mixing the sample with phenol. The bulk preparation, however, did show an increase in activity after the removal of material insoluble in 1 *M* NaCl from the sample. The RNA remaining after removing the RNA insoluble in 1 *M* NaCl from the non-nuclear and whole-cell samples of RNA showed a higher ability to take up amino acids than our best sample of soluble RNA obtained by differential centrifugation. This is probably a reflection of the fact that much less time elapsed before these samples were treated with phenol to stop enzymic action.

These results will allow the easy bulk preparation of these biologically active ribonucleic acids without the necessity of the slow ultracentrifugation technique which can result in the isolation of a partially degraded product.

These studies were supported by funds from the National Cancer Institute (Grant C-2896).

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Received December 3rd, 1959

Biochim. Biophys. Acta, 40 (1960) 360–361

The isolation of androsterone and aetiocholanolone from the urine of oophorectomised-adrenalectomised women

There have been several reports that patients with metastatic mammary cancer may continue to excrete various steroid hormones or their metabolites in the urine after the removal of the ovaries and the adrenal glands (for reviews, see ref. 1–3). The amounts of steroids found in the urine of such patients are very small and evidence for the identity of the compounds in question has generally been based on their chromatographic properties or, in the case of the oestrogens, on their biological activity[4].

Rigorous proof is needed for the identity of androsterone and aetiocholanolone since Δ 9–(11) artefacts produced by the dehydration of cortisone metabolites are very difficult to separate from androsterone and aetiocholanolone unless chemical modification is carried out[5]. PLANTIN *et al.*[6] found only Δ 9–(11) aetiocholenolone in urine obtained from adrenalectomized patients, a result which did not agree with the findings of KELLIE AND WADE[7], BULBROOK, GREENWOOD AND THOMAS[8] and HOBKIRK[9,10] that such patients continue to excrete androsterone and aetiocholanolone.

An attempt was therefore made to isolate and identify the compounds in the

androsterone and aetiocholanolone fractions prepared from urine from oophorectomized-adrenalectomized patients.

A pool of urine (70 l) was collected from women with metastatic breast cancer who had been subjected to oophorectomy and adrenalectomy at least one month previously. The urine was stored at —20° and thawed immediately before extraction. The methods used for the isolation of androsterone (3α-hydroxy-5α-androstan-17-one) and aetiocholanolone (3α-hydroxy-5β-androstan-17-one) were based as closely as possible on methods used for the detection and estimation of these compounds in urine. Conjugates were extracted and hydrolysed by the method of KELLIE AND WADE[7] and separated into a non-ketonic and a ketonic fraction by the method of MASON[11]. The total amount of Zimmermann-reacting material in the ketonic fractions was 37.4 mg, calculated in terms of dehydroepiandrosterone. After separating this fraction into 11-oxy and 11-deoxy-17-oxosteroids, using the ligroin–propylene glycol system of SAVARD[12], 3.2 mg of the latter were obtained. The compounds in this fraction were further resolved by running 500-μg portions (in strips across the paper) on a variant of the BUSH A paper chromatographic system[13], using isooctane–80 % aq. methanol. Compounds with the same mobility as standard androsterone and aetiocholanolone were eluted separately with ethanol. The pooled androsterone eluates were finally purified by gradient-elution chromatography on alumina[7]. Most of the elution solvents (ethanol; benzene) were removed at room temperature under a steam of nitrogen. The last traces of solvent were allowed to evaporate slowly *in vacuo*. A yield of 500 μg of white crystalline material was obtained. The aetiocholanolone fraction was treated in the same way, giving a final yield of 1200 μg of white crystals. Identification was by infrared spectrometry which was kindly carried out by Dr. T. F. GALLAGHER of the Sloan-Kettering Institute for Cancer Research, New York City. The spectra were identical with those of authentic androsterone and aetiocholanolone and there was no indication of the presence of unsaturated analogs.

The isolation of these compounds in a pure form in no way alters the previous conclusions of BULBROOK, GREENWOOD AND THOMAS[8] concerning the quantitative estimation of these compounds. Neither gradient elution from alumina nor paper chromatography on the BUSH A system is sufficient, used alone, for the separation of pure androsterone or aetiocholanolone in low-titre urine. If column chromatography alone is used, small amounts of other Zimmermann-reacting substances may be found in the androsterone eluate, and less frequently, in the aetiocholanolone eluate. Similarly, if paper chromatography is used alone, a compound with the same R_F as androsterone, but which is separable from it on alumina, may be found. In both cases, an over-estimate of the amounts of androsterone or aetiocholanolone is obtained.

There has been considerable controversy concerning the continued excretion of steroid hormones (or their metabolites) by women with cancer of the breast subjected to oophorectomy and adrenalectomy[3]. KELLIE AND WADE[7] found androsterone and aetiocholanolone in urine from such patients. BULBROOK, GREENWOOD AND THOMAS[8] found that most adrenalectomised patients continued to excrete aetiocholanolone but androsterone was rarely found. In all these cases, evidence for the identity of androsterone and aetiocholanolone was based on their behaviour on alumina columns and on paper-chromatographic systems. HOBKIRK[9,10] found androsterone in urine from 1 of 7, and aetiocholanolone in urine from 6 of 7 adrenalectomised women. Further characterisation of androsterone was carried out using a variety of paper-

chromatographic systems and by examination of the absorption curve of the compound in H_2SO_4. The substance extracted from urine appeared to be authentic androsterone and no evidence was found for the Δ 9–(11) artefact. However, PLANTIN *et al.*[6] found only Δ 9–(11) aetiocholenolone in urine obtained from adrenalectomised women but it appears probable that this compound arose as a dehydration artefact, caused by the method of hydrolysis used for for the steroid conjugates by these workers. The isolation and characterisation of androsterone and aetiocholanolone by methods similar to those used for the determination of these compounds confirms the previous reports on their continued excretion by women subjected to oophorectomy and adrenalectomy. Furthermore, GALLAGHER[14] and his colleagues have also isolated these two steroids from similar sources.

The mean 24-h urine volume for the adrenalectomised patients was 1600 ml. From this figure it can be calculated that the mean daily excretion of androsterone was approximately 10 μg and of aetiocholanolone, 30 μg. These levels agree well with those found by HOBKIRK[9].

The source of these compounds is still obscure. There is little likelihood that the 11-deoxy-17-oxosteroids arise from the maintenance doses of cortisone since BRADLOW AND GALLAGHER[15] have found that 11-oxysteroid hormones appear in the urine as compounds in which the 11-oxygen atom is retained, regardless of other changes in the molecule. The only report of the removal of the 11-oxygen atom is that of MILLER AND ALEXROD[16], who make the surprising claim that cirrhotic rat liver converts cortisone to 6β, 21-dihydroxy-Δ^4-pregnene-3,20-dione (among other metabolites). However, since oestrogens and pregnanediol have also been found in urine from adrenalectomized patients the most likely source for so many steroids is adrenal tissue either left at operation or in the form of adrenal rests[3].

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Received December 21st, 1959