UDC. 547.789'854

58. **Shigeru Yoshida and Mitsuru Kataoka**: Studies on Allied Compounds of Vitamin B_1. XXI.[1] The Structure of Dihydrothiamine.* (2).

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In the preceding paper of this series,[1] It was shown that the structural formula for normal- and iso-dihydrothiamine should not be the heretofore believed 3-(2-methyl-4-amino-5-pyrimidylmethyl)-4-methyl-5-(2-hydroxyethyl)-4-thiazoline (I) but should be 3-(2-methyl-4-amino-5-pyrimidylmethyl)-4a-methylperhydrofuro[2,3-*d*]thiazole (II). On the other hand, pseudo-dihydrothiamine[1,2] has properties far different from either normal- or iso-dihydrothiamine, such as being sparingly soluble in chloroform, while the

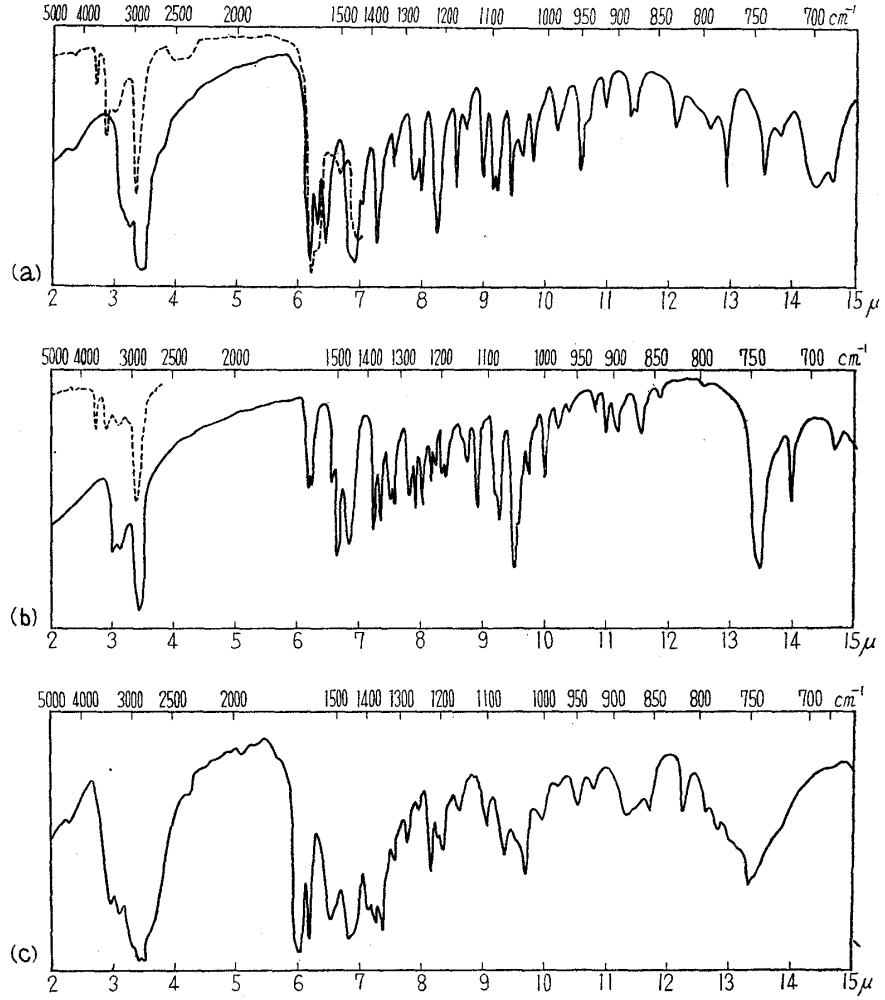

Fig. 1.

(a) pseudo-Dihydrothiamine —— Nujol ------ $CHCl_3$ solution (cell thickness, 1.0 mm.)
(b) Substance of m.p. 142° —— Nujol ------ $CHCl_3$ solution (cell thickness, 1.0 mm.)
(c) pseudo-Dihydrothiamine hydrochloride (Nujol)

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1) Part XX. S. Yoshida, M. Kataoka: This Bulletin, **5**, 176(1957).

2) T. Iwatsu: J. Pharm. Soc. Japan, **75**, 677(1955).

normal and iso compounds are soluble, the shift of its ultraviolet absorption to a longer wave length range, showing maximum absorptions (in ethanol) at 243 mμ (ε 10.3×10^3) and 288 (7.4×10^3)(cf. Fig. 4), and the entirely different absorption characteristics in its infrared spectrum from those of the normal and iso compounds, exhibiting absorptions (in Nujol) at 3160~3060 cm^{-1}(br) and (in chloroform) at 3650, 3450, and 3310 (w) cm^{-1}(cf. Fig. 1a).[3] The absorption at 3650 cm^{-1} is that due to the O—H stretching vibration and those at 3450 and 3310 cm^{-1} are considered to be the NH vibration of the imino or amino group.

3-*o*-Nitrobenzyl-4-methyl-5-(2-hydroxyethyl)thiazolium chloride[1] (III) was reduced with stannous chloride to the amino compound (IV), which was not obtained in crystalline state, and its reduction, *per se*, with lithium aluminum hydride afforded a substance melting at 142°. Similarly as in the previous case,[1] a formation of 3-(2-aminobenzyl)-3a-methylperhyrofuro[2, 3-*d*]thiazole (V) was anticipated from the above substance but it was assumed from its infrared spectrum that it was a compound with a structure like pseudo-dihydrothiamine. formed by the change of (V). The spectrum exhibited maximum absorptions (in Nujol) at 3340 and 3210 cm^{-1}, and (in chloroform) at 3650, 3450, and 3230 cm^{-1} (w), indicating the presence of a hydroxyl and imino or amino group (cf. Fig. 1b). It is still obscure why (V) was not formed in this reaction.

In order to determine whether the pseudo compound has the imino or the amino group, the normal and pseudo compounds were deuterated and the infrared absorption of the products was examined. The normal compound exhibited absorption maxima (in Nujol) at 2525 and 2300 cm^{-1}, while the pseudo compound showed maxima at 2400 (sh) and 2296 cm^{-1}(cf. Fig. 2a and b). From past studies on deuterated amines and imines,[4] it is assumed that the pseudo compound possesses an imino group. Further, the pseudo compound and the substance of m.p. 142° do not show absorptions at 1030 and 840 cm^{-1}, which were considered to be the characteristic vibration of the perhydrofurothiazole ring.[1] These facts indicate that the pseudo compound and the substance of m.p. 142° do not possess the structures formulated as (II) and (V), have undergone ring cleavage, and changed into compounds possessing a hydroxyl and imino groups. However, the

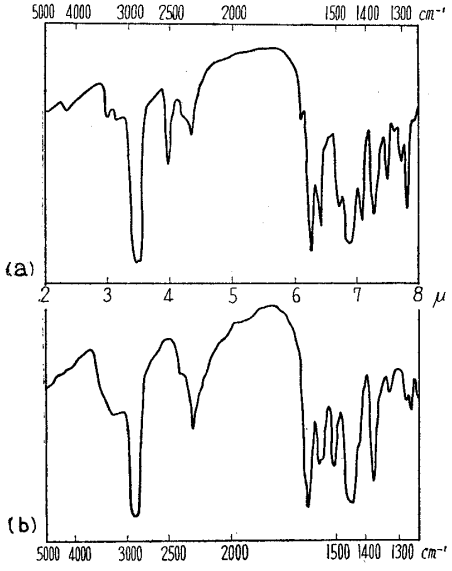


Fig. 2. Infrared Spectra of Deuterated Compounds (in Nujol)
(a) Deuterated normal-dihydrothiamine
(b) Deuterated pseudo-dihydrothiamine

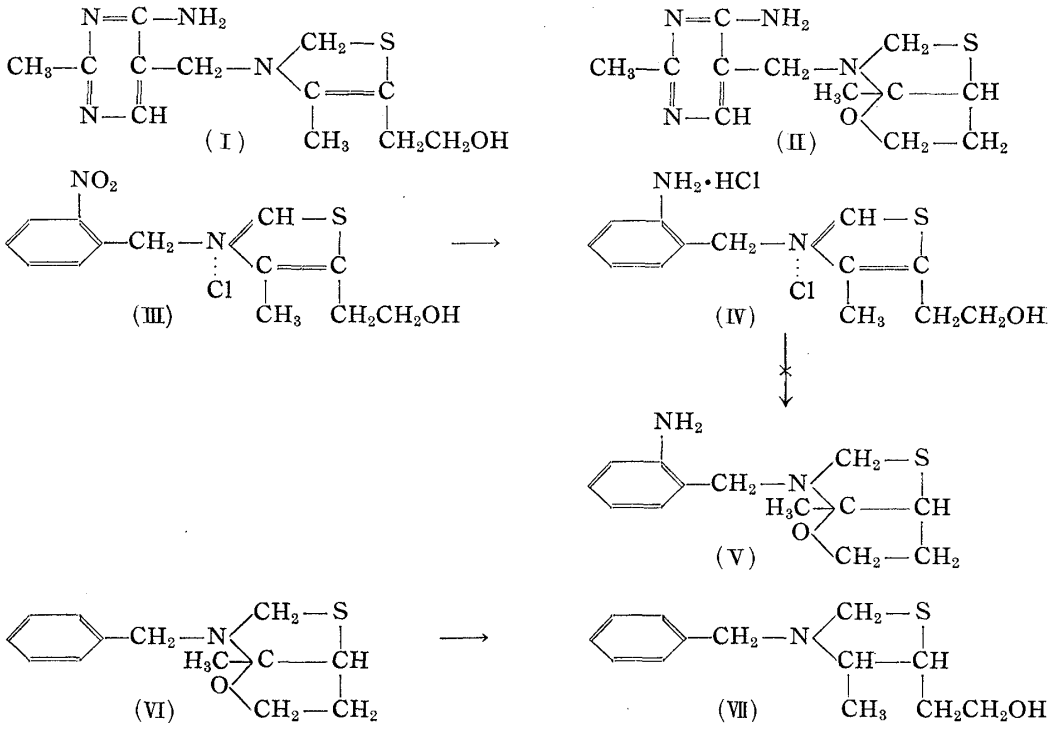
Displacement factors for normal-dihydrothiamine is 1.35 and 1.37, and that for pseudo-dihydrothiamine is 1.37.

3) Hirano reported absorption maxima of pseudo-dihydrothiamine as at 3390 and 3257 cm^{-1}, differing entirely from the above data (cf. J. Pharm. Soc. Japan, **76**, 1332(1956)).

4) H. J. Becker : Ber., **89**, 1953, 1951(1956); T. Miyazawa : J. Chem. Soc. Japan, **76**, 341, 1018(1955), **77**, 171, 321, 366, 381, 526(1956); T. Miyazawa, T. Shimanouchi, S. Mizushima : J. Chem. Phys., **24**, 408(1956).

pseudo compound shows the same Rf value as those of the normal and iso compounds in paper partition chromatography developed either with butanol–acetic acid–water, butanol–water, or pyridine–butanol mixture. It is hard to understand this identical Rf value in paper chromatography in spite of the assumption that the structure of the pseudo compound is different from that of normal and iso compounds, becuase of the different melting point and different infrared and ultraviolet spectral data.

All the normal–, iso–, and pseudo–dihydrothiamines form the same hydrochloride, m.p. 182~184°, on passing dry hydrogen chloride gas through their solution in dehydrated ethanol or chloroform, and neutralization of the salt with sodium hydrogen carbonate gives the pseudo compound. Therefore, this dihydrochloride must be the salt of the pseudo compound and will henceforth be termed pseudo–dihydrothiamine hydrochloride. The infrared spectrum of the hydrochloride exhibits absorption maxima (in Nujol) at 3350, 3200, 1670, and 1650 cm^{-1}, these being entirely different from the absorptions of the normal and pseudo compounds (cf. Fig. 1c). On the contrary, the infrared absorptions of 3–benzyl–3a–methylperhydrofuro[2, 3–*d*]thiazole (VI) and its hydrochloride are almost identical. This indicates that the amino group in 4–position of the pyrimidine ring takes part in the change effected on derivation to the hydrochloride.



Reduction of (VI) in tetrahydrofuran with lithium aluminum hydride affords an oily substance, whose analytical values correspond to $C_{16}H_{19}ONS$ and whose infrared absorption maximum lies at 3320 cm^{-1}(liquid). It is therefore considered that this oily substance is 3–benzyl–4–methyl–5–(2–hydroxyethyl)thiazolidine (VII) formed by the opening of the tetrahydrofuran ring.[5]

Application of benzoyl chloride to the normal, iso, and pseudo compounds in pyridine affords the same substance melting at 140°, whose infrared spectrum exhibits absorption maxima (in Nujol) at 3300 and 1720 cm^{-1} and (in chloroform) at 3450, 3300 (w), and 1720 cm^{-1}, indicating the presence of an imino group and a benzoyloxycarbonyl group (Fig. 3a). Its analytical values agree with $C_{19}H_{22}O_2N_4S$ and the substance is considered to be a benzoate of the pseudo compound, that it will be designated as benzoyl–pseudo–

5) N.G. Gaylord : Experientia, 8, 351(1954).

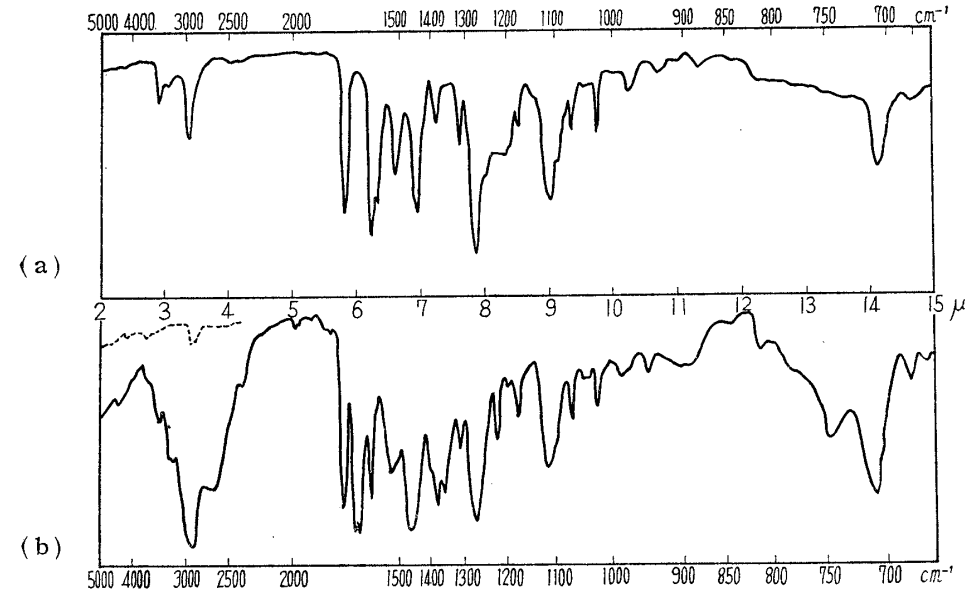


Fig. 3.

(a) Benzoyl-pseudo-dihydrothiamine (0.5% $CHCl_3$ solution; cell thickness 0.1 mm.)
(b) Benzoyl-pseudo-dihydrothiamine (Nujol)

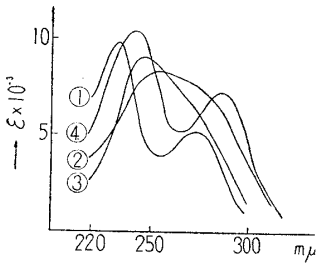


Fig. 4.

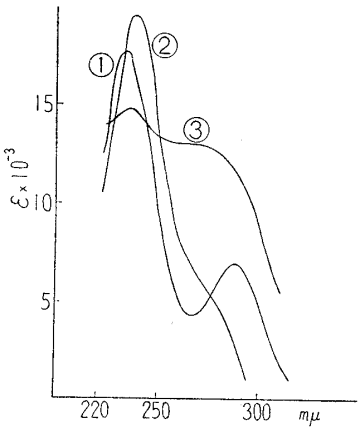


Fig. 5.

① normal-Dihydrothiamine (in EtOH)
② pseudo-Dihydrothiamine hydrochloride (in EtOH)
③ Dihydrothiamine (in 0.01*N* HCl)
④ pseudo-Dihydrothiamine (in EtOH)

① Benzoyl-pseudo-dihydrothiamine (in EtOH)
② Benzoyl-pseudo-dihydrothiamine (in 0.01*N* HCl)
③ Benzoyl-pseudo-dihydrothiamine (in EtOH)

dihydrothiamine.[6] Its ultraviolet absorption maxima lie at 230 mμ (ε 17.7×10^3) and 285 (7.2×10^3)(Fig. 5) and infrared spectrum of its hydrochloride exhibits absorption maxima at 3400, 3200, 1725, 1670, and 1656 cm^{-1}(in Nujol), similar to those of pseudo-dihydrothiamine hydrochloride (Fig. 3b).

From the examination of the pseudo-dihydrothiamine and its benzoyl derivative, several formulae can be forwarded for the structure of pseudo-dihydrothiamine, based on the mechanism of the formation and structure of normal and iso compounds, details of which will be discussed in a forthcoming paper.

Deep gratitude is expressed for valuable advices from Prof. S. Uyeo of the University of Osaka, for kind encouragements from Mr. M. Matsui, the Director of this Laboratory, to Messrs.

6) Hirano and others reported the formation of a benzoate of dihydrothiamine (see Footnote 3).

H. Shindo and O. Amakasu for infrared spectral measurements, and to Misses C. Furukawa and H. Ohtsuka for elemental analytical data.

Experimental

Infrared absorption spectra were measured by the Perkin-Elmer Model 21 spectrophotometer.

pseudo-Dihydrothiamine—Prepared by the method of Iwatsu.[2]

pseudo-Dihydrothiamine Hydrochloride—To a solution of 0.5 g. of normal-dihydrothiamine dissolved in a small amount of dehyd. EtOH with warming, 0.5 cc. of 25% ethanolic HCl was added and the mixture was allowed to stand. The crystals that precipitated out were collected by filtration and dried. Yield, 0.4 g. *Anal.* Calcd. for $C_{12}H_{18}ON_4S \cdot 2HCl \cdot H_2O$: C, 40.11; H, 6.12; N, 15.59. Found : C, 40.70; H, 6.54; N, 16.00.

The same hydrochloride is also obtained on passing dry HCl gas through the $CHCl_3$ solution or by the use of iso- or pseudodihydrothiamine as the starting material.

Neutralization of pseudo-Dihydrothiamine Hydrochloride—The solution of 1 g. of the foregoing hydrochloride dissolved in 5 cc. of water was neutralized with $NaHCO_3$, the filtrate was concentrated under a reduced pressure, cooled, and the crystals that separated out were recrystallized from EtOH to crystals of m.p. 175°, undepressed on admixture with pseudo-dihydrothiamine.

3-*o*-Aminobenzyl-4-methyl-5-(2-hydroxyethyl)thiazolium Chloride Hydrochloride[7] **(IV)**—To the solution of 7 g. of (III)[1] dissolved in 250 cc. of 2*N* HCl, 12 g. of Sn was added and the mixture was boiled gently for 1 hr. To this mixture, 17 g. of $SnCl_2$ was added gradually and the mixture was stirred at 50° for 24 hrs. The reaction mixture was diluted with 700 cc. of water, H_2S was bubbled through the solution until the precipitation no longer formed, and SnS was filtered off. The filtrate was evaporated under a reduced pressure and an oily residue was obtained. After drying over P_2O_5 in a vacuum desiccator, the substance was used immediately for the next step.

Reduction of 3-*o*-Aminobenzyl-4-methyl-5-(2-hydroxyethyl)thiazolium Chloride Hydrochloride (IV) with $LiAlH_4$—To a stirred mixture of 2 g. of $LiAlH_4$ and 30 cc. of dehyd. tetrahydrofuran, 4 g. of the above hydrochloride (IV) was added and the mixture was stirred at room temperature for 4 hrs. This was decomposed with 1 cc. of water, precipitate was filtered off, and the filtrate was evaporated under a reduced pressure. The residue was allowed to stand in a refrigerator and crystalline solid was recrystallized from MeOH to 2 g. of crystals, m.p. 142°. *Anal.* Calcd. for $C_{13}H_{18}ON_2S$: C, 62.40; H, 7.20; N, 11.20. Found : C, 62.32; H, 7.51; N, 11.40.

Benzoyl-pseudo-dihydrothiamine—To a solution of 1 g. of normal-dihydrothiamine dissolved in 15 cc. of pyridine, 0.6 g. of BzCl was added under stirring with ice cooling and the mixture was allowed to stand over night. The crystals that separated out were removed by filtration, the filtrate was evaporated under a reduced pressure, and the residue was rendered alkaline with 10% Na_2CO_3. This was extracted with $CHCl_3$, the $CHCl_3$ extract was washed with water, dried over anhyd. Na_2SO_4, and the solvent evaporated. The residual oil was left to stand with dehyd. Et_2O by which it crystallized gradually. Recrystallization from benzene gave crystals melting at 147~148°, that from 95% EtOH gave crystals of m.p. 89°, and the substance dried at 50° *in vacuo* melted at 147~148° The infrared spectrum of the substance of m.p. 89° in Nujol is different from that of the substance with m.p. 147~148°, but the spectra in $CHCl_3$ are identical in these substances. *Anal.* Calcd. for $C_{19}H_{22}O_2N_4S$: C, 61.62; H, 6.00; N, 15.11. Found : C, 61.60; H, 5.73; N. 15.19.

The same benzoyl derivative is obtained from iso- and pseudo-dihydrothiamine by the same reaction.

Benzoyl-pseudo-dihydrothiamine Hydrochloride—One gram of the above benzoyl compound was dissolved in 10 cc. of dehyd. EtOH with warming, cooled, and 0.8 cc. of 25% ethanolic HCl solution was added. Crystals precipitated on standing and were collected to 0.7 g. of m.p. 193~194°. *Anal.* Calcd. for $C_{19}H_{22}O_2N_4S \cdot 2HCl$: C, 51.46; H, 5.41; N, 12.64. Found : C, 50.75; H, 5.40; N, 12.99.

Reduction of 3-Benzyl-3a-methylperhydrofuro[2, 3-*d*]thiazole (VI) with $LiAlH_4$—To a solution of 2 g. of (VI) dissolved in 20 cc. of dehyd. tetrahydrofuran, 0.6 g. of $LiAlH_4$ was added and the mixture was boiled for 3 hrs. on a steam bath. This was decomposed with 0.5 cc. of water, insoluble matter filtered off, and the filtrate was evaporated under a reduced pressure. The residue was distilled *in vacuo* and an oily substance of $b.p_{0.005}$ 165~170° (bath temp.) was obtained. Yield, 0.5 g, *Anal.* Calcd. for $C_{13}H_{19}ONS$: C, 65.80; H, 8.01; N, 5.90. Found : C, 66.15; H, 8.13; N, 6.00.

Hydrochloride of (VI) was obtained in exactly the same manner as for pseudo-dihydrothiamine hydrochloride. m.p. 158°. *Anal.* Calcd. for $C_{13}H_{17}ONS \cdot HCl$: C, 57.45; H, 6.63; N, 5.15. Found : C, 57.00; H, 6.80; N, 5.10.

Deuteration of normal- and pseudo-Dihydrothiamine—The base was dissolved in a small amount

7) A.H. Livermore, R.R. Sealock : J. Biol. Chem., **167**, 699(1947).

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).

1) Part Ⅷ: Y. Ito, B. Tamaoki, M. Egusa, H. Sakamoto: J. Pharm. Soc. Japan, **72**, 1290(1952).
2) C. Hamburger: "Hormone Assay," ed. C.W. Emmens, Academic Press Inc., New York, 190(1950).
3) Y. Ito, B. Tamaoki, M. Egusa, H. Sakamoto: J. Pharm. Soc. Japan, **72**, 1282(1952).
4) R.C. Grauer, H.S. Strikler, J.J. Wolken, E. Cutuly: Proc. Soc. Exptl. Biol. Med., **75**, 651(1950).
5) W. Fleischmann, S. Fleischmann: J. Mt. Sinai Hosp., N.Y., **19**, 228(1952).
6) G. Nati, E. Odelblad: Acta Endocrinol., **19**, 43(1955).