Summary

The relationship between the weight of target organs (ovary and uterus) and the uptake of radiophosphorus under the influence of gonadotropin was examined and the response was expressed as the counts per organ reduced by the analysis of covariance with the organ weight. Periodical change of radioactivity of the target organs was traced after the administration of radiophosphorus. The phosphorus-accumulating activity of the target organs after the administration of gonadotropin was studied and the maxima of those of ovaries and uterus were found respectively at the 72nd and 96th hour.

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60. Bun-ichi Tamaoki: Studies on Sexual Hormones. X.[1] Hormonal Influence on the Uptake of Radiophosphorus by Endocrine Organs. (2).

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In the preceding paper,[1] it was shown that the uptake of radiophosphorus at the target organs was accelerated by the administration of gonadotropin. Fleischmann and Fleischmann[2] had analysed the phosphates in the seminal vesicle of castrated rats treated with or without testosterone propionate, and reported that mitotic activities of the target organs could be explained by the ratio of RNA-P to DNA-P. As a preliminary study, the analysis of phosphate in ovaries and uterus was studied and the results were compared between the gonadotropin-treated group and the control.

Endocrinologically, one of the factors which promoted the mitosis of uterus, accordingly its weight, and the uptake of radiophosphorus could be progesterone (gestogen), when pretreated with estrogen. In this paper, the influence of progesterone upon the uptake is described. On the basis of fundamental results shown in the preceding paper,[1] this method of using radiophosphorus was applied to Aschheim-Zondek test of pregnancy diagnosis in order to examine the relationship between the uptake of radiophosphorus and other types of response. The experimental methods and material were almost the same as in the preceding paper.[1]

Experimental Results

Biochemical Analysis of Radiophosphates in the Target Organs—As a preliminary analysis of the phosphate in the organs, the method of Schmitt and Thannhauser[3] modified by Friedkin and Lehninger[4] was employed for the nucleic acid analysis. In this experiment, immature female albino rats (body weight, 30~40 g.) were used and 50 I.U. of gonadotropin was subcutaneously administered to each rat in the treated group and normal saline to the control. Then, 48 hrs. later, 151 μc per head of radiophosphorus was given (72 hrs. after the administration of saline or gonadotropin solution), the animals were sacrificed, and their ovaries and uterus were biochemically analysed as shown in Table I.

As shown by Table I, the injection of gonadotropin caused weight increase and promotion of the mitotic activities of ovaries, and secondarily of uterus, which could be suggested by the decreased ratio of RNA-P fraction to DNA-P.

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1) Part IX: B. Tamaoki: This Bulletin, **5**, 325(1957).
2) W. Fleischmann, S. Fleischmann: J. Mt. Sinai Hosp. N. Y., **19**, 228(1952).
3) G. Schmitt, S. J. Thannhauser: J. Biol. Chem., **161**, 83(1945).
4) M. Friedkin, A. L. Lehninger: *Ibid.*, **177**, 775(1949).

Table I. Result of Nucleic Acids Analysis

Organ	Treatment	No. of animals	Organ wt. (mg.)	Radioactivities in Cts*: Acid-sol. fraction	Phopholipid fraction	Phospho-protein fract.	Total nucleic acid fraction	RNA-P / DNA-P
Ovarium	Gonadotropin	5	84.0	7109	3611	75	648	3.93
	Control	6	10.7	956	365	7	76	40.07
Uterus	Gonadotropin	5	102.4	6595	3470	77	512	4.30
	Control	6	18.8	543	141	25	36	45.82

* Counts for 3 mins.

Influence of Progesterone upon the Weight and Uptake of Radiophosphorus of the Uterus activated by Estrogen—In order to demonstrate the influence of gestogen (progesterone) upon the estrogen-treated uterus, the following experiments were carried out. In the first experiment, the castrated immature female rats were divided into three groups, such as PE, E, and C. Ten μg. of estradiol was administered to each animal in PE and E groups and 72 hrs. later, 0.5 mg. of progesterone was subcutaneously given to each rat in the PE group, while the animals in the C group received only the solvents. Then, 24 hrs. later, all animals received radiophosphorus, 59 μc a head, and 4 hrs. later, animals were sacrificed. The organ weight and uptake of radiophosphorus were measured as shown in Table II. The radioactivity of the organs was measured by G-M counter, after treating them with conc. HNO_3 and then HCl, and drying.

Table II. Effect of Progesterone upon Estrogen-pretreated Uterus

Animal No.	Treatment: C y	C x	E y	E x	PE y	PE x
1	32	182	92	310	151	538
2	57	179	56	233	144	609
3	29	142	101	281	87	484
4			52	223	104	610
5					102	557
Total	118	503	301	1047	588	2798

Table III. Analysis of Variance and Covariance of Table II

Factor	S_{x^2}	S_{y^2}	C_{xy}	Δ	f'
Treatment	348539	11936	62747		2
Error	17374	5505	5369	12138	8
Total	365913	17441	68116	99885	10

Δ Treatment=87747, $F_0=28.92 (p<0.01)$

Table IV. Means Reduced by Covariance

Treatment	y	$y-\bar{y}$	$b_E(y-\bar{y})$	x	$x-b_E(y-\bar{y})$
C	39	−45	−43	168	211
E	75	−9	−9	262	271
PE	118	34	+33	560	527

$b_E=0.975$

As the Table IV shows, even after the analysis of covariance, the uptake of radiophosphorus in the PE group was remarkably increased in comparison to the uptake either in the P or C group. However, 48 hrs. after the administration of progesterone, the uterine uptake of phosphorus reduced by covariance could not be found significant, although both the weight of the uterus and counts per organ themselves showed highly significant difference among those three treatments.

Application of this Method to Aschheim-Zondek Test—As the radioactivity of the ovary seemed to be relatively influenced by variation in its weight by 72 hrs. after the administration of gonadotropin, the relationship between the uptake of radiophosphorus and the weight was examined at the 24th and 48th hr. The mice received subcutaneously 0.5 cc. of the specimen a head, and 24 hrs. later, the weight and radioactivity of the ovaries were examined. In Tables V, VII, and X, x and y respectively denotes $10\times\sqrt{\text{cts. per 3 mins.}}$ and $10\times$(weight in mg.).

Here, the ovarian uptake of radiophosphorus at the 24th hr. could be considered as significant, even after the reduction by covariance with its weights. The above statistical procedures were also applied to the analysis of results on the ovarian response at the 48th hr. and the uterine responses at the 24th and 48th hr. after the administration of pregnancy and non-pregnancy

Table V. Application of Radioisotope to Aschheim-Zondek Test (1)

	Treatment			
	Pregnancy		Non-pregnancy	
	y	x	y	x
	56	469	40	419
	52	480	40	382
	51	482	36	396
	41	431	39	400
	47	431	47	349
	68	528	38	368
Total	315	2821	240	2314

Table VI. Analysis of Covariance of Table V

Factor	S_{x^2}	S_{y^2}	C_{xy}	Δ	f'
Treatment	21421	469	3169		1
Error	9744	487	1258	6494	9
Total	31165	956	4427	10665	10

Δ Treatment = 4171, $F_0 = 5.78$ ($P < 0.05$)

urine, and the ovarian response at the 24th hr. was found to be the most suitable among them. This result would suggest that Aschheim-Zondek test could be shortened by the use of radiophosphorus. Next, the pregnancy and non-pregnancy urine specimens obtained from gynecologists after clinical diagnosis were confirmed were examined.

Table VII. Application of Radiophosphorus to Aschheim-Zondek Test (2)

	Non-pregnancy urine								Pregnancy urine							
	A		B		C		D		E		F		G		H	
	y	x	y	x	y	x	y	x	y	x	y	x	y	x	y	x
1	40	127	33	144	32	144	27	133	55	225	47	212	49	217	78	260
2	47	155	47	166	32	121	33	144	44	211	72	198	49	226	54	194
3	43	146	40	148	39	199	33	173	50	223	55	188	52	220	50	218
4	41	123	41	152	31	144	32	145	48	166	38	142	40	192	62	236
5	39	149	38	146	41	169	35	132	53	200	35	156	52	219	47	217
6	40	158	43	155	36	130	39	164	64	255	33	146	52	219	45	204
7	36	130	21	106	48	177	37	140	71	257	32	145	52	220	59	244
8	41	127	49	175	45	156	48	174	47	206	46	154	47	207	62	261
9	39	160	28	133	38	155	42	150	46	223	39	170	59	209	43	189
Total	366	1275	340	1325	342	1395	326	1355	478	1966	397	1511	452	1929	500	2023

Table VIII. Analysis of Covariance of Table VII

Factor	S_{x^2}	S_{y^2}	C_{xy}	Δ	f'
Treatment	79144	3599	16200		7
Error	29724	7177	8262	20213	63
Total	108868	10776	24462	53338	70

Δ Treatment = 33125, $F_0 = 14.75$ ($p < 0.01$)

Table IX. The Means reduced by Covariance

Treatment	y	$y-\bar{y}$	$b_E(v-\bar{y})$	x	$x-b_E(y-\bar{y})$
A	41	−3	−3	142	145
B	38	−6	−7	147	154
C	38	−6	−7	155	162
D	36	−8	−9	151	160
E	53	9	10	218	208
F	44	0	0	168	168
G	50	6	7	214	207
H	55	11	13	225	212

$b_E = 1.151$

As Tables VIII and IX show, the F group (pregnancy urine at term) could not be found significantly different from the 4 groups treated with non-pregnancy urine, but the E, G, and H

(5th, 6th, and 5th month pregnancy urine, respectively) were significantly different. Furthermore, to confirm this finding, a similar type of the experiment was performed after reducing the number of control group, as the previous experiment showed small variation among the control groups. The animals were treated with the same procedure as before.

TABLE X. Application of Radiophosphorus to Aschheim-Zondek Test (3)

Animal No.	Pregnancy urine H y	H x	I y	I x	J y	J x	Non-pregnancy urine K y	K x
1	71	215	68	205	22	101	21	113
2	16	111	57	222	17	122	16	98
3	22	128	30	137	35	153	24	99
4	37	186	30	182	27	139	22	97
5	84	241	25	140	25	151	22	98
6	17	109	51	182	43	130	13	77
7	14	109	14	117	27	125	15	76
8	25	128	52	226	47	168	20	110
Total	286	1227	327	1411	243	1089	153	768

TABLE XI. Analysis of Covariance of TABLE X

Factor	S_{x^2}	S_{y^2}	C_{xy}	Δ	f'
Treatment	27617	2083	7573		3
Error	35887	8391	15580	6959	27
Total	63504	10474	23153	12324	30

Δ Treatment$=5365$, $F_0=6.94(p<0.01)$

TABLE XII. The Means reduced by Covariance

Treatment	y	$y-\bar{y}$	$b_E(y-\bar{y})$	x	$x-b_E(y-\bar{y})$
H	36	4	7	153	146
I	41	9	16	176	160
J	30	−2	−4	136	140
K	19	−13	−24	96	120

$b_E=1.857$

As Tables XI and XII show, the pregnancy urine accumulated more radiophosphorus in the ovary than the non-pregnancy urine, even after the analysis of covariance.

Discussion

Referring to Caspersson's work[5] on the interrelationship between DNA synthesis and mitosis, the great increase in radioactivity of DNA-P due to the treatment of gonadotropin is of interest. The ratio of RNA-P to DNA-P of seminal vesicles was found[1] to be 3.97, 3.77, and 3.74 in the testosterone-treated group, while 63.5 and 37.5 in the control, and those were comparable with the experimental result in this paper. From the relationship between this ratio and the mitotic activity, the low ratio found in the gonadotropin-treated group suggests active mitosis, while the high ratio in the control means low mitosis of the resting organs of immature rats.

The administration of progesterone to the estrogen-pretreated castrated animal induced a remarkable increase of radiophosphorus uptake in comparison to the estrogen singly-treated group or the control. This suggests that, after the administration of gonadotropin (FSH and LH) to the immature female animal, the peak of radiophosphorus uptake could be expected first by estrogen and secondarily by gestogen secreted from the ovary.

Previously, it had been found by the author that the uptake of radiophosphorus per ovary and the ovarian weight were increased 72 hrs. after the administration of pregnancy urine, but not by non-pregnancy urine, and this result agreed with the diagnosis

5) T. O. Caspersson: "Cell Growth and Cell Function," W. W. Norton Co., New York (1950).

of Aschheim–Zondek test such as the existence of corpus leuteum, blood spots, and white spots in the enlarged follicles.* However, whether the response was expressed as the counts per mg. of ovarian weight, or as the counts reduced by the analysis of covariance, the difference between these responses of pregnancy urine–treated group and non–pregnancy urine–treated could not be found as being significant.

As the previous experience shows, the uptake of phosphorus was presumably accelerated prior to the peak of mitosis, or before the weight of the target organ increases significantly in comparison to the control. Therefore, this result seemed to the author very important in the following respects. 1) The histological change of ovarian function caused by the administration of pregnancy urine was accompanied by the increase of radiophosphorus uptake as well as the increase of ovarian weight. 2) Qualitative, or all–or–none type response of ovary could be changed to quantitative one, that would increase the statistical reliability and also economize the number of animals used.

The application of radiophosphorus to Aschheim–Zondek test would be fruitful as far as shortening of the assay period is concerned, because the routine assay requires 5 days. As the weak points of this method, the following could be mentioned. Radioisotope technique, instruments, and statistical analysis necessary, and the control group with non–pregnancy urine were always needed. For the compensation, the diagnosis would be objectively determined by this test, within 48 hrs. with the level of its reliability. Therefore, this would be very useful, when a rapid diagnosis is urgently desired.

On the contrary, Aschheim–Zondek test as well as Friedman test for pregnancy were routinely used, but, as the response was all–or–none type, the reliability of diagnosis calculated from the result itself was not so high as the one calculated from the graded response obtained by this radioisotope method.

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Summary

The ratio RNA–P/DNA–P was compared between the gonadotropin–treated group and the control, and the former showed a low ratio, suggesting a great mitotic activity while the latter showed a high ratio or low activity. Progesterone was found to be a hormone which accelerated the accumulation of the phosphorus at the estrogen–pretreated uterus. The radioisotope technique was applied to Aschheim–Zondek test and could shorten the assay period with a level of significance.

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