dermis was comparable to that found during chromatography of mouse liver extract by the same procedure. When the positions of various peaks of activity were expressed relative to chloride concentration required to elute the major peak of nucleic acids (Table I), the β-glucuronidase activity appeared in each chromatogram in the same relative position.

In liver extracts glucose-6-phosphate dehydrogenase activity appeared as 3 peaks (Table I). Each peak of activity of this enzyme in tumor and epithelium chromatograms corresponded to one of the 3 of liver.

Summary. These results show that the above chromatographic procedure can be used to separate different protein extracts into a number of fractions and to investigate the distribution of various enzyme activities to pick out qualitative enzymic differences between malignant tumors and the normal tissues from which they arise.

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Free Phenolic Substances in Blood Plasma.*† (25508)

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Veldhuis(1), Nakao and Aizawa(2), Aitken and Preedy(3), Slaunwhite and Sandberg(4), and Oertel *et al.*(5) have described chemical methods for determination of estrogens in blood. The methods of Nakao and Aizawa, and Aitken and Preedy employ column chromatography for final separation of the estrogens prior to estimation by fluorometry. In our laboratory an alumina column similar to that described by Nakao and Aizawa was employed. However, instead of eluting the column with 1% ethanol in benzene for the estrone fraction, elution was carried out with 0.25, 0.50 and then 1.0% ethanol in benzene. When this was done, it was found that the free "estrone" fraction actually consisted of a substance slightly less polar than estrone. This phenolic substance less polar than estrone would be estimated as estrone if elution were performed with only 1.0% ethanol in benzene. The following is a description of results of estimations of free estrone and estradiol-17β fractions in plasma. Some properties of a substance less polar than estrone are also described.

Method. Heparinized blood was centrifuged immediately after withdrawal and the plasma separated. The method of Brown (6) was adapted for extraction. Plasma (20 ml) was extracted by partition once with 4 volumes of ether for 30 min. and then twice with 2 volumes for another 15 min. The ether extract was washed with 16 ml of concentrated sodium carbonate at pH 10.5 and partitioned with 4 ml of 2 N sodium hydroxide. The sodium hydroxide was adjusted to pH 10.5 by addition of 16 ml of 8% sodium bicarbonate,

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shaken with the ether, and the aqueous phase discarded. The ether was successively washed with 4 ml of 8% sodium bicarbonate, and twice with 2 ml of water and evaporated to dryness. An alumina column that gave consistent separation and recovery was developed. The alumina column described by Stimmel (7) and more recently by Nakao and Aizawa (2) was modified using Harshaw alumina "catalyst grade" which was deactivated by the addition of 6 ml of water per 100 g of alumina. One end of a glass tube (0.5 x 30 cm) was plugged with glass wool and filled with benzene. Two grams of alumina were allowed to settle through the benzene. The alumina was then washed with 12 ml of benzene and the extract transferred to the column with 32 ml of benzene in 4 ml aliquots. This was followed by 12 ml of 0.25% ethanol in benzene. The estrone was eluted by 24 ml of 0.50% ethanol in benzene, and the estradiol-17β with both 1% and 5% ethanol in benzene. Four ml fractions were collected. The solvents were evaporated in a vacuum oven at a temperature of 55-60°C. For fluorometry 4 ml of 88% sulfuric acid was added to each fraction, agitated thoroughly and allowed to stand for 30 minutes. The acid solution was then transferred to Farrand tubes (10 x 70 mm), heated in an oven for 6 minutes at 100°C and allowed to cool for 20 minutes to room temperature. The fluorescence was determined in a Farrand Model A photofluorometer with a primary filter of maximum transmission at 436 mμ and a secondary filter with maximum transmission of 480 mμ. Spectrophotofluorometric studies have shown that these wavelengths were optimal for the estrogens under discussion, when sulfuric acid solutions were used.

Results obtained with a plasma extract separated by the alumina column using 1% ethanol in benzene for elution of estrone, compared with an equivalent plasma separated with the 0.25 and 0.50% ethanol in benzene eluting mixtures showed that there appeared a substance less polar than estrone with the latter procedure. A peak in the estradiol-17β fraction, and a peak between this and the "estrone" were found. Free estrone was not always present in plasma extracts separated by

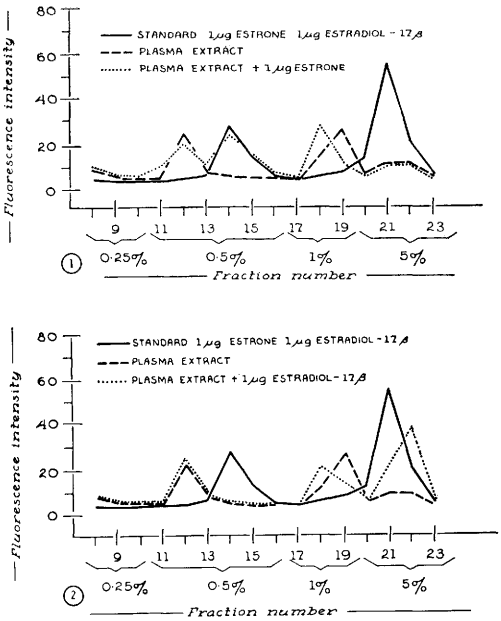

FIG. 1. Behavior of 1 μg of estrone added to an extract before chromatography.

FIG. 2. Behavior of 1 μg of estradiol-17β added to an extract before chromatography.

the column. In order to determine whether the peak less polar than estrone was an artefact caused by the extraction procedure, estrone and estradiol-17β were added to individual plasma before and after extraction, prior to chromatography. After chromatography the material less polar than estrone was always present. The added estrone and estradiol-17β appeared in the same fraction as did the estrogens of the reference column. The same estrogens extracted from aqueous solution did not show the substance less polar than estrone after chromatography. Fig. 1 shows the results of chromatography of a plasma extract to which estrone had been added. Fig. 2 gives the result after addition of estradiol-17β. No appreciable peaks were found in a solvent blank.

In later work phenolic separation between toluene and 1 N sodium hydroxide as described by Engel(8) prior to chromatography on alumina was done. The substance less polar than estrone was phenolic. The material between estrone and estradiol-17β was not always present. The fraction corresponding to estradiol-17β was not affected by the phenolic separation and is phenolic.

The absorption spectrum of the substance less polar than estrone when dissolved in sulfuric acid showed maxima at 400 and 420 mμ which are not shown by any estrogen available to us. The fluorescence of this compound in sulfuric acid was found to be maximal at 480 mμ with maximal excitation at 440 mμ which is characteristic of the estrogens.

Studies with the fraction corresponding to estradiol-17β have shown that the fraction may contain phenols other than estradiol-17β. The absorption spectrum of the fraction in sulfuric acid showed maxima at 420 and 490 mμ with an inflection at 455 mμ which estradiol-17β does not show. In sulfuric acid fluorescence properties were the same as those of estradiol-17β. However, this fraction when studied for estrogenic potency produced a positive vaginal smear in castrate female mice.

The amounts of estrone in 2 of 15 blood samples from pregnant women were 7.6 and 2.3 μg per 100 ml of plasma. The amounts of the fraction corresponding to estradiol-17β in the 15 samples averaged 6.5 μg/100 ml blood, calculated as estradiol-17β. It must be emphasized that this figure includes the increment that is not estradiol-17β.

These results agree with the preliminary report of Purdy, Engel and Oncley(9) that estrone is not found in free form in blood. They showed that estrone sulfate was the main metabolite found in blood after administration of estradiol-17β. The nature of the unknown materials is being investigated.

Summary. A detailed examination of the free estrone fraction of alumina column chromatograms of extracts of blood plasma has shown that when elution is performed with 0.25, 0.50 and then 1% ethanol in benzene instead of only using 1% ethanol in benzene, the free "estrone" fraction actually consisted of a substance slightly less polar than estrone. Free "estrone" is seldom found in plasma. Two of 15 samples contained 7.6 and 2.3 μg/100 ml plasma. The fraction which presumably contained estradiol-17β contained other material. The so-called estradiol-17β fraction averaged 6.5 μg/100 ml plasma.

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Factors Affecting Drug Metabolism by Liver Microsomes. IV. Starvation.* (25509)

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We have studied effects on drug metabolism of factors which affect liver structures, especially liver microsomes(1). One such factor is starvation, since electron micrographs of liver tissue showed that differences do exist between normal and fasted animals. Cytoplasmic basophilia decreases sharply after starvation of a few days, and most hepatic cells lose ergastoplasm(2) and undergo significant change in dispersion of this structure(3). We believed that these cellular changes in liver structure: namely, reduction in amount of ergastoplasm, and changes in microsome subunits, might have an effect on certain microsomal drug metabolisms. A variety of drugs are metabolized through the action of en-

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