hormone, organ weight change, and changes in iron and porphyrin metabolism.

Summary. Serum iron levels were determined on individuals in the following categories (average value indicated in parenthesis): controls (81.0), cancer with no metastases (77.1), cancer with regional metastases (64.2), cancer with widespread metastases (47.8), nonmalignant disease (outpatients) (85.2), nonmalignant disease (inpatients) (83.0) and tuberculosis (54.7). Serum iron levels in patients with advanced breast cancer were not significantly different from the levels observed in cancer patients without metastases. The data indicate that the serum iron levels may be related to the extent of metastases, but that they are not a factor in determination of the presence of cancer *per se.*

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20α-hydroxypregn-4-en-3-one, an Interfering Fluorogen in Assay of Corticosterone.* (29957)

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The quantitative measurement of serum corticosterone by fluorimetric techniques(1,2, 3) has been widely used as an estimate of adrenal function in rodents under various experimental conditions. The reliability of these methods is based on the assumption that the rat and mouse secrete no steroids which have fluorescence spectra similar to corticosterone under standard conditions in sulphuric acid. Interference from estrogen fluorescence is minimized in the extraction procedure(4).

Recently 20α-hydroxypregn-4-en-3-one was identified in rats in ovarian venous(5) and peripheral blood(6). Thus it seemed necessary to investigate the fluorescence properties of this steroid.

Materials and methods. All chemicals were of analytical reagent grade except for chloroform and methylene dichloride which were 'Chromatoquality.' 20α-hydroxypregn-4-en-3-one, its 20β isomer and corticosterone were obtained as gifts from Ciba (Basle), E. R. Squibb, and the National Institutes of Health Cancer Chemotherapy Section respectively. Fluorescence reagent was 55, 65, 75, 85 or 95% concentrated sulphuric acid in ethanol (v/v) or concentrated sulphuric acid alone. Fluorescence spectra were determined in an Aminco-Bowman Spectrofluorometer. All

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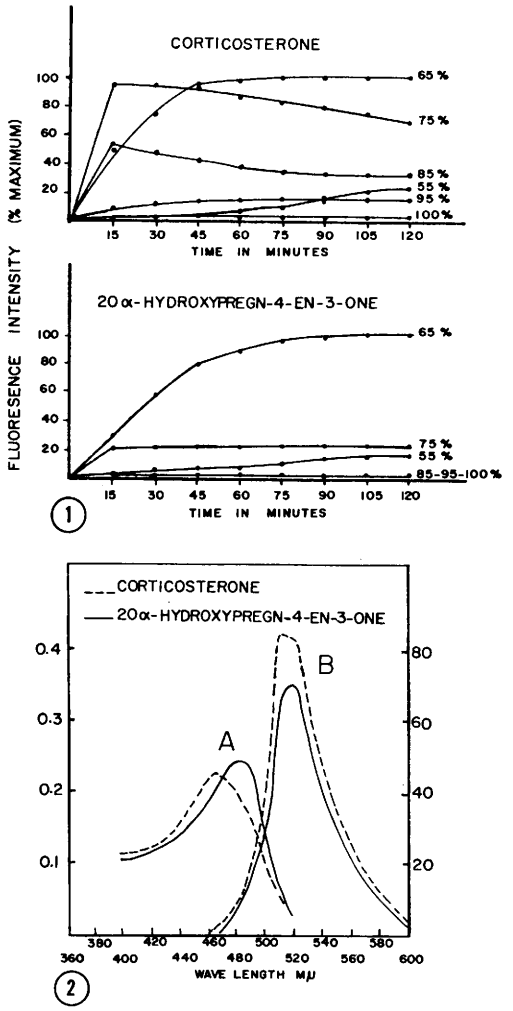

FIG. 1. Fluorescence intensity of corticosterone and 20α-hydroxypregn-4-en-3-one in 55, 65, 75, 85, 95 and 100% sulphuric acid/ethanol.

FIG. 2. Spectral properties of corticosterone and 20α-hydroxypregn-4-en-3-one (10 μg/2 ml) in 65% sulphuric acid/ethanol: A—absorbance, B—fluorescence with exciting light at 470 mu.

other fluorescence values were measured in a Farrand Model A fluorometer with a Corning 5113 primary filter (470 mμ) and Wratten 16 and 74 secondary filters (520-580 mμ). Absorption spectra were measured in a Carey Recording Spectrophotometer.

Steroid dissolved in 4 ml of chloroform: methylene chloride (2:1) was extracted with 2 ml of the fluorescence reagent. For estimation of recovery, steroid was dissolved in 1 ml of male rat serum and extracted according to the method of Hilf *et al*(6) employing a petroleum ether wash, extraction with chloroform:methylene dichloride, and removal of phenols with 0.1 N sodium hydroxide.

Results. Development of fluorescence with time in acid/ethanol mixtures. Both corticosterone and 20α-hydroxypregn-4-en-3-one exhibit the greatest fluorescence in 65% sulphuric acid/ethanol and development of fluorescence during a 2-hour period is similar for both steroids (Fig. 1). However, after addition of 75% acid/ethanol, the development of fluorescence with time is different for the two steroids. Maximum fluorescence for corticosterone is reached after 15 minutes but begins to decline after 45 minutes. Intensity of fluorescence of 20α-hydroxypregn-4-en-3-one is only about 20% of that of corticosterone in 75% acid, is fully developed within 15 minutes and is stable for 2 hours. Fluorescence intensity is low for both steroids in acid/ethanol concentrations of 55, 85, 95 and 100% (Fig. 1).

Spectral characteristics in 65% acid/ethanol (Fig. 2). 20α-hydroxypregn-4-en-3-one exhibits maximal absorption at 482 mμ while that for corticosterone is 466 mμ. With exciting wavelength at either 470 or 480 mμ, 20α-hydroxypregn-4-en-3-one emits maximum fluorescence at 520 mμ while the fluorescence peak for corticosterone is 510-520 mμ. Fluorescence intensity of 0.5, 1.0 and 2.0 μg of 20α-hydroxypregn-4-en-3-one was only 78% the intensity of the same quantities of corticosterone. 20β-hydroxypregn-4-en-3-one was found to have spectral properties similar to the 20β isomer.

Recovery after extraction. Recovery of 0.5-2.0 μg of both steroids from 1 ml of male rat serum at various stages of the extraction procedure was examined. It was found that after washing serum once with 3 volumes of petroleum ether (B.P. 40-60°), 93.8% ± 2.8 (n,18) corticosterone remained in the serum *versus* 43.2% ± 2.8 (n,24) 20α-hydroxypregn-4-en-3-one. Recovery at other steps in the extraction procedure was the same for both steroids.

Discussion. 20α-hydroxypregn-4-en-3-one, a naturally occurring ovarian secretion in rats (5) and ovarian product *in vitro* in mice(7) can interfere appreciably in the widely used fluorimetric method for assay of corticoster-

one in serum. The extent of the interference is decreased by the use of petroleum ether during the extraction procedure and is less in 75% than in 65% acid/ethanol mixtures. The presence of 20α-hydroxypregn-4-en-3-one in peripheral circulation of female rats may contribute in part to the reported sex differences in concentration of serum corticosterone when measured fluorimetrically(8). Further, since 20α-hydroxypregn-4-en-3-one secretion is greatest during late proestrus(5), observed changes in corticosterone level during the estrous cycle(9,10) may be attributed to interference from this progestin.

A sensitive fluorimetric method can be adapted for measurement of small quantities of 20α-hydroxypregn-4-en-3-one present in ovarian tissue and venous blood. Differences between corticosterone and 20α-hydroxypregn-4-en-3-one in development time in 75% acid/ethanol suggests that these two steroids may be determined simultaneously in one sample by the use of calculations suggested by Stewart *et al*(11) for simultaneous measurement of corticosterone and cortisol.

Summary. A comparison of 20α-hydroxypregn-4-en-3-one and corticosterone reveals that their fluorescence properties are similar in concentrated sulphuric acid. Since rodent ovaries secrete 20α-hydroxypregn-4-en-3-one, this steroid may interfere with the assay of corticosterone by fluorimetric techniques; however, interference may be reduced by the use of a petroleum ether wash. It is suggested that a fluorescence method may be used as a sensitive assay for 20α-hydroxypregn-4-en-3-one.

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Secretion of Sr^{85} and Ca^{47} in Human Saliva.* (29958)

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Several investigations have shown(1,2) that radiocalcium and radiostrontium have different rates of passage through various membranes and in different secretions of the body. Little is known about the relative secretion of the two radioisotopes in saliva. It has been reported recently(3) that a decrease of the body burden of Sr^{85} in cats and mice can be attained more readily by using pilocarpine to stimulate the flow of saliva than by increasing the excretion of radiostrontium in urine. The increased secretion of Sr^{85} in saliva is said to take place(3) with less loss of calcium than the excretion of Sr^{85} in urine. The literature contains conflicting accounts concerning the effects of stimulating the secretion of saliva on concentration of calcium, some cited by Dreisbach(4). One group of investigators reported that concentration of calcium in saliva rose with an increase in flow rates(5). In his own work, Dreisbach(4) found that the concentration of calcium in saliva, obtained by pilocarpine stimulation in rats, decreased from an initial level of 6.4 mg% to less than a third of this value in one hour. He also found that rat salivary glands discriminated slightly in favor of Ca and against Sr^{85} in secretion of

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