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TECHNICAL METHODS

ULTRAFILTRATION OF PLASMA URIC ACID

BY

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In view of the possibility that the uric acid of the blood might be partly "bound" to protein and partly "free" (Benedict and Behre, 1931) and that this might provide some explanation for the phases of gout, it was decided to investigate various methods of ultrafiltration of the plasma uric acid in gouty and normal individuals.

Lambie (1940) reported a case of leukaemia with subsequent development of gout in which the ultrafiltrable fraction of the plasma uric acid was only 28%, but changed after splenectomy to normal, i.e., 100%. Lambie used collodion membranes for the ultrafiltration. His explanation was a change in the colloidal state of the blood urates.

Adlersberg, Grishman, and Sobotka (1942) reviewed the literature on the solubility of uric acid, reporting Minkovski's view on the free and bound uric acid in body fluids and the different results hitherto gained by ultrafiltration of serum and plasma and the explanation given by the different authors. Adlersberg *et al.* ultrafiltered serum, using 80 lb. nitrogen per sq. in. (positive pressure) and a "600" cellophane membrane for 18 to 24 hours. They reported that in normal individuals 76–96% (average 84%) of the uric acid was filtrable. They claimed further that, in contrast to normal individuals, gouty patients show a considerable fluctuation in the uric acid partition, even when the total blood uric acid level remains constant. In three out of 10 gouty patients 80% was the average.

Wolfson, Levine, and Tinsley (1947) used the anaerobic ultrafiltration method of Lavietes (1937), and found in normal individuals (13 filtrations in two patients) an ultrafiltrability of plasma uric acid of 79%. They used "600" cellophane membrane, which they soaked for three days at 0° C. before use.

Our first attempt was made with the positive pressure method of Adlersberg *et al.*, but we found that we could not filter more than 20–30% of the uric acid, possibly because of shortcomings in our filtration arrangements. We therefore adopted the method of Lavietes, used by Wolfson and his colleagues, which is as follows.

Method

Two pyrex half-cells (Fig. 1) blown from capillary tubing of 1.5 mm. internal bore 25 mm. deep, with rims ground to form an accurately fitting junction, were used. Between the two half-cells the soaked cellophane membrane was placed and held by pressure applied by a clamp and an elastic band. One limb (1) of half-cell A (Fig. 2) was connected by approximately 40 mm. long pressure tubing to a bent capillary tube

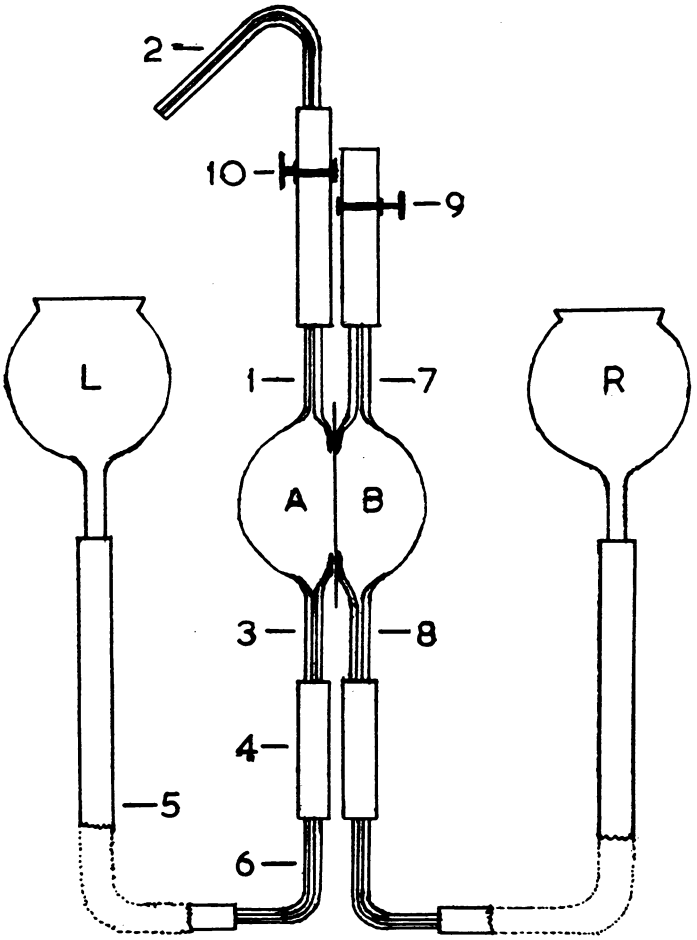


FIG. 2.

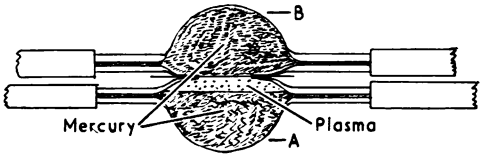


FIG. 3.

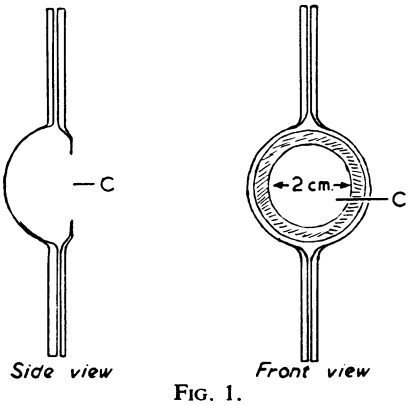


FIG. 1.

(2). The other limb (3) was connected by pressure tube (4 and 5) and an angle piece capillary tube (6) to a glass funnel (L). One limb (7) of half-cell (3) was closed by a pressure tube and screw clip (9). Limb (8) was connected in the same way as (3) to a glass funnel (R). Both systems, including the bent capillary tube, were completely filled with mercury through the funnels so that no air was trapped. Both screw clips were tightened. A funnel (L) was then lowered about 10 cm. below the level of the half-cells. Plasma was then sucked into half-cell A through the capillary tube (2) by loosening the screw clip, no air being allowed to enter. The screw-clip was then closed and funnel (L) lifted about 10 cm. over the half-cell level. The half-cells were then turned through 90° to the position shown in Fig. 3, so that cell (A) should be at the bottom and the plasma should be completely sealed by mercury. The funnel was then raised 35 cm. above the plasma level. To remove the filtrate the half-cells were turned back to the position shown in Fig. 2. Funnel (L) was then lowered to 10 cm. above the half-cell level, a pipette placed into the pressure tube of short length (B), the screw clip opened, and funnel (R) carefully raised in order that all filtrate should enter the pipette. The mercury level in (R) was 2–3 cm. above plasma level during the filtration.

To obtain the optimal conditions for the ultrafiltration of the urates, we chose, after several trials, cellophane membranes " 300 " (moisture proof). This type of cellophane yielded a protein-free ultrafiltrate, but, because of its large pore size, afforded the minimum resistance to the filtration of the urates. The cellophane was soaked in distilled water at 0° C. for 24 hours.

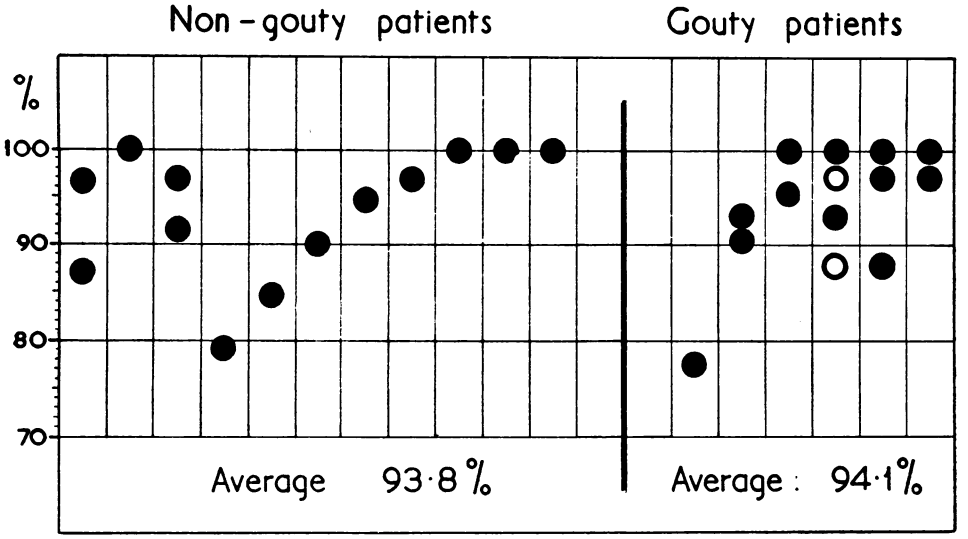
The filtration occupied 17 hours or more. Although we obtained in two instances an ultrafiltrability of 100% even after five hours, we came to the conclusion that usually a longer time is required to obtain the maximum results.

The plasma uric acid and uric acid in the ultrafiltrate were estimated by the method of Benedict as modified by Bidmead (see page 370). The determination was carried out simultaneously in both plasma and ultrafiltrate. The plasma was kept during the ultrafiltration under the same conditions as the ultrafiltrate.

Our results are presented in Fig. 4. We carried out 13 filtrations in 11 non-gouty patients, and 14 in six patients with classical gout. The plasma uric acid in the non-gouty group varied between 3.1 and 6.3 mg.% (average 4.3 mg.%), and in the gouty group from 3.5 to 8.7 mg.% (average 6.4 mg.%). The average ultrafiltrability for the non-gouty group was 93% and for the gouty ones was 94%. In both groups, with three exceptions, all the results were between 90 and 100%.

Discussion

The method of ultrafiltration employed was a modification of that used by Wolfson *et al.*, but ' 300 " moisture-proof cellophane was used instead of " 600," as it was considered that, in spite of the conditions being anaerobic, the more quickly the filtration was carried out the less chance there was of change in equilibrium in the solution. In spite of this, the time of ultrafiltration was considerable—17 hours—and the percentage of uric acid that was ultrafiltrable was on an average as high as 93% (in eight samples 100%). There was no change between the percentage ultrafiltrable from gouty and non-gouty patients. It was felt that the filtrable fraction was so large that no conclusion about the state of the urates in the blood could be drawn from this experiment. The method was considered of no value in differentiating between gouty and non-gouty individuals, contrary to previous claims.



No. of patients = 11. No. of filtrations = 13. P.U.A.: maximum = 6.1 mg.%, minimum = 3.1 mg.%, average = 4.3 mg.%.

No. of patients = 6. No. of filtrations = 14. P.U.A.: maximum = 8.7 mg.%, minimum = 3.5 mg.% (after sod. sol.), average = 6.4 mg.%. O denotes acute gouty attack.

FIG. 4.

Summary

An attempt has been made to see whether the ratio of plasma uric acid to ultrafiltrable uric acid might throw any light on the cause of undue retention or deposition of uric acid in gout.

A satisfactory method of ultrafiltration was finally found, but both in gouty and non-gouty individuals the ultrafiltrable fraction averaged between 93 and 94%. Ultrafiltration, therefore, appears of no value in differentiating patients suffering from gout from normal individuals.

We should like to thank Professor Garner for the assistance of his workshop in preparing apparatus, and Messrs. Colodense, Ltd., Bristol, for the generous supply of cellophane membranes.

We are also indebted to Dr. Pearson and Mr. Bidmead for assistance with some of the estimations, and to the Empire Rheumatism Council for some financial help.

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