The Extraction of a Carcinogenic Fraction from Human Urine*†
(Preliminary Report)

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The structural characteristics of synthetic carcinogenic substances, estrogens, and androgens have suggested to many the possibility that an abnormal metabolism of sterols or a disturbed elimination or destruction of carcinogenic steroid intermediates might be involved in human cancer. The fact that human urine contains relatively large amounts of conjugated androgens and estrogens in water-soluble forms led us to investigate human urine as a possible source of carcinogenic substances, possibly steroid in type. Accordingly, a systematic extraction of human urine was undertaken and the crude fractions obtained were tested for carcinogenic activity in mice.

Studies by others on various urinary preparations have not led to definite conclusions. Bischoff and Maxwell (1) reported that a "Kallikrein" or depressor fraction, which is difficult soluble in 50 to 80 per cent ethanol may augment the number of takes after tumor inoculation in rats. Turner (6) and Bowman and Mottshaw (2) studied the effects of the very crude alcohol-soluble growth-stimulating and the difficulty alcohol-soluble growth-inhibiting fractions (4) from urine on tumor growth. The former (6) concluded that the growth-stimulating fraction from normal urine has no effect on the rate or type of tumor produced by carcinogenic agents. He indicated a slight inhibiting effect from the growth-inhibiting fraction. Bowman and Mottshaw (2) reported that neither of the fractions above, nor ether, nor benzene extracts from the urine of cancer patients produced tumors in mice in 250 days. Brikker and Timofejewa (3) and Sobotta and Bloch (5) reported negative results with benzene and butyl ether extracts from the urine of cancer patients in tests observed for 5 months. Sobotta and Bloch (5) also reported a 50 to 75 per cent recovery of 100 milligrams phenanthrene and dibenzanthracene added to 20 liters of urine.

Sarcomas Developed at the Site of Injection in Mice Injected with an Extract of Human Urine

The percentage yield of the tumors was low so that repetition and extension of the work is necessary and is under way. Pending the conclusion of these new experiments, which because of the long induction time (minimum of 17 months) will be about 2 years, we are making this preliminary report. Also it is important that others who might be working with similar urine extracts appreciate this long induction time so as not to end their experiments prematurely.

Preparation of Urine Extracts

All the urine was from men. The normal urine was collected daily from urinals. The cancer urine was from patients suffering from cancer not involving the endocrine system as far as could be determined. As this was necessarily a preliminary study all the extracts were very crude mixtures. A brief description of the various types of extracts follows:

All the extractions were conducted under diminished pressure in a modification of the extractor used by the Department of Biochemistry, University of Chicago, for the quantitative extraction of sex hormones from urine.

Benzene extract without hydrolysis.—The urine at pH 3.4 to 3.8 was extracted at room temperature with benzene until at least 12 volumes of benzene had passed through the urine. In order to diminish the foaming, large amounts of sodium chloride were added to the urine. This extract should contain all the free androgens, estrogens, other steroids, phenols, fatty acids, fats, some pigments, etc. By shaking with saturated aqueous sodium bicarbonate, acids and much pigment were removed.

Butyl alcohol extract.—The urine after this treatment was extracted with normal butyl alcohol in the same apparatus. This extract should contain conjugated forms of steroids, estrogens, and androgens, and some water-soluble nitrogenous extractives.

Benzene extract after acid hydrolysis.—The urine after the butyl alcohol extraction was acidified by HCl to pH 1.0, boiled for 15 minutes, cooled, and re-extracted with benzene in the same way.

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Neutral fraction of the first benzene extract.—The first benzene extract was very toxic, hence it was fractionated by shaking an ether solution of the solids three times with saturated aqueous sodium bicarbonate. The ether solution left is referred to as the “neutral fraction.”

50 per cent alcohol-insoluble fraction from the first benzene extract.—The ether solution above was evaporated to dryness, dissolved in ethanol, and then diluted with an equal volume of distilled water. The tarry precipitate which separated is the fraction referred to here.

Petroleum ether-soluble fraction from the 50 per cent alcohol-soluble fraction.—This was obtained by extracting the 50 per cent alcoholic solution above with petroleum ether.

Experiment B. Carcinogenicity of the butyl alcohol urine extracts.—This extract was injected in doses of 100 mgm. per mouse given once. Seventeen male mice were injected with the extract from cancer urine, and 17 females were injected with the noncancer urine extract. The mice were from 37 to 59 days old.

The urine extracts had a slight primary toxicity, one animal in each group of 17 dying within 24 hours. The delayed caustic effects of the injected extracts were slight, only an occasional small slough being noted. Soft, fluctuant masses persisted at the site of injection of some mice for many months after injection. The

<table>
<thead>
<tr>
<th>Description of urine extract</th>
<th>Sex of mice</th>
<th>Survival time in months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral fraction of the first benzene extract—normal.</td>
<td>Female</td>
<td>11 11 10 8 6 5 5 2</td>
</tr>
<tr>
<td>Neutral fraction of the first benzene extract—cancer.</td>
<td>Female 2</td>
<td>7 6 6 4 3 3 2 1</td>
</tr>
<tr>
<td>Petroleum ether-soluble fraction from the 50 per cent alcohol-soluble fraction of the first benzene extract—normal.</td>
<td>Male 5</td>
<td>5 5 5 4 3 3 3</td>
</tr>
<tr>
<td>50 per cent alcohol-insoluble fraction of the first benzene extract—normal.</td>
<td>Female</td>
<td>7 5 4 3 3 1 0</td>
</tr>
<tr>
<td>50 per cent alcohol-insoluble fraction of the first benzene extract—cancer.</td>
<td>Male 2</td>
<td>8 8 8 6 5 5 3</td>
</tr>
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<td>Female 6</td>
<td>8 8 8 6 5 5 3</td>
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* The doses per mouse represent the equivalent of 5 to 15 liters of urine.

Method of Testing the Extracts for Cancer-Producing Ability

The urine extracts were dissolved in sesame oil. Some of them remained in solution poorly and it was necessary to warm them to the boiling point of water to get them into a syringe for injection. Each mouse was injected once subcutaneously in the interscapular region with the desired amount of urine extract contained usually in 0.5 cc. of sesame oil. The mice used were of our own albino stock. They were about 8 weeks old, and of the sex indicated in Tables I to III. Spontaneous sarcomas, other than lymphosarcomas, have never been seen in this partly inbred colony in several thousand individuals over a period of nearly 3 years, although mammary gland tumors, lung tumors, and leukemic diseases are spontaneous in them.

Experiment A. Carcinogenicity of various fractions of the benzene extract of urine (without hydrolysis).—Various fractions of human urine, normal and cancer, as indicated in Table I, were injected in one dose of 100 mgm. per mouse. The sex of the mice and the survival time in months up to the end of the second year are shown. No sarcomas occurred at the site of injection in these experiments. Despite the negative results these experiments are included in this report in order that unplanned duplication of effort may be avoided by others, and because they serve as control experiments for our stock of mice and for our specimen of sesame oil.

Table II: Tumor Production in Mice by Butyl Alcohol Extracts of Human Urine *

<table>
<thead>
<tr>
<th>Time in months</th>
<th>Number mice living</th>
<th>Number mice dead with sarcoma</th>
<th>Number mice living</th>
<th>Number mice dead with sarcoma</th>
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<tbody>
<tr>
<td>0</td>
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<td>26</td>
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* The dose per mouse was the equivalent of 80 to 125 cc. urine.

Unfortunately high mortality in the mice injected with the cancer urine extracts was due mostly to renal infection, the result of fighting wounds around the genitalia in these males. The results of these experiments are summarized in Table II.
Three tumors have occurred at the site of injection and 6 mice are still alive and without visible tumors at 26 months. One of these tumors was present in a mouse which had been injected with cancer urine extract, dying in the 17th month. At this time only one other mouse was alive in this group and he died subsequently without tumor. Two tumors have appeared in the mice injected with noncancer urine extracts. The first of these died with tumor in the 21st month, at which time 11 mice were alive in the experiment, and the second died in the 23rd month. Considering the induction time as 7 months, the effective total tested was 16 mice, and the percentage yield was 18.7 per cent (3 tumors in 16 mice).

These 3 tumors are fibrosarcomas in which the cells are pleomorphic and the collagen abundant (Fig. 1). Grossly they were first recognized as tiny, hard masses immediately beneath the skin to which they were adherent. They showed early ulceration. They grew slowly and spread laterally beneath the skin to form flat, fixed, infiltrating tumors which measured 12 x 10 x 6 mm.; 24 x 22 x 16 mm.; and 25 x 21 x 10 mm. at the time of death. One metastasized to the lungs. Because of the ulceration with infection attempts at transplantation were not made.

Tumors also occurred in the lungs, the mammary glands, and the lymphatic system of some mice in this experiment. They are not counted as induced tumors because at the present time it is not certain that their number exceeded the expectancy of spontaneous tumors for this stock of mice.

The three sarcomas which occurred at the site of injection of the urine extracts are different in several respects from the sarcomas which are usually induced by carcinogenic hydrocarbons. They were different in their long induction time, slow rate of growth, flat infiltrating type of tumor with early ulceration, and by their scirrhous, pleomorphic microscopical appearance. Whether this behavior is due to low dosage or low potency of carcinogen, or to other factors we cannot say. It is not attributable to any peculiarity inherent to the mice used, as is indicated by the following experiment.

Experiment C. Tests of the butyl alcohol extracts from human urine for factors which modify tumor production by methylcholanthrene.—Male mice of the same stock as those used in the previous experiments were each injected once with 100 mgm. of the butyl alcohol-urine extracts and to which 1 mgm. of methylcholanthrene was added. Another group of mice was injected with methylcholanthrene alone. This experiment was planned to see if there might be either acceleration or retardation of tumor production by the methylcholanthrene when the urine extract was added to it.

The results are given in Table III. The tumors listed there are all spindle or mixed cell sarcomas occurring at the site of injection. There is no important difference in the induction time or the percentage yield of tumors in these three groups of mice, or in the gross and microscopical features of these tumors, which resemble those found in other experiments in which the chemical carcinogens are used. Most of the deaths in nontumor-bearing mice were due to fighting wounds and their sequelae.

This experiment shows that tumor induction by methylcholanthrene was not inhibited or accelerated by a co-injection of an extract of human urine from cancer and noncancer persons, in the dosages used. It further shows that this stock of mice is reasonably susceptible to tumor induction by methylcholanthrene (a high percentage of those alive when the first tumors

![Fig. 1.—Photomicrograph of section of pleomorphic cell fibrosarcoma infiltrating muscle in a mouse injected subcutaneously with butyl alcohol extract of urine from noncancerous persons. Mouse died 21 months after injection. Mag. ×285.](#)
occurred developing tumors), and that the explanation for the long induction time, the low percentage yield of tumors, and the peculiar morphology of the three tumors reported in experiment B does not lie in the stock of mice used.

**Table III: Effect of Butyl Alcohol Extracts from Human Urine on Tumor Production by Methylcholanthrene in Mice**

<table>
<thead>
<tr>
<th>Time in months</th>
<th>Number of mice</th>
<th>Number of mice living</th>
<th>Number of mice living with tumor</th>
<th>Methylcholanthrene alone</th>
<th>Number of mice living with tumor</th>
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* Each mouse received one injection of the equivalent of 80 to 125 cc. urine.

**DISCUSSION**

Special comment will be made on the possible nature of the material found in the butyl alcohol extract. In view of the preliminary exhaustive extraction by benzene we would expect the butyl alcohol extract to be relatively free from free androgens and estrogens. Hence the carcinogenic action, if it is confirmed later, might be due to conjugated forms of one or both of these groups, to other steroid combinations having these peculiar solubilities, or to nonsteroid water- and butyl alcohol-soluble extractives of varying chemical character. It would be very remarkable if the water-soluble conjugated estrogens and androgens from 80 to 100 cc. of human urine were found responsible for these tumors, first because of the water solubility and second because of the low dosage involved. Hence the indications are that the carcinogenic action probably is due to other constituents of which there are many kinds in this crude extract. The fact that only 100 mgm. of this crude material showed some carcinogenic activity encourages us to attempt the confirmation of these observations and the identification of the carcinogen.

**SUMMARY**

1. Benzene extracts from unhydrolyzed human normal or cancer urine, whether injected as such or after further fractionation, did not produce tumors in albino mice.
2. The butyl alcohol extracts from such urines previously extracted with benzene produced 3 fibrosarcomas in 16 to 23 months after one injection into 34 mice. The effective total was 16 mice, and the percentage yield of tumors was 18.7. One tumor occurred after the injection with extract of cancer urine and 2 after injection of extract of normal urine.
3. Such butyl alcohol extracts, when combined with methylcholanthrene, did not retard or accelerate the tumor induction in white mice. The tumors produced by methylcholanthrene alone or with butyl alcohol extracts of urine were morphologically identical, but differed from those produced by the urine extract alone.

**REFERENCES**

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