hence without thymidine uptake but this is unlikely in the case of human syncytiotrophoblast in view of the near-diploid DNA valves found by Galton(1) in these nuclei using microspectrophotometry.

On the basis of these experiments it now seems likely that the syncytiotrophoblast is a mitotic end stage that is continually derived from another cell type, possibly cytotrophoblast. Experiments are under way to investigate this possibility and to extend our findings to other species and other experimental conditions.

Summary. Portions of human placental tissue have been studied by radioautography utilizing tritiated thymidine. Thymidine uptake could be demonstrated in cytotrophoblast, villous mesenchyme and vascular endothelium but not in the syncytiotrophoblast. This finding suggests that amitotic division of the syncytiotrophoblast does not play a prominent role in the derivation of this cellular layer in the human placenta of 14-18 weeks gestational age.

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Effects of Time and Steroid Concentration on Binding of C^{14}-Estrogens in Human Plasma.* (26491)

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We have reported(1-3) on the distribution of radioactive steroids and their metabolites among the various plasma protein fractions following intravenous injection of C^{14}-steroids into human subjects. Thus, it was shown that most unconjugated and conjugated steroids and their metabolites were bound principally to the plasma fractions IV-1 and V. In the case of C^{14}-estrone and C^{14}-estradiol the bloods were drawn 20 and 45 minutes after injection, respectively. This study deals with the effects of time of blood withrawal and prior administration of large amounts of non-radioactive steroids, either identical to or different from the radioactive steroids, on distribution of radioactivity among the fractionated plasma proteins.

Experimental. The materials, methods and experimental procedures were similar to those previously reported(1,2) with the following exceptions: 1) *ca.* 250 ml of blood was withdrawn 30 minutes after injection and an equal volume 90-150 minutes later and 2) two subjects, in addition to receiving radioactive steroid, were infused a second time with the same radioactive steroid plus 50 mg of non-radioactive steroid. The solution for infusion was prepared by dissolving 50 mg of crystalline U.S.P. steroid in 10 ml of ethanol, which was added dropwise with stirring to 240 ml of 1% HSA (human serum albumin) resulting in the appearance of a very fine microcrystalline suspension of the steroid. This solution was infused intravenously over 30 minutes. The C^{14}-steroid (2.7 μc/mg) dissolved in 50 ml of 4% ethanol in saline, was injected intravenously over a period of 2-3 minutes through the tubing halfway through the infusion. The timing commenced with injection of the radioactive steroid.

Before discussing the results, it may be worthwhile to comment on the state of the carrier and radioactive steroids injected in

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TABLE I. Distribution of Bound and Unbound Radioactivity in Human Plasma after Injection of Ca 1 μc of C^{14}-labeled Steroids with and without Unlabeled Steroid.

Steroid inj.	Phlebotomy time (min.)	Radioactivity	Bound (fractions I-V) Total	Conjugated	Unconjugated	Unbound (supernatant fluid V) Total	Conjugated	Unconjugated
16-C^{14}-estrone (M.F.)	30	c/m/100 ml	7140	4370	2770	7470	4400	3070
		%	49	61*	39*	51	59*	41*
	180	c/m/100 ml	5530	5260	270	4980	4530	450
		%	53	95	5	47	91	9
16-C^{14}-estrone + 50 mg progesterone (M.F.)	30	c/m/100 ml	5870	3920	2050	5650	3000	2650
		%	51	65	35	49	53	47
	180	c/m/100 ml	4750	4440	310	5160	4680	480
		%	48	93	7	52	91	9
16-C^{14}-estradiol-17β (R.C.)	30	c/m/100 ml	4820	4130	690	7330	6340	1090
		%	40	86	14	60	85	15
	120	c/m/100 ml	3390	3280	110	3780	3580	200
		%	48	97	3	52	95	5
16-C^{14}-estradiol-17β (J.J.)	30	c/m/100 ml	5150	3900	1250	4720	3170	1550
		%	52	76	24	48	67	33
	120	c/m/100 ml	2510	2250	260	2830	2490	340
		%	47	90	10	53	88	12
16-C^{14}-estradiol-17β + 50 mg estradiol-17β (J.J.)	30	c/m/100 ml	3630	2460	1170	2650	1670	980
		%	58	69	32	42	65	35
	120	c/m/100 ml	710	600	110	700	510	190
		%	50	85	15	50	73	27

* Percentages are calculated by using the value in "Total" column as 100%.

the 1% HSA (in 4% ethanol in saline). From previously published results(1) and from unpublished data in our laboratory it can be calculated that a maximum of 10 mg of estradiol-17β and 5 mg of progesterone, out of the 50 mg administered, were bound by the administered HSA. Hence, when the C^{14}-labeled steroid was injected half-way through the infusion, the preponderant part of the radioactive steroid was not associated with the HSA, but was in the aqueous phase. Since the amount of HSA administered over a period of 30 minutes represents about 1/50 of total plasma albumin, it can be safely presumed that the binding of most of the carrier and radioactive steroids occurred to endogenous plasma protein.

Results and discussion. The distribution of protein bound radioactive C^{14}-estrone or C^{14}-estradiol and their metabolites is shown in Table I and is very similar to our previous results(1). Percentage of radioactivity bound and unbound was not materially affected by time of blood withdrawal, even as late as 3 hours after injection of the radioactive steroid. In all cases the percentage of radioactive conjugated metabolites increased with time. Since previous *in vitro* work(1,3) indicated that the binding site (or sites), at least on albumin, for progesterone was different from that for C^{14}-estrone, 50 mg of the former steroid were given by infusion before injection of C^{14}-estrone to ascertain whether progesterone would affect the metabolism and distribution of the C^{14}-estrone *in vivo*. Prior injection of the large amount of unlabeled progesterone produced no pronounced changes in the binding of radioactive C^{14}-estrone and its metabolites when compared to that following injection of C^{14}-estrone alone.

Injection of a large amount of estradiol resulted in more rapid disappearance of conjugated, radioactive metabolites of C^{14}-estradiol in both the bound and unbound fractions. Since very little difference was seen in concentration of either bound or unbound unconjugated radioactivity following administration of 50 mg of estradiol and C^{14}-estradiol, the more rapid clearance of the conjugates of these steroids may be related to the saturation of the protein system responsible for the binding of such conjugates. This

TABLE II. Distribution of Radioactivity Bound to Human Plasma Fractions after Injection of Ca 1 μc of C^{14}-labeled Steroids with and without Unlabeled Steroid.

Steroid inj.	Phlebotomy time (min.)	Radioactivity	Conjugated Plasma fraction No. I	II+ III	IV-1	IV-4	V	Unconjugated Plasma fraction No. I	II+ III	IV-1	IV-4	V
16-C^{14}-estrone (M.F.)	30	c/m/100 ml	34	370	140	3830		91	510	450	1720	
		%	1	8	3	88		4	18	16	62	
	180	c/m/100 ml	72	380	370	460	3980	5	55	26	15	169
		%	1	7	7	9	76	2	20	10	5	63
16-C^{14}-estrone + 50 mg progesterone (M.F.)	30	c/m/100 ml	110	710	320	150	2530	38	310	360	66	1280
		%	3	19	8	4	66	2	15	18	3	62
	180	c/m/100 ml	130	210	720	87	3290	22	39	44	3	202
		%	3	5	16	2	74	7	13	14	1	65
16-C^{14}-estradiol-17β (R.C.)	30	c/m/100 ml	130	300	200	160	3340	65	140	76	19	390
		%	3	7	5	4	81	9	20	11	3	57
	120	c/m/100 ml	76	240	160	130	2670	5	23	13	8	56
		%	2	7	5	4	82	5	21	12	7	51
16-C^{14}-estradiol-17β (J.J.)	30	c/m/100 ml	43	180	690	580	2400	91	180	180	56	740
		%	1	5	18	15	61	8	14	14	5	59
	120	c/m/100 ml	64	120	89	140	1840	11	40	17	12	175
		%	3	5	4	6	82	4	16	7	5	68
16-C^{14}-estradiol-17β (J.J.)	30	c/m/100 ml	47	210	480	74	1650	37	180	210	21	821
		%	2	9	19	3	67	3	15	18	2	62
	120	c/m/100 ml	16	75	83	15	410	7	8	25	3	68
		%	3	12	14	3	68	6	7	22	3	62

Percentages represent breakdown of the total radioactivity of Fractions I to V.

would result in more rapid clearance of the conjugates from the plasma and was evidenced by the more rapid excretion of radioactivity in the urine when large amounts of carrier were given in addition to C^{14}-estradiol (4). It would also seem to indicate that the binding system for the unconjugated metabolites was not saturated by the large amount of estradiol given. On the basis of previously published data(1) in the case of estradiol it would require 5-6 times (250-300 mg or more) of the amount of estradiol injected in the experiments reported to saturate the binding capacity of the serum albumin. Since the injected steroid is metabolized rather rapidly, it would require a relatively enormous amount of estradiol to saturate the protein binding system for this particular steroid.

Distribution of the bound radioactivity among plasma fractions is shown in Table II. The preponderant part (over 60% of the unconjugated steroid, over 70% of the conjugated steroids) of the radioactivity was in Fraction V and did not vary much with the phlebotomy time. Prior administration of unlabeled steroid produced no pronounced changes in distribution of the radioactive steroids among the various plasma fractions. Mild increases in the amount of radioactivity associated with Fraction IV-1 at the longer phlebotomy time can be seen in 3 instances, when compared to similar circumstances without administration of unlabeled compound.

In regard to the concentration of "unbound" radioactivity in plasma (Table I), one should keep in mind that under the conditions of the fractionation techniques employed in these studies loosely protein-bound C^{14}-estrogens might be dissociated from the protein. At least 3 factors could influence the binding: ionic strength, pH, and ethanol concentration. The conditions for separation of Fraction I, II, III and IV-1 are moderate. The conditions for separation of Fractions IV-4 (40% ethanol, pH 5.8) and V (40% ethanol, pH 4.7) might cause real concern. As we have stated(1,2), "the major error in interpretation which results from this effect of the fractionating technique has been the suggestion that an appreciable portion of infused steroid may circulate in plasma un-

bound to protein." The importance of Fraction V (albumin) in the binding and transport of estrogens in human plasma has been demonstrated(1,3,5,6). The results of these studies point to albumin as the major protein concerned with transport of estrogens and their metabolites in human plasma.

Summary. 16-C^{14}-estrone and 16-C^{14}-estradiol-17β were injected intravenously into normal human subjects. C^{14}-estrogens were injected either alone or following infusion of 50 mg unlabeled progesterone or estradiol-17β. Blood samples were drawn at 30 minutes and at 120 or 180 minutes after injection. The blood plasma was fractionated by the cold ethanol fractionation techniques and plasma fractions were examined for radioactivity. The delay of time of phlebotomy resulted in an increase in percentage of conjugated metabolites. Injection of a large amount of estradiol-17β resulted in a more rapid disappearance of conjugated radioactive metabolites of C^{14}-estradiol-17β in both protein-bound and unbound forms. No pronounced changes in distribution of radioactivity among the various plasma fractions occurred as a result of prior administration of large amounts of unlabeled steroid.

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Evidence for a Central Mechanism in Angiotensin Induced Hypertension.* (26492)

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Ever since Goldblatt(1) successfully produced sustained hypertension in dogs by partially constricting both renal arteries, investigators have been trying to isolate pressor substances from the blood of hypertensive animals and patients. Angiotensin II(2), a pressor octapeptide has been isolated in renal hypertensives(3) and has been synthesized by Bumpus *et al.*(4,5). Most investigators (4-6) have felt that the hypertensive properties of Angiotensin II were due entirely to the peripheral actions of the compound and have inferred that this agent did not act through the central nervous system. The purpose of this study was to determine if Angiotensin II possessed central hypertensive properties, and if so determine the possible mechanism of action.

Method. Six dog cross circulation preparations, modified after that described by Taylor and Page(7), were utilized in this study. Mongrel dogs were anesthetized by an intravenous injection of 35 mg/kg of pentobarbital sodium. The external jugular veins and common carotid arteries were isolated and covered with gauze moistened in normal saline solution. The internal jugular veins were doubly ligated and cut between the ligatures. The neck musculature was separated into sheets and removed, utilizing electrocautery, to expose the vertebral column from C-2 to C-5. A dorsal laminectomy, which included removal of the spinous processes of the more

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