CXLIX. IDENTITY OF URINARY ALBUMIN.

By LESLIE FRANK HEWITT.

From the Hale and Dunn Clinical Laboratories, London Hospital, E. 1.

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In general albuminuria is accompanied by a decrease in the proportion of albumin in the blood, and the object of this investigation is to determine whether blood-albumin passes through the kidney unchanged or if there is a difference between blood- and urinary albumin.

It was shown in a recent paper [Hewitt, 1927] that proteins when purified possess a characteristic optical rotatory power and dispersion, and this provides a sensitive method for their identification. Hynd [1925] has examined albumins isolated from urine but the figures obtained for the specific rotatory powers of the specimens vary within very wide limits, probably due to non-removal of lipins and other substances which seriously affect the rotation.

Albumins excreted by nephritic patients have been isolated from urine, purified, and their rotatory power and dispersion determined.

Isolation and purification of urinary albumins.

Urine was collected in bottles containing toluene and worked up as soon after collection as possible. In some experiments the albuminous urines were first concentrated by ultrafiltration. For this purpose flat collodion discs were used, mounted with rubber washers in a large Seitz filter. The membranes were prepared by pouring a filtered 6 % solution of pyroxylin in a mixture of alcohol (50 vols.), ether (50 vols.) and acetic acid (10 vols.) on a flat glass plate. After leaving for a few minutes for the ether to evaporate, the glass plate was plunged into cold water, when the membrane became readily detachable. After washing thoroughly in water, the membrane was cut to shape and fixed in the Seitz filter. If the membranes were allowed to dry, they shrank and became impermeable. Experiments with samples of cellophane kindly supplied by the British Cellophane Company were not successful, filtration being much too slow. Negative pressure was applied to the filtrate side of the apparatus and a positive pressure up to 10 atmospheres was applied to the other side using a reservoir constructed by Mr Macarthy in this Hospital.

To the filtered (or concentrated) urine saturated ammonium sulphate solution was added to make the mixture at least 60 % saturated with ammonium sulphate and acetic acid was added until the reaction was about p_H 4.7. After allowing to stand the precipitated proteins were filtered off on a Büchner

funnel, dissolved in a small quantity of water, the solution neutralised, and cooled to 0° and then added drop by drop to about 8 times its volume of a mixture of alcohol (7 parts) and ether (3 parts) kept at -15°. After standing at low temperature the precipitated proteins were filtered on a chilled Büchner funnel in the ice-chest, thoroughly washed with cooled alcohol-ether mixture, and with cold ether, and then rapidly transferred to a Soxhlet thimble and extracted for 20 hours with ether, metallic sodium being placed in the extraction flask to prevent any alcohol present from vaporising. After the first two hours' extraction, the ether and sodium were renewed. The extracted proteins were transferred to a vacuum desiccator and dried *in vacuo* over sulphuric acid for 48 hours, and then allowed to remain exposed to laboratory air for 24 hours, when the powdery solid was no longer readily electrified and was more easily "wetted," and hence dissolved in water more rapidly. The solid was pulverised, dissolved in water, any insoluble material (*e.g.* uric acid) filtered off or removed by centrifuging, and the solution made half saturated with ammonium sulphate by the addition of saturated ammonium sulphate solution. After allowing to stand overnight the precipitated globulins were filtered off, and the albumins precipitated in the mother-liquor by adding a further quantity of saturated ammonium sulphate solution and adjusting the reaction to about p_H 4.8 by adding 10 % acetic acid. After allowing to remain overnight the albumin was filtered off, dissolved in water, the solution neutralised and the above precipitation repeated. After one further repetition of the process the albumin was dissolved in a small quantity of distilled water, the solution placed in a collodion sac and dialysed against distilled water which was renewed frequently for at least 10 days. The clear dialysed solution was used for optical work.

Optical rotatory power and dispersion of urinary albumins.

The optical rotations of the solutions of the albumins obtained as described were determined in 1 dm. tubes, a Schmidt and Haentzsch polarimeter being used. The amount of protein present in the solutions was determined by Dr J. R. Marrack using the Kjeldahl method. In the following table specific rotatory powers for the mercury green line ($\lambda = 5461 \text{ \AA.}$) are quoted.

Patient	$[\alpha]_{5461}$	Diagnosis
1	-76.9°	Chronic nephritis with oedema
2 <i>a</i>	-78.9	Chronic nephritis with oedema
2 <i>b</i>	-76.9	
2 <i>c</i>	-79.6	
3	-78.0	Subacute chronic nephritis, oedema and uraemia
4	-74.8	Chronic nephritis, oedema and uraemia
5	-77.5	Albuminuria of pregnancy; chronic nephritis
6	-77.3	Albuminuria of pregnancy
Urinary albumin (mean)	-77.6	
Serum-albumin [Hewitt, 1927]	-78.1	

It will be seen that the mean values for urinary albumin (-77.6°) and serum albumin (-78.1°) are, within the limits of error, identical, so that it

is probable that urinary and serum-albumins are the same protein. The specific rotatory powers of the albumins were determined also for light of different wave lengths. The results were

Albumin	$\lambda = 6660$	5780	5461	4358 Å.
Urinary (mean of 8)	-47.7°	-67.3°	-77.6°	-148.1°
Serum	-47.3°	-67.0°	-78.1°	-151.5°

The rather low figure $[\alpha]_{4358} - 148.1^\circ$ for urinary albumin can be explained, since some of the specimens were somewhat dark in colour and absorbed a considerable amount of blue light, rendering accurate measurements impossible. Apart from this, very close agreement is observed. It is therefore rendered extremely probable that serum-albumin passes through the kidney unchanged.

SUMMARY.

The albumins obtained from the urine of patients with chronic nephritis and albuminuria of pregnancy possess the same optical rotatory power and dispersion as serum-albumin, and are therefore probably identical with it.

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REFERENCES.

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