A RAPID EXTRACTOR FOR URINARY STEROIDS

BY E. B. HERSHBERG* AND JOHN K. WOLFE†

(From the Converse Memorial Laboratory, Harvard University, Cambridge, and the Medical Clinic of the Peter Bent Brigham Hospital, Boston)

(Received for publication, February 19, 1940)

This work was undertaken in connection with an investigation being conducted by one of us (J. K. W.) with L. F. Fieser and H. B. Friedgood of the androgens of pathological urines. A continuous extractor has been constructed which meets the requirements of clinical assay work and which appears to have certain advantages over other forms of apparatus designed for the extraction of hormones from urine. This paper includes a description of the extractor and a report of orienting experiments conducted to test its efficiency and rapidity of action as applied to a few urines of low and of high androgen content. Its suitability for general assay work is being investigated further by Dr. Friedgood.

To increase the efficiency of extraction with a solvent lighter than water over that attained in an extractor of the type described by Smith and Smith (1), Gallagher, Koch, and Dorfman (2) provided for the dispersion of the organic solvent (benzene) by causing it to pass through a sintered glass disk, while Talbot and Langstroth (3) introduced an ingenious mechanical stirring device to disperse the solvent benzene into small droplets. The present apparatus utilizes the principle of dispersion with a porous disk as applied to a solvent heavier than water, a combination embodied in an early extractor for general laboratory purposes devised by Friedrichs (4), although this is not mentioned by Wehrli (5) in his review of extractors in which solvents heavier than water

* Research Fellow on grants from the National Cancer Institute and Eli Lilly and Company to Professor L. F. Fieser.
† Research Fellow on a grant from the Milton Fund of Harvard University to Dr. H. B. Friedgood.
Extractor for Urinary Steroids

are used. The solvent selected was carbon tetrachloride, which has the practical advantage over benzene or ether of being non-inflammable and comparatively non-toxic and which is regarded by Callow, Callow, Emmens, and Stroud (6) as being superior to benzene as a solvent for the extraction of steroids. The heavy solvent is passed through a sintered glass plate and the fine droplets allowed to fall through a long, narrow column of urine.

Apparatus (Fig. 1)—The extractor body \( J \), which contains the urine and a lower layer of carbon tetrachloride, is connected by means of the standard taper joint \( D \) to a unit \( G \) consisting of a spiral condenser \( F \) and a sealed-on tube carrying a sintered plate \( H \). The boiling flask \( A \) is fitted to the apparatus by means of a spherical joint \( B \), which requires no lubricant and is not easily frozen. The joint is kept closed with an arm type ball clamp with which the flask is supported on a ring-stand. The extractor body is fastened to a ring-stand by a large universal clamp placed near the level of \( H \). Stop-cock \( K \) does not require a lubricant other than the solvent used and permits draining the spent urine remaining after extraction without dismantling the apparatus.

Heating of the boiling flask is best done with a Bunsen burner with a direct, soft flame (an electrically heated aluminum block did not give a sufficiently high boiling rate or permit as easy adjustment of the temperature). The vapors ascend through the tube \( C \) and enter the condenser chamber through a hole drilled in the male section of joint \( D \). The condensate then falls through tube \( I \) and is dispersed by the sintered glass plate \( H \) which dips under the surface of the urine. The solvents separate in the lower part of the chamber; the carbon tetrachloride containing hormone returns to the boiling flask through stop-cock \( K \) and tube \( L \). Tube \( E \) equalizes the pressure between the extractor body and the condenser system and also allows accumulated water to overflow and return to the main body of the urine.

The efficient and compact spiral type of condenser adequately takes care of the vapor at the desired maximum boiling rate. In trials with a spiral having only sixteen turns it was possible to maintain a flow of more than 10 liters of carbon tetrachloride per hour with cooling water at either 5° or 30°.

From a trial of dispersion plates of different porosity, it was concluded that a coarse plate is better than a fine one. With a
Fig. 1. Assembly of urine extractor. A, 1 liter flat bottom boiling flask; B, spherical joint 35/20 (Ace Glass, Inc., Vineland, New Jersey; Scientific Glass Apparatus Company, Bloomfield, New Jersey); C, vapor tube, 20 to 22 mm. inside diameter; D, special 50/50 standard taper joint with an 18 to 20 mm. hole drilled in the side of the male section; E, overflow-equalizing tube, 7 mm. outside diameter. The outlet hole is 7 to 9 mm. below the vapor inlet; F, coiled tube condenser of tubing of 8 mm. outside diameter with both inlet and outlet at the top and facing to the back. The diameter of the spiral is 36 to 38 mm. and it is centered by three small knobs at the lower end of the coil. The clearance between the coil and side wall is 3 to 4 mm.; G, condenser jacket, 42 to 46 mm. inside diameter; H, porous glass disk, 41 to 43 mm. outside diameter, with average pore diameter of 85 to 145 μ (Ace Glass, Inc.); I, liquid connector tube, 13 to 15 mm. outside diameter. The length is such that plate H is 130 to 140 mm. below the vapor inlet hole; J, extractor body, 60 to 62 mm. inside diameter, 64 to 66 mm. outside diameter; K, 3-way 4 mm. bore stop-cock with interchangeable No. 17-F plug (Scientific Glass Apparatus Company, No-Lub catalogue No. C-880). This permits the extraction to be carried out without contamination by stop-cock grease. The same result can be obtained with an ordinary stop-cock by a final light grinding with jewelers’ rouge suspended in either oil or water; L, return tube, 9 mm. outside diameter. See the calibration procedure for the vapor tube connection.
fine plate it is necessary to provide a greater fluid head by lengthening tube I, with consequent decrease in the effective volume available for the urine sample, and even so the solvent flow cannot be maintained at the maximum boiling rate. A fine plate also gives rise to more trouble with the emulsions which tend to form in the extraction of urine with a dispersed solvent at a high rate of flow, and this may necessitate an increase in the space provided at the bottom of the extractor for the separation of layers. The water content of such emulsions, to be sure, is low, and in one test with a fine plate the emulsion was allowed to siphon over with no apparent harm. The tendency to form persistent emulsions is greatly reduced by using a coarse plate, and at the resulting high rate of flow the droplets are still very small and appear in a shower extending to all parts of the tube. Another important factor is the temperature, emulsions being more prone to form in the cold than at the temperature of about 60° which is reached after the extractor has been in operation. If the urine is introduced cold, the combination of an unfavorable temperature and a high initial steroid content may cause considerable trouble, but the difficulty is easily met by first warming the urine to 60°. Under these conditions, the emulsion usually breaks a short distance below the interface, and even with dark pathological urines very rich in steroids it is only necessary to moderate the boiling in the early stages of the extraction in order to prevent the emulsion from being carried over.

Prausnitz (7) recommends that a sintered glass plate be wet first with the organic solvent, while Friedrichs (8) states that smaller drops are obtained by wetting it with the aqueous solution. In our experience with the present extractor, the particle size appeared to be the same in either case after a short period of operation.

Assembly of Apparatus—The extractor is designed to accommodate a fixed volume of hydrolyzed urine in each operation, for this makes for simplicity of construction. The volume decided upon, in relation to the total capacity of the extractor tube, fixes the level at which the return tube L should enter the vapor tube C, and since this is the last connection to be made in assembling the apparatus, the proper point of entrance must be determined by experiment. The extractor body J, with the vapor tube C and
the stop-cock $K$ sealed in place but not yet directly connected, is fitted with the condenser unit and charged with 1175 cc. of water at room temperature and enough carbon tetrachloride is added to cause the sintered glass plate $H$ to dip 10 to 15 mm. into the water. A rubber tube is connected to one of the outlets of stop-cock $K$ and slowly lowered from a vertical position until a point is reached where carbon tetrachloride begins to run out. This is selected as the proper point for the entrance of the return tube $L$ into $C$.

Operation—In the usual case a 1 liter sample of urine is treated with 150 cc. of concentrated C.P. hydrochloric acid and the mixture boiled for 10 minutes under a reflux. The extractor is charged with 250 cc. of carbon tetrachloride and the hydrolyzed urine is cooled to 50-60° and introduced through the open joint $D$, a funnel being used to prevent spilling of the liquid into $C$ or onto the ground joint, for this may cause the joint to stick. The condenser unit is inserted and a rapid stream of cooling water started. Flask $A$ is charged with 500 cc. of carbon tetrachloride and heated with the direct flame of a Bunsen burner. With normal urines boiling can be conducted at the maximum rate from the start, but with pathological urines containing suspended solid the heating should be moderate at the outset, with a gradual increase in the course of about 15 minutes, when the initial emulsion coagulates and full heat can be applied.

In the test experiments the extraction was interrupted at suitable periods and the extract in the boiler transferred with rinsing to a distillation flask and replaced by fresh solvent. The solvent was completely removed from the extract by distillation at diminished pressure and the residue was dissolved in 200 cc. of ether and the solution washed in a separatory funnel with five 25 cc. portions of 10 per cent sodium hydroxide solution, the washings being discarded. The ethereal solution was evaporated to dryness at reduced pressure and the residue dissolved in 95 per cent ethanol and transferred to a small volumetric flask of size proportional to the estimated sterone content.

We are indebted to Dr. H. B. Friedgood and Miss R. A. Berman of the Endocrine Laboratory of the Peter Bent Brigham Hospital for making colorimetric assays of the extracts and for carrying out parallel extractions of some of the urines with benzene in the apparatus of Smith and Smith (1). The assays were con-
ducted by a modified Zimmerman procedure (9) similar to that of Callow et al. (10) and the results are expressed in mg. equivalents of androsterone.

The results listed in Table I show that with urines of widely varying androgen content the new apparatus extracts somewhat more sterone in \(\frac{1}{2}\) hour than the Smith and Smith benzene extractor does in 22 hours. With the urines in or near the normal range of hormone content, the material removed in the half hour period is about 95 per cent of that extracted in a period twice as long and doubtless represents nearly the entire amount present. This performance is somewhat more rapid than that attained in the extractor of Talbot and Langstroth (3). The apparatus has the added advantage of being rugged in construction and can be conveniently refilled. Since spent urine can be replaced by a fresh charge without dismantling the apparatus, a large quantity of urine can be extracted easily and the total hormone accumulated in the boiling flask.

With the specimen of urine (Sample J) of extremely high androgen content the steroid extracted in \(\frac{1}{2}\) hour is 80 per cent of

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance of Extractor; Mg. of Sterone Extracted in Successive Periods (Calculated As Androsterone)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine sample</th>
<th>Total extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min.</td>
</tr>
<tr>
<td>A</td>
<td>16.9</td>
</tr>
<tr>
<td>B</td>
<td>18.9</td>
</tr>
<tr>
<td>B₁ Benzene extractor</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>21.0</td>
</tr>
<tr>
<td>D</td>
<td>21.0</td>
</tr>
<tr>
<td>E</td>
<td>21.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>21.7</td>
</tr>
<tr>
<td>E₁ Benzene extractor</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>23.2</td>
</tr>
<tr>
<td>G</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>235</td>
</tr>
<tr>
<td>H₁ Benzene extractor</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>394</td>
</tr>
</tbody>
</table>
that removed in 1 hour and 62 per cent of the total accumulated in 17 hours, which may still be somewhat short of the actual total present. The comparative slowness of extraction may be due both to the high steroid content and to occlusion of hormone in the pigmented solid dispersed in the urine.

Since in our apparatus the rapidly flowing solvent maintains the urine at a steady temperature of about 60°, it may be practicable to combine the hydrolysis step with the extraction and so minimize a possible destructive action of the hot acid on certain of the hormones. This possibility is being investigated.

SUMMARY

A rapid and efficient apparatus is described for the extraction of steroids from urine embodying the principle of causing carbon tetrachloride to pass through a porous disk and fall in fine droplets through a long column of urine. Colorimetric androgen assays of normal and abnormal urines demonstrate the satisfactory performance of the apparatus. With urines in the normal range, the bulk of the androgen fraction is extracted in 3 hour.

BIBLIOGRAPHY

4. Friedrichs, J., Chem. Fabrik, 2, 90 (1929); 5, 199 (1932); Chem.-Ztg., 55, 519 (1931).
A RAPID EXTRACTOR FOR URINARY STEROIDS
E. B. Hershberg and John K. Wolfe

J. Biol. Chem. 1940, 133:667-673.

Access the most updated version of this article at http://www.jbc.org/content/133/3/667.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/133/3/667.citation.full.html#ref-list-1