of $CHCl_3$ and a mixture of this $CHCl_3$ solution and heavy water in 1:1 ratio was shaken thoroughly. $CHCl_3$ was evaporated under a reduced pressuse. The heavy water used was the product of Norsk Hydro (99.77%).

Summary

1. Pseudo-dihydrothiamine was found to possess hydroxyl and imino groups from the infrared absorption spectrum.

2. Normal-, iso-, and pseudo-dihydrothiamines give the same benzoyl-pseudo-dihydrothiamine.

3. It was concluded from foregoing results that pseudo-dihydrothiamine possessed a structure entirely different from that of normal- and iso-dihydrothiamine.

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

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Growth of ovarium is controlled mainly by the internal secretion of pituitary gland and growth of the uterus is controlled by the hormones secreted from thus stimulated ovarium. Weight increase of ovary and uterus of intact immature rat or mouse has been used as the most reliable response metameters for the bioassay of gonadotropin[2] and estrogen.[3] The weight increase caused by the hormone suggests mitosis of the target organ which could be used as a possible index for hormonal activity. It has been reported that the uptake of radiophosphorus at the target organ increased in accordance with the growth of organs and by the administration of hormones such as estrogen,[4] androgen,[5] and gonadotropin.[6] In this paper, a relationship between the weight increase of organs and the uptake of radiophosphorus is discussed.

Experimental Methods and Materials

1) Experimental Animals—Immature female mice (*DD* strain), weighing 6.0~8.0 g., were raised at room temperature and sampled at random from the colony.

2) Colony Diet—The animals received a cooked mixture of wheat and dried fish meat *ad libitum*.

3) Hormone and Radiophosphorus—As gonadotropin, pregnant mare serum preparation (PMS) (Antex by Leo) was used after dissolving it in normal saline solution. Carrier-free ^{32}P in the chemical form $H_3{}^{32}PO_4$ was diluted with 0.0002*M* K_2HPO_4 solution to certain concentrations after heating in a boiling water bath for 1 hr. Both were administered subcutaneously on the back of mice.

4) Measurement of Radioactivity—Radioactivity of the organ was measured for 3 mins. by the Geiger-Müller Counter at the same geometrical position.

* Hongo, Tokyo (玉置文一).

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Experimental Results

Qualitative Assay of Gonadotropin by the Uptake of Radiophosphorus—The immature female mice were divided at random into four groups which received single injection of normal saline only (control), and the saline solution of gonadotropin 4, 8, and 16 I.U. per head, respectively. At the 72nd hr. after the injection, the animals received 1.4 μc per head of radiophosphorus. Thereafter, the animals were sacrificed, ovaries and uterus were isolated, and their weight and radioactivity were measured as shown in Table I.

Table I. Relationship between the Dosage of Gonadotropin and Responses

P.M.S.(I.U.)		0	4	8	16
Ovarium	Cts	186	245	261	322
	Wt (mg.)	2.7	6.3	4.8	8.2
Uterus	Cts	128	325	343	321
	Wt (mg.)	5.2	26.1	28.1	31.6

* Cts in this paper were expressed in (the square root of the counts for 3 mins.) multiplied by 10, in order to normalize its Poisson distribution.

Ovaries—After the analysis of variance, difference among the treatments was found to be significant ($P=0.05$), but a further analysis showed that the difference of the group treated with 16 I.U. of gonadotropin from the control was significant, but that of the others from control was not. By analysis of covariance for reducing the effect of the weight of organ upon the uptake, the groups treated with 4, 8, and 16 I.U. of gonadotropin were found significantly different from the control. Therefore, by this method, 4 I.U. of gonadotropin at least could be detected by the analysis of covariance.

Uterus—The significant difference of the treated groups from the control was found either in the uptake of ^{32}P or in the weight, and that was further confirmed by the analysis of covariance.

Relationship between Dosage of Gonadotropin and Uptake of Radiophosphorus by Ovaries and Uterus—Besides the control group, the four groups were respectively treated with 1, 2, 4, and

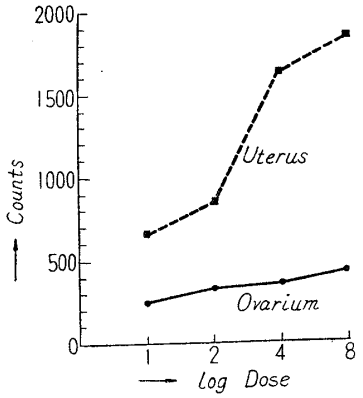

Fig. 1.
Relationship between Dosage of Gonadotropin and Uptake of Radiophosphorus

Table II. Uptake of Radiophosphorus and Dosage of Gonadotropin (Cts.)

Animal No.	P.M.S.(I.U.) 1	2	4	8
1	549	1197	2005	1403
2	937	721	1153	2054
3	706	758	1700	2123
4	508	743	1658	2025
5	602	851	1681	1604
6			1727	
Mean	660	854	1654	1842

Table III. Analysis of Variance of Table II

Factor	*SS*	*f*	*V*	F_0
Regression	4839583	1	4839583	
Residue	403212	2	201606	3.22 (N.S.)
Treatment	5242795	3	1747598	27.93**
Error	1063668	17	62569	
Total	6306463	20		

Table IV. Summarized Result of Regression Analysis

Response	Organ.	F_0^1	F_0^2	F_0^3	s/b
Organ Weight	Ovarium	2.39	<1	<1	12.07
	Uterus	61.67**	<1	5.03*	2.63
Uptake of ^{32}P	Ovarium	40.67**	<1	2.31	3.86
	Uterus	24.01**	3.22 (N.S.)	27.93**	1.14

$F_0^1 = V_{Reg}/V_{Res}$, $F_0^2 = V_{Res}/V_{Error}$, $F_0^3 = V_{Treat}/V_{Error}$

8 I.U. of gonadotropin, and then the weight and radioactivity were measured by the same procedure, 72 hrs. after the administration of gonadotropin (Fig. 1, Tables II, III, and IV).

The analysis of regression on the uptake of radiophosphorus by ovaries and uterus to the dosage of gonadotropin respectively shows that there was no evidence to deny the linearity of the dose-response curve (Table III and IV). In this case, it was found that this method was valid for qualitative assay, since the significant difference of the reduced uptakes of radiophosphorus was found after the analysis of covariance between the control and the group treated with more than 2 I.U. In order to confirm the linearities of these curves, the above scheme of the experiments were repeated to obtain the same conclusion.

Relationship between Uptake of Radiophosphorus by the Activated Target Organs and the Period after the Administration of Radiophosphorus—All the animals received 10 I.U. of gonadotropin and 72 hrs. later, 1 μc of radiophosphorus per head was given. Then the periodical changes of radioactivities taken up by ovaries and uterus were examined.

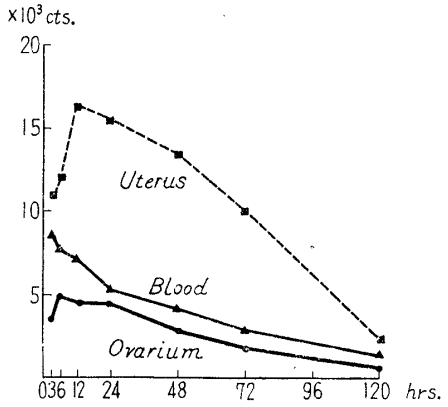

Fig. 2. Relationship between the Uptake of Radiophosphorus and the Period after Administration of Radiophosphorus

Radioactivity of ovaries and uterus was expressed as an averaged response (counts for 3 mins.) of 8 animals in each group. Radioactivity of the blood was also expressed as averaged counts for 3 mins. in 0.4 cc. of the blood.

In the previous experiments, the animals were sacrificed 4 hrs. after the administration of radiophosphorus, but the present experiment shows that there was no significant difference among the uptake of radiophosphorus at the 6th, 12th, and 24th hr., while the radioactivity decreased at the 48th hr. and thereafter.

Relationship between Uptake of Radiophosphorus by the Activated Target Organs and the Period after Administration of Gonadotropin—The immature female mice received 10 I.U. per head of gonadotropin and then 5.4 μc per head of radiophosphorus was administered 4 hrs. prior to death, and sacrificed at the 24th, 48th, 72nd, 96th, 120th, 144th, and 168th hr. after the administration of gonadotropin (Fig. 3, 4, and 5; Tables V and VI).

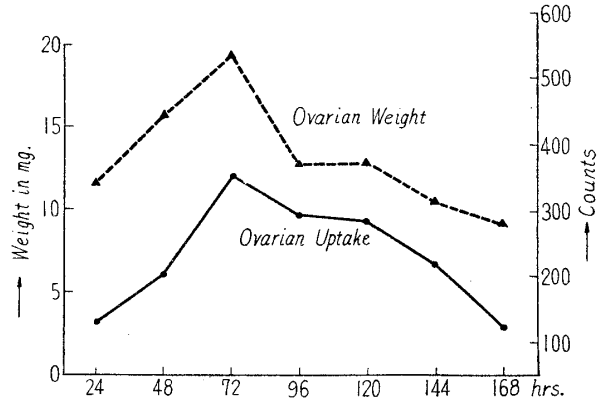

Fig. 3. Change of Radiophosphorus Uptake and Weight of Ovaries

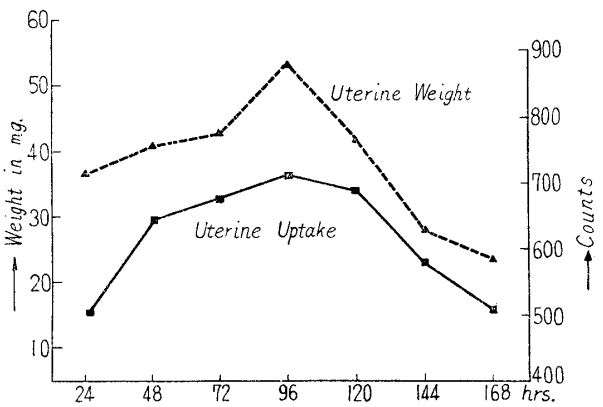

Fig. 4. Change of Radiophosphorus Uptake and Weight of Uterus

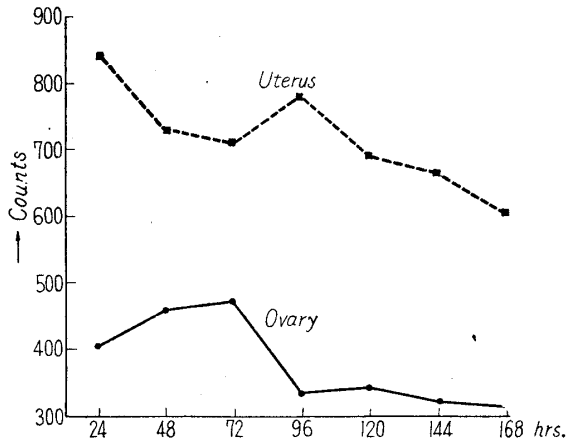

Fig. 5.
Change of the Uptake after
Analysis of Variance

TABLE V. Relationship between the Responses of Target Organs and the Period

No. of animals	Hours after administration of gonadotropin													
	24		48		72		96		120		144		168	
	Cts	Wt	Cts	Wt	Cts	Wt	Cts	Wt	Cts	Wt	Cts	Wt	Cts	Wt
1	401	42	462	56	363	43	443	131	349	78	317	78	273	41
2	311	27	349	35	671	248	385	56	333	58	319	68	313	45
3	282	19	527	68	523	91	410	122	329	68	356	64	279	47
4	300	22	427	56	527	114	429	139	452	163	313	64	255	43
5	257	20	455	85	516	127	266	100	382	135	307	75	264	60
6	394	38	461	75	472	76	314	61	318	51	257	56	333	68
7	461	60	440	62	642	145	332	73	433	99	312	62	258	70

Cts : $10 \times \sqrt{\text{counts}/3 \text{ mins.}}$ Wt : Weight in mg. $\times 10$.

TABLE VI. Analysis of Variance and Covariance of Table V

1) Analysis of variance of ovarian counts

Factor	SS	f	V	F_0
Time	299564	6	49927	12.47**
Error	168115	42	4003	
Total	467679	48		

2) Analysis of variance of ovarian weight

Factor	SS	f	V	F_0
Time	37806	6	6303	5.56**
Error	47587	42	1133	
Total	85393	48		

3) Analysis of covariance

Factor	S_{x^2}	S_{y^2}	C_{xy}	A	f'
Time	299564	37806	68790		
Error	168115	47587	66783	74393	41
Total	467679	85393	135573	252438	47
			Error	74393	41
	$F_0 = 16.33$**		Time	178045	6

The reduced responses as well as the initial data showed that the peak of ovarian responses (weight and uptake of radiophosphorus) appeared 72 hrs. after the administration of gonadotropin, whereas the peak of uterine responses appeared at 96th hr., in other words, 24 hrs. after the peak of ovarian response.

Discussion

Growing tissue, in general, accumulates more radiophosphorus than fully grown tissue. This was explained as the formation of labeled phosphorus compounds in the growing

tissue by Hevesy.[7] In the field of endocrinology, it is known that the growing target organs under hormonal influences show an increase in the uptake of radiophosphorus. Nati and Odelblad[5] studied the uptake of radiosulfate and -phosphate by the mouse ovary after the administration of PMS, and found the interesting difference in the uptake of radiosulfate from that of radiophosphate. However, they did not refer to the relationship between the organ weight and the uptake, and expressed only as counts per ovary. Besides, the physiological significance of the sulfate and phosphate taken up by the organs is quite different from each other. On the other hand, other workers expressed the uptake of radioisotope as counts per mg. of organ weight. As gonadotropin promoted simultaneously an increase in the weight of the target organs as well as an increase in counts per organ, it seemed interesting to the author how to express this response. First, the correlation coefficient was examined between the weight and radiophosphorus uptake of individual organs, and it was found that the counts per organ were not so closely influenced by the weight increase of the organs as expected from the expression as the counts per mg. of the organ. Therefore, the counts per organ could be expressed as follows :

$$C = R + I + \varepsilon$$

where C, R, I, and ε respectively denote the total counts per organ, the part of counts influenced linearly by the weight, the other part of the count independent of the weight, and the part of error. As gonadotropin influenced positively the increase of R as well as I, the total count would be increased in accordance with its weight increase. In comparison to this static accumulation, the factor I, or the dynamic accumulation of radiophosphorus, could be examined by the analysis of covariance, reducing the R factor from the C.

As qualitative assay, 2 I.U. of gonadotropin could be detected 72 hours after its administration, and for quantitative assay, the uptake of phosphorus by uterus was more suitable than the one by ovary at the 72nd hour after the administration of gonadotropin, especially concerning the slope of dosage-response curve and s/b, and that agreed with the results of the weight increase of these organs.[8]

The periodical change at ovaries and uterus was found to be different from the change of radioactivity in blood, suggesting that the uptake of radiophsphorus at these organs was not wholly influenced by the radioactivity of the blood which was contained in these organs, and possibly that the phosphorus taken up would be utilized for the mitosis of the organs.

The ^{32}P-accumulating activity of the ovaries and uterus changes with time after the administration of gonadotropin and the maximum uptake of the ovaries appeared at the 72-nd hour, while the one of the uterus at the 96th hour. This would explain endocrinologically that the administered gonadotropin stimulated the ovaries, causing the secretion of its endogenous estrogen as a result, and the estrogen secreted reached the uterus, stimulating its mitosis. The time lag between the maxima for these two organs would imply the period from the peak stimulation of the first target organ (ovary) by the gonadotropin to the most functional state of the second target organ (uterus) through thus produced estrogen. The effect of progesterone was not considered here, as no corpus luteum was found in the ovaries stimulated by the gonadotropin under this experimental condition. Though uterine uptake of radiophosphorus at the 24th hour itself was not so much, the adjusted uptake was found unexpectedly large, after the analysis of covariance, mostly owing to the very light weight of the uterus at the 24th hour.

The author is grateful to Prof. Y. Ito for his guidance and encouragement during this work.

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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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