THE BIOGENESIS OF PRIMARY SEX HORMONES

I. The Fate of Estrins Injected into the Rabbit*

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The rabbit normally excretes scarcely detectable quantities of estrogenic material (Pincus, Wheeler, Young, and Zahl (1936)), but injected estrin may be recovered in the urine (Smith and Smith (1931)). Smith and Smith have in fact shown that 600–700 r.u. (rat units) of theelin injected into non-pregnant rabbits results in the excretion of about 30 r.u. over a period of 4 days after injection, whereas the simultaneous injection of 600–700 r.u. of theelin plus progestin into an ovariectomized rabbit doe results in the recovery of about 500 r.u. of estrogenic material in the urine. This difference may be due to the transformation of theelin (estrone) into a more active estrogen in the progestin-treated animal, or to a greater destruction of the estrone in the non-pregnant animal with no change in its chemical constitution.

Estrone might conceivably be converted into four native estrogenic compounds in the organism, estradiol, estriol, equilin (and its isomer hippulin), and equilenin (Fieser (1936)). Of these, by ordinary test, estradiol is more active than estrone, estriol and equilenin less active, equilin and hippulin about as active. Since estradiol is the ovarian hormone and estrone a urine component it seems likely that the latter is an excretion product and not normally converted to the former; furthermore, estradiol has been recovered from the urine of mares in small amount only (Wintersteiner, Schwenk, and Whitman (1935)) while estrone is obtained in large amount. The equilinic compounds have been obtained from members of the horse family only, so that it seems unlikely that rabbits would produce them. Nonetheless since the nature of rodent estrogens is unknown we cannot ignore the possibility.

Fortunately there exists a specific color test for estriol which should

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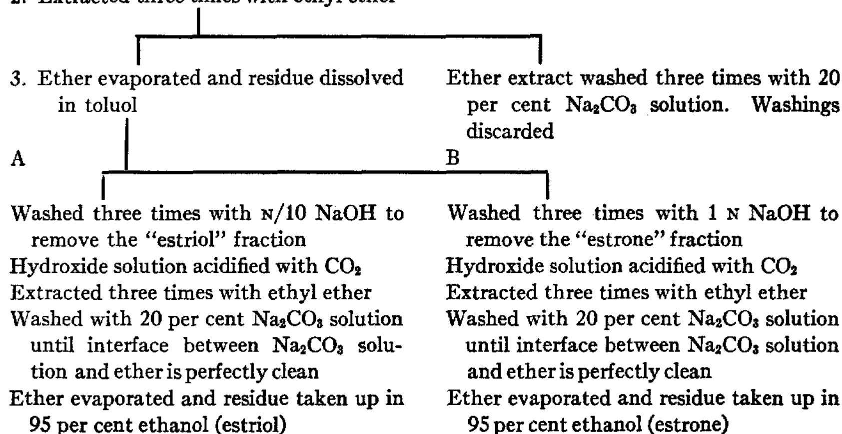
make possible its detection in urine extracts. This is the production of a blue color when the hormone is heated with concentrated H_2SO_4 followed by cooling, dilution, and further heating with arsenic acid (David (1934)). In a previous paper (Pincus, Wheeler, Young, and Zahl (1936)) we demonstrated that when human urines are separated into estrone and estriol fractions after the method of Cohen and Marrian (1934) the David test cannot be employed for estriol determinations because of the presence of inactive interfering substances. Furthermore, due to the inactive materials in rabbit urines all ordinary colorimetric tests give a positive value for both estrone and estriol when neither are in fact present. By modifying the extraction procedure we are now able to free rabbit urines from the interfering chromogens so that the David test can be employed as a quantitative measure of estriol and other tests as a measure of the hormone content of the estrone fraction.

Methods

Urine collections have been made from rabbits (1) in heat, (2) pseudopregnant, (3) pregnant, (4) hysterectomized in heat, (5) hysterectomized pseudopregnant, (6) ovariectomized. 48 hour samples are taken since we have found that these give comparable volumes. Two 48 hour urine samples are taken preceding the injection of estrogenic material and urine is collected after injection as long as hormone is excreted. The urine is extracted and fractionated according to the following procedures:

1. Acidified to pH 2.0 with HCl and placed in an autoclave at 15 lbs. pressure for 15 to 30 minutes.
2. Extracted three times with ethyl ether



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This is essentially the procedure of Cohen and Marrian (1934) except that the hydrolysis under pressure is carried on for a very short interval of time. Washing is made twice with 20 per cent Na_2CO_3 rather than once with 10 per cent Na_2CO_3 and fractionation is made from a toluol extract (A) rather than the ethereal extract they use. Acid hydrolysis for the 2 hours they employ almost completely destroys the hormone in rabbit urines. The washing with 20 per cent Na_2CO_3 removes interfering chromogens and pigmented materials, so that our final solutions are colorless (Schmulovitz and Wylie (1935)). The fractionation is made from toluol because this reagent retains certain pigmented chromogens that are not retained by ethyl ether.

For rapid determinations of hormone content we employ the red color obtained by diazotization with sulfanilic acid. The reagents are: (1) 5 gm. sulfanilic acid dissolved in 50 cc. concentrated HCl, diluted to 500 cc. with distilled H_2O; (2) 25 gm. $NaNO_2$ in 500 cc. distilled H_2O; (3) 15 per cent NaOH in aqueous solution. The color reagent is made up by adding 1.5 cc. of the $NaNO_2$ solution to 5 cc. of the sulfanilic acid solution and diluting to 50 cc. with H_2O. This reagent gives a constant color reaction with the hormones at between 1/4 and 2 hours after it is made up. Fresh reagent must be made after 2 hour intervals or longer. The color reaction is obtained by adding exactly 2 cc. of the color reagent to 2 cc. of alcoholic hormone extract. After thorough mixing 3 drops of 15 per cent NaOH are added. The red color resulting remains constant for 1/2 hour and then grows somewhat more intense, so that readings of color absorption are made within 1/2 hour using the S-51 filter of the Pulfrich photometer. The hormone content is determined from a standard curve made with crystalline hormone. We make up a new standard curve for each set of determinations since the color reaction varies from day to day. These standard curves do not differ by more than 25 per cent, but this is too wide a variation to justify the use of a single standard.

The colorimetric assay of the estriol fraction is checked by the David test (Table II). We do not employ this test for rapid determination since it is much more time-consuming. It should be used, however, to make certain of the estriol content since the sulfanilic acid test will reveal the presence of phenolic compounds other than the hormone.[1] There is no specific test for estrone comparable to the David test, but the sulfanilic acid determinations have been checked by the modified Cohen and Marrian phenolsulfonic acid test described by Pincus, Wheeler, Young, and Zahl (1936). This test is about five times as sensitive as the sulfanilic acid test and will reveal small quantities of material not detectable by the sulfanilic acid test. On the other hand it will give positive tests for inactive materials not revealed by the sulfanilic acid test.

Equilenin is determined by the color reaction described by Sandulesco, Tschung, and Girard (1933).

[1] It should be noted that inactive chromogens are rarely encountered in our extracts. Their presence is always denoted by slight pigmentation of the extracts. Removal of the pigment results in correct colorimetric titers.

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TAB

The Estrogen Content of Various Rabbit Urines before and after the Injection of Estro

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
			Estrone content				
Rabbit No.	Condition of rabbit	Amount of hormone injected	Days 4–3 before injection	Days 2–1 before injection	Days 1–2 after injection	Days 3–4 after injection	Days 5–6 after injection
			micrograms	*micrograms*	*micrograms*	*micrograms*	*micrograms*
1	Estrus	300 micrograms estrone	0	0	20	20	0
2	Pseudopregnant (1st 10 days of pseudopregnancy)	" "	0	0	38	20	0
3	Pregnant (1st 10 days of pregnancy)	" "	0	0	55	20	0
4	Pregnant (last 10 days of pregnancy)	" "	0	0	35	20	0
5	Hysterectomized (unmated)	" "	0	0	35	0	0
6	Hysterectomized (mated 1st 10 days of pseudopregnancy)	" "	0	0	105	38	20
7	Ovariectomized (1st 10 days after ovariectomy)	" "	0	0	35	10	0
8	Ovariectomized (2 mos. after ovariectomy)	" "	—	—	55	0	0
9	Estrus	300 micrograms estriol	0	0	0	0	0
10	Pseudopregnant (1st 10 days of pseudopregnancy)	" "	0	0	0	0	0
11	Hysterectomized (unmated)	" "	0	0	0	0	0
12	Hysterectomized (mated 1st 10 days of pseudopregnancy)	" "	0	0	0	0	0
13	Ovariectomized (1 mo. after ovariectomy)	" "	0	0	0	0	0
14	Pseudopregnant (1st 12 days of pseudopregnancy)	600 micrograms estrone benzoate	0	0	130	51	37
15	Hysterectomized (mated)	" "	0	0	168	125	75
16	Ovariectomized (1½ mos. after ovariectomy)	" "	0	0	110	100	75

* Positive David test

† Negative David test.

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striol, and Estrone Benzoate, Using the Sulfanilic Acid Test for Urinary Hormone

	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
	Estriol content						Total hormone excreted		Total estrin (estrone plus estriol)	Injected hormone recovered
-8 n	Days 4-3 before injection	Days 2-1 before injection	Days 1-2 after injection	Days 3-4 after injection	Days 5-6 after injection	Days 7-8 after injection	Estrone	Estriol		
ms	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*per cent*
	0	0	37	0	0	—	40	37	77	25.7
	0	0	100*	40*	0	—	58	140	198	66.0
	0	0	45	35	0	—	75	80	155	51.7
	0	0	25	60	0	—	55	85	140	46.7
	0	0	0	0	0	—	35	0	35	11.7
	0	0	0	0	0	—	163	0	163	54.3
	0	0	33*	0	0	—	45	33	78	26.0
	—	—	0†	0†	0†	—	55	0	55	18.3
	0	0	33	0	0	—	0	33	33	11.0
	0	0	110*	42	0	—	0	152	152	50.7
	0	0	33	15	0	—	0	48	48	16.0
	0	0	125*	50	8(?)	—	0	183	183	64.3
	0	0	45	15	0	—	0	60	60	20.0
	0	0	126*	62	0	0	218	188	406	93.8
	0	0	0†	0	0	0	423	0	423	97.9
	0	0	0	0	0	0	340	0	340	78.7

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RESULTS

The data on urinary estrin as determined by the sulfanilic acid test are presented in Table I. It is immediately deducible that estrone as such or in the form of the benzoate is converted to estriol in the rabbit and that estriol is not converted but excreted unchanged. But the conversion of estrone does not occur in hysterectomized rabbits (Nos. 5, 6, 15), nor in rabbits long ovariectomized (Nos. 8, 16). Shortly after ovariectomy a limited amount of conversion occurs (No. 7). This implies that the conversion takes place in the uterus, but that it will not take place in a uterus lacking ovarian control. Since in no instance have detectable amounts of hormone been obtained before injection (Table II) it seems safe to conclude that the excreted material is derived from the injected material unless one postulates that the injected estrogens stimulate the endogenous production of excretion products. Such stimulation is scarcely credible in view of the excretion by ovariectomized animals since the ovary is presumably the hormone precursor; secondly, one would have to assume further that estriol stimulates only estriol excretion and estrone the excretion of both in certain animals and not in others, a most unlikely situation.

The data demonstrate also that a two- to fourfold increase in excretion of injected estrone and estriol excretion products occurs in animals with corpora lutea in the ovaries (Nos. 2, 3, 4, 6, 12). This may occur if the presence of a corpus luteum (1) prevents destruction or (2) hastens excretion.

The injections of estrone monobenzoate were undertaken to test this point. That the benzoylation of the estrone prevents its destruction in the liver has been demonstrated by Zondek (1934) working with mice. The estrone benzoate-injected animals received 600 micrograms of the compound of which 433 micrograms were estrone. The data demonstrate again that the conversion to estriol occurs only in an animal with a functional uterus. The mean percentage of hormone recovered for the three animals of this group is 90.1 with the lowest value (78.7 per cent) for the ovariectomized animal (No. 16). This lowest value in the ovariectomized female would indicate that the corpus luteum may prevent destruction, since about 21 per cent of the protected molecules in this case disappeared in this animal compared with 6 per cent in the pseudopregnant female (No. 14), and 2 per cent in the hysterectomized pseudopregnant animal (No. 15).

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It will be noted, however, that the complete excretion of the estrone took place within 6 days in the pseudopregnant animal (No. 14) and in 8 days in the other two (Nos. 15 and 16). This would indicate more rapid excretion by the pseudopregnant animal. It seems possible, therefore, that the presence of a corpus luteum increases the excretion of injected hormone and thus prevents its destruction. This conclusion is not especially supported by the data on the "free" hormone injections, since excretion is ordinarily completed in 4 days in animals without as well as with corpora lutea, the sole difference seeming to reside in the absolute quantities of hormone excreted. Thus in the estriol-injected animals the mean quantity of hormone excreted during the second 48 hour period after injection is 10 micrograms per animal for the three animals without corpora lutea; the ratio of excreted hormone in the two groups is 1:4.6, whereas for the preceding 48 hour period it is 1:3.2. The corresponding quantities in the estrone-injected series are 7.5 and 58 micrograms for the second 48 hours and 53 and 98 micrograms for the first 48 hours with ratios of 1:7.7 and 1:1.8 respectively, indicating in both estrone and estriol recipients relatively more hormone excretion in the second 48 hour period, *e.g.*, therefore less destruction.

In Table II we present the data on a number of these extracts, comparing the hormone content as determined by various colorimetric tests and by bioassay. Since the David test is specific for estriol the presence of other materials giving a positive sulfanilic acid test will be revealed by the sulfanilic acid test, giving a higher titer than the David test. In five extracts (Nos. 1, 3, 10, 12, 18) the sulfanilic acid test gives a higher titer; in three (Nos. 2, 2, 11) the David test titer is higher. On the average the sulfanilic acid test gives a slightly higher titer. This may be due to (1) inactive chromogen, (2) small amounts of estrone in the estriol fraction, (3) small amounts of estradiol. The bioassays should reveal the presence of the latter since by our tests it is many times as active as estriol. The three estriol fractions (Nos. 2, 14, B_2) titered by bioassay indicate more hormone than either the sulfanilic acid or David tests reveal. If we deduce that there is estradiol present in these extracts the amounts of estradiol would be 6, 14, and 2 micrograms respectively.

In the case of the estrone fractions the sulfanilic acid titers and phenolsulfonic acid titers agree fairly well, except that the latter tend

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TABLE II

The Hormone Content of Various Rabbit Urine Extracts As Determined by Several Colorimetric Tests and by Bioassay

Rabbit No.	Extract* No.	By sulfanilic acid test	By phenolsulfonic acid test	By bioassay	By David test	
		micrograms	*micrograms*	*micrograms*		
1	5	0	0	—		
1	6	20	31	—		
2	4	0	0	—		
5	5	0	0	—		
6	4	0	0	—		
6	6	105	164	154		
7	4	0	0	—		
8	6	55	42	—		Estrone
10	4	0	0	—		
13	4	0	0	—		
14	6	130	—	150		
15	4	0	0	—		
16	9	55	50	—		
B_2†	—	0	—	0		
1	11	0	0	—	0	
1	12	37	—	—	25	
2	10	0	0	—	—	
2	12	100	—	167	120	
2	13	40	—	—	45	
3	10	0	0	—	—	
3	12	45	—	—	30	
5	11	0	0	—	—	
6	10	0	0	—	—	
7	10	0	0	—	—	Estriol
10	10	0	0	—	—	
10	12	110	—	—	105	
11	12	33	—	—	40	
12	12	125	—	—	105	
13	10	0	0	—	—	
14	12	126	—	233	—	
15	10	0	0	—	—	
B_1†	—	—	—	0	0	
B_2†	—	—	—	120	102	

* The extract No. refers to the column number of Table I, *e.g.* rabbit No. 1, extract No. 6, refers to the estrone determination made on rabbit No. 1 extract taken 1–2 days after estrone injection.

† These extracts are not included in the data of Table I; B_1 is the unheated extract from a rabbit receiving 300 micrograms of estrone, B_2 is from the same batch of urine heated according to our regular technique.

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to give slightly higher values. This is to be expected since the phenolsulfonic acid test reveals small amounts of inactive materials that act as chromogens. Comparison of colorimetric values with bioassay values reveals an excess of activity in one case (No. 14) and approximate equivalence in two others (Nos. 6 and B_2). If the excess is due to estradiol, No. 14 contains less than 4 micrograms of estradiol.

We have tested a number of these extracts for equilenin by the Girard test. The result is invariably negative. Therefore the rabbit produces no equilenin. Since equilenin in urine is invariably accompanied by equilin or hippulin (Fieser (1936)) we may deduce that these compounds are not formed.

The conversion of estrone to estriol that these experiments demonstrate occurs only in a functional uterus. Two possibilities seem evident: (1) the ovary produces an hitherto unidentified hormone which acts upon the uterus to effect the conversion, or (2) the corpus luteum hormone stimulates the uterus in such a way as to make this conversion possible. It can scarcely be estrogenic hormone since we would expect a larger conversion than observed from the estrus female (No. 1) and conversion in the ovariectomized animal No. 8 which received estrogen. The second alternative mentioned above is on the face of it rendered unlikely by the conversion observed in the estrus rabbit (No. 1), and in the recently ovariectomized rabbit (No. 7), unless we postulate that the estrus rabbit's ovary produces corpus luteum hormone in the absence of formed corpora lutea and that in the recently ovariectomized animal the effect of this luteal hormone on the uterus lasted for some days after ovariectomy.

To test these alternatives we performed the experiments the data of which are given in Table III. The five rabbits of these experiments were given a preliminary injection of 300 micrograms of estrone. The first 48 hour collection was made from the end of the 2nd to the end of the 4th day after this injection in order to determine the hormone excretion occurring just before a second injection of 300 micrograms of estrone given simultaneously with 0.5 mg. of progesterone[2] in oil. The first injection of estrone served also to prime the uterus of the ovariectomized females. In one ovariectomized rabbit (No. 21) the second injection contained no progesterone, so that it might

[2] We are indebted to Dr. Erwin Schwenk of the Schering Corporation for this progesterone (Proluton synthetic).

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TABLE III

The Effect of the Injection of Progesterone upon the Excretion of Urinary Estrin in Estrone-Injected Animals

300 micrograms of estrone were injected 48 hours before the collection of urine was begun, and 48 hours after the first collection an additional 300 micrograms of estrone were injected simultaneously with 0.5 mg. of progesterone.

Condition of rabbit	Estrone content in urine specimen				Estriol content in urine specimen				Total hormone excreted after injection		Total estrogen (estrone + estriol)	Increase of excretion over animal not receiving progestin
	1	2	3	4	1	2	3	4	Estrone	Estriol		
	micrograms	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*micrograms*	*per cent*
17 Estrus	20	35	25	0	0	85	0	0	70	85	135	75.3
18 Pseudopregnant	45	38	20	0	65	90	0	0	58	90	148	25.3*
19 Hysterectomized (unmated)	0	33	25	0	0	0	0	0	58	0	58	65.7
20 Ovariectomized (4 mos.)	25	20	15	12	0	45	0	0	47	45	92	95.7
21 Ovariectomized† (2 mos.)	15	47	0	0	0	0	0	0	47	0	47	14.5*

* Per cent decreased excretion.

† No progesterone in second injection.

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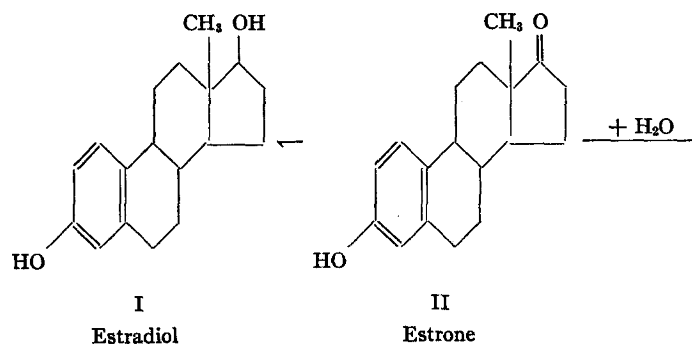
serve as a control for animal No. 20. Urine specimens Nos. 2, 3, and 4 in Table III represent urines wherein the effect of progesterone is evidenced. The estriol data of Table III were checked by the David test so that there can be no doubt that animal No. 20 did convert estrone to estriol whereas its control did not. This supports the notion that the conversion takes place in a uterus influenced by progesterone. The data on the hysterectomized rabbit (No. 19) in which no conversion occurred indicate again that the uterus is necessary.

In addition we see from these data that progesterone facilitates the excretion of additional estrogen. In the last column of Table III the increased percentage excretion is based upon the data of Table I. Thus animal No. 17 (Table III) excreted a total of 135 micrograms of estrin whereas animal No. 1 (Table I) excreted 77 micrograms, indicating an increase of 75.3 per cent in the former over the latter. The increase in the hysterectomized rabbit (No. 19) was 65.7 per cent, in the ovariectomized rabbit (No. 20) 95.7 per cent (over No. 21). The pseudopregnant female (No. 18) showed small decrease when comparison is made with the control rabbit (No. 5) but this is comprehensible when we consider that the initial estrone injection presumably inhibited or suppressed the effect of the animal's own corpus luteum secretion. The decrease in animal No. 21 (compared with No. 8) is within the limit of error of these determinations.

DISCUSSION

The data of these experiments support the scheme of estrogenic hormone conversion *in vivo* that follows:



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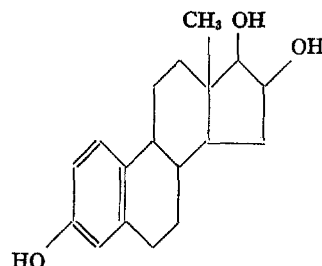

III
Estriol

The step from II to III is an irreversible hydration, and is the chief result of estrone injection. The step from II to I occurs presumably only in limited amount, if at all, or if this is an extensive conversion only small amounts of the diol are excreted. The former statement would seem most probable since the animals receiving estrone benzoate and excreting most of the injected hormone excrete chiefly estrone and estriol (see Nos. 14, 15, 16 especially, in Table II).

The conversion II to III has not been accomplished in the laboratory, although Butenandt and Hildebrandt (1931) have obtained estrone by the dehydration of estriol. The conversion II to I has been made by Schwenk and Hildebrandt (1933) and Girard, Sandulesco, and Fridenson (1933) by catalytic reduction. According to MacCorquodale, Thayer, and Doisy (1936) estradiol is the ovarian hormone and we would therefore expect I to II to occur more easily than the reverse since estrone is an excretion product, estradiol not.

These data leave no doubt that the conversion II to III requires a functional uterus, and that the action of progesterone on the uterus facilitates the reaction. That corpus luteum secretion normally promotes the conversion is somewhat indicated by the fact that estrone-injected animals with corpora lutea and uteri (Nos. 2, 3, 4) excreted more estriol (305 micrograms total) than estrone (188 micrograms total), whereas those in that group without corpora lutea (Nos. 1 and 7) excreted more estrone (85 micrograms total) than estriol (70 micrograms total). Animal No. 14, however, reverses the expected excretion on this basis. This may be due to the possibility that estrone benzoate has a low kidney threshold compared to the

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kidney threshold of the bound form of estrone excreted by estrone-injected animals or to a lesser reactivity of the molecule in the benzoate compound.

It should be clearly understood that the actual nature of the compound involved in the steps II to III or II to I is not known; *e.g.*, whether "free" or "bound" hormone is the reactant. Since we must employ a mild hydrolysis to obtain full recovery from the urine we know that bound hormone is excreted and to judge by analogy with human urines probably as a glycuronic acid compound (Cohen and Marrian (1936)). But the binding appears to be different than in the compounds in human urine since the rabbit urines require a much less drastic hydrolysis.

These experiments support the notion that corpus luteum hormone causes increased estrin excretion by preventing destruction of the hormones rather than by lowering the kidney threshold, and lend credence to the opinion of Smith and Smith (1936) that "there is no renal threshold for estrin." We are thus led to the apparently paradoxical situation in which one of two competing hormones protects its competitor. At the same time it should be noted that progesterone facilitates the conversion of estrone into the less active estriol. During early pregnancy, for example, when a high concentration of estrin might very well lead to abortion the sterilizing potency of estriol is about one-fourth to one-fifth that of estrone (Pincus and Kirsch (1936)). Furthermore, we do not know just what chemical form these hormones take *in vivo*, and it may well be possible that the protection afforded by progesterone action involves the production of an estrin compound which is physiologically inactive. Since the liver is the presumable site of estrin destruction, and the liver enzyme system presumably acts chiefly upon the phenolic portion of the estrin molecule (*i.e.*, benzoylation prevents destruction) progesterone might act to induce something like benzoylation of the phenolic ring. Alternatively (or perhaps at the same time) progesterone might replace estrin in the liver system due to a higher partition coefficient of the progesterone for the particular system involved. That progesterone may be more quickly destroyed than the estrins is indicated by the data of Table III which show no excretion of estriol in urine specimens 3 and 4 whereas the estrone continues to be excreted 48 to 96 hours after estriol excretion is completed. If the estriol excretion be taken

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as an index of the presence of active progesterone (particularly in animals Nos. 17 and 20), it would seem that the 0.5 microgram progesterone injected was more rapidly destroyed than the 0.3 microgram of estrone injected at the same time.

SUMMARY

1. A method is given for the extraction and fractionation of rabbit urines which frees these urines of inactive chromogens but permits a quantitative recovery of estrone and estriol for the colorimetric determination of these compounds.

2. Estrone and estriol content of rabbit urine extracts can be determined by the concentration of the colored compound they form upon diazotization with sulfanilic acid and by the modified phenolsulfonic acid test of Cohen and Marrian. Estriol can be determined by the specific reaction first described by David. The technique for these tests is presented.

3. Estriol (300 micrograms) injected into rabbits (*a*) in heat, (*b*) pregnant, (*c*) pseudopregnant, (*d*) hysterectomized in heat, (*e*) hysterectomized pseudopregnant, (*f*) ovariectomized, is excreted in the urine as estriol. Rabbit does in the luteal phase (*b*, *c*, and *e*) excrete 3 to 4 times the amount of estriol excreted by females without corpora lutea (*a*, *d*, and *f*).

4. When estrone (300 micrograms) is injected into the same types of rabbit does types *a*, *b*, and *c* excrete both estrone and estriol, type *f* excretes both estrone and estriol shortly after ovariectomy, but only estrone at 2 months after castration. Hysterectomized animals (types *d* and *e*) never excrete estriol after estrone injection. The total urinary estrin (estrone plus estriol) in estrone-injected animals is increased 2 to 3 times in animals in the luteal phase (*b*, *c*, and *e*).

5. It is concluded that the uterus is the site of conversion of estrone to estriol, and that the conversion cannot take place in a uterus completely free of ovarian control (*e.g.*, in long time ovariectomized animals).

6. In neither estrone-injected nor estriol-injected females is all the injected hormone recovered in the urine. The maximum recovery is 66 per cent. When estrone-benzoate (600 micrograms) is injected 94–98 per cent of the hormone is recovered from animals in the luteal phase (types *c* and *e*) and about 79 per cent in an ovariectomized

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female (type *f*). These data are taken to indicate that luteal secretions give partial protection against destruction to the hormones.

7. The observation that in certain of the urine extracts the hormone titer by bioassay is somewhat higher than the colorimetric titer may indicate that there is a slight conversion of estrone to estradiol, particularly since no equilenin was found in any of the extracts by colorimetric test.

8. The simultaneous injection of 300 micrograms of estrone and 500 micrograms of progesterone 4 days after an initial injection of 300 micrograms of estrone results in: (1) an increased estrin excretion in females in heat, hysterectomized unmated, and ovariectomized, and a slight decrease in the pseudopregnant female; (2) the appearance of estriol in the urine of the long time ovariectomized animal with no urinary estriol in a control ovariectomized animal receiving no progesterone. These findings are taken to prove that the conversion of estrone to estriol occurs in the uterus under the influence of progesterone. Since animals in heat produce small amounts of estriol after estrone injection it is inferred that the ovaries of estrus rabbits produce small amounts of corpus luteum hormone in the absence of formed corpora lutea.

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