THE ADSORPTION OF ESTRIOL BY THE PLASMA PROTEINS [1]

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A number of foreign substances when injected into the blood stream are partially adsorbed by the plasma proteins. As a result of this adsorption only a portion of the total concentration is freely filterable through the glomeruli of the kidney. During a study of the mechanism by which estrogens are excreted it became necessary to know whether such normal body products are adsorbed in a similar manner.

The relation between bound and free estriol was investigated in human plasma by in vitro ultrafiltration. Estriol was used in these experiments because of its greater solubility in water compared with other estrogens and the ease with which it may be chemically determined. The filtration procedure used was essentially the same as that of Shannon ('35), and of Smith and Smith ('38). Some difficulty was encountered in finding suitable membranes for ultrafiltration. Those of collodion and similar material strongly adsorbed estriol. Commercial sausage casings were found to be satisfactory and were used in all the experiments reported here.

Standard estriol solutions were made up in absolute alcohol from pure dried crystals. Portions of this solution were measured into test tubes and the alcohol evaporated off in a water bath. The estriol was dissolved in a dilution fluid of 0.2% $NaHCO_3$ with the aid of gentle heating. Plasma was then

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added to give different final concentrations of plasma proteins. It was necessary to vary the plasma rather than the estriol as adsorption was of a high order and with pure plasma little estriol could be recovered. In a few cases the estriol was dissolved in pure plasma.

The estriol-plasma solution was equilibrated with 40 mm partial pressure of CO_2 to adjust the pH. Thirty cubic centimeters were then placed in a bag made from the sausage casing and filtered under 150–200 mm Hg pressure. Care was taken to prevent evaporation of the filtrate during the 3 hours required for the collection of 4 or 5 cm^3. The pressure was then reduced to 30–40 mm Hg pressure and the filtrate dialyzed against the contents of the bag for another 3 hours. Filtration and dialysis were carried out at 38°C.

The filtrate was extracted four times, each time with 3 cm^3 of ether, previously purified with ferrous sulphate and redistilled. The ether extract was placed in a pyrex test tube and the ether evaporated off in a water bath. Estriol was determined colorimetrically by the method of Bachman ('39). The phosphoric acid reagent was added directly to the tubes containing the extract residue. Controls were run with ether alone and on ether extracts of plasma free of added estriol (table 1). Properly purified ether gave no appreciable color with the reagent. Recovery experiments using ultrafiltered solutions of estriol in dilution fluid were made to demonstrate that estriol was not adsorbed by the membrane and that filtration was complete under the conditions in the experiments reported.

Table 1 gives the percentage of the total estriol that is free when different quantities of plasma proteins are present and the total estriol concentration is 1 mg/100 cm^3 of plasma solution. Estriol is shown to be very strongly adsorbed by plasma proteins.

The adsorption of substances by the plasma proteins is expressed by the adsorption isotherm

$$X = mk \cdot C\, I/N$$

where X is mg of the substance bound by m grams of protein, k and N are constants and C is the mg of free substance per 100 cm^3 of plasma water. In the following analysis, no attempt was made to correct the concentrations to mg per 100 cm^3 plasma water. Concentration per 100 cm^3 of solution was considered sufficiently accurate in view of the small amount of data obtained. Circumstances did not allow a more complete study.

TABLE 1

Free estriol in plasma solutions.

PER CENT PLASMA	TOTAL ESTRIOL MG/100 CM^3	FREE ESTRIOL MG/100 CM^3	PER CENT FREE ESTRIOL
0	0.90	0.96	..
0	0.90	0.96	..
0	0.87	0.90	..
100	0.00	0.10	..
100	0.00	0.09	..
100	0.00	0.13	..
100	1.00	0.10 [1]	10
33	1.00	0.32 [1]	32
17	1.00	0.36 [1]	36
7	1.00	0.42 [1]	42
3	1.00	0.59 [1]	59
1	1.00	0.70 [1]	70

[1] The free estriol values have been corrected for the color due to the plasma chromogens.

As log X/% plasma = log mk + I/N log C, the slope of the straight line obtained by plotting log X/% plasma against log C is I/N and the value of log X/% plasma when log C equals zero is log mk. When these figures are obtained the equation becomes:

$$X = 10.5\ C^{.93}$$

and can be used to calculate free and bound estriol for various estriol and plasma concentrations. That this equation is only approximate is shown in table 2 where a comparison is made between actual and calculated results for four experiments. The range of concentrations in these experiments was 1.00 to 6.43 mg/100 cm^3.

TABLE 2

Comparison of total estriol added and total estriol calculated from free estriol.

PER CENT PLASMA	TOTAL ESTRIOL ADDED MG/100 CM³	FREE ESTRIOL [1] MEASURED MG/100 CM³	TOTAL ESTRIOL CALCULATED MG/100 CM³	PER CENT DIFFERENCE
100	6.43	0.40	4.87	— 24
50	1.67	0.28	1.87	13
50	1.83	0.33	2.20	— 20
33	1.61	0.36	1.70	6

[1] The free estriol values have been corrected for the color due to the plasma chromogens.

DISCUSSION

A possible figure for the total estrogen in plasma is 0.00003 mg per 100 cm³. Fluhmann ('34) found about three mouse units of estrogen per 100 cm³ human female plasma; one mouse unit is approximately 0.01 gamma. These early experiments were crude and give no more than the order of magnitude of the blood estrogens. At this level according to the above equation for estriol only 3% is free and therefore filterable at the kidney glomeruli. If 100 cm³ are filtered per minute in the kidneys, 144 liters per 24 hours, 3% of 0.00003 mg will be filtered with each 100 cm³ or 1 gamma (0.001 mg) in 24 hours. This is approximately the quantity found to be excreted per day in the free unconjugated form during certain periods of the menstrual cycle (Palmer, '37). There is some question, however, whether the free estrogen comes directly from the plasma or is formed in the urine from conjugated estrogens.

The relationship between the quantities of estrogen in blood and urine is complicated by the fact that estriol, and perhaps other estrogens, is adsorbed by the plasma proteins. Free urinary estriol is not directly proportional to total plasma estriol. As it has been shown that the aglomerular kidney of the toadfish is capable of excreting estrogens (Boettiger, '46) one cannot rule out the possibility that the kidney tubules play an important part in the elimination of estrogens from

the body. The assumption that the urinary excretion is a function of blood concentration may be questioned.

SUMMARY

Estriol is strongly adsorbed by the plasma proteins so that only a small fraction of the total plasma concentration is filterable at the glomeruli of the kidney. Such an adsorption process may play an important role in the distribution of estrogens throughout the body and in the protection of estrogens from enzymatic action within cells and tissues.

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