

Generally sulfaguanidine does not seem to penetrate through the small intestine *in vivo*; thus the above results suggest that there may be some differences between studies on the penetration *in vitro* and these on the absorption *in vivo*.

The authors thank Dr. T. Matsuzawa for his kind advices on the experimental technique and Mr. H. Toguchi for his technical assistance in the experiment. This work was supported by the Grant-in-Aid for Scientific Research provided by the Ministry of Education, to which they are also grateful.

### Summary

1. The penetration of sulfonamides through the rat small intestine was investigated from the physicochemical standpoint *in vitro*.

2. Theoretical equations for the penetration mechanism of sulfathiazole were derived from the assumption that it penetrates through intestinal barrier in its three forms at the respective penetration rates.

3. From the data obtained, the respective permeability coefficients for the undissociated, alkaline and acid forms of sulfathiazole,  $P_1$ ,  $P_2$ , and  $P_3$  were statistically determined. The estimated values of  $P_1$ ,  $P_2$ , and  $P_3$  were 0.00064, 0.00979, 0.00132 (cc./cm./min.), respectively.

4. The experiment at the equilibrium state was attempted in order to examine the validity of the theoretical equation. Satisfactory results are obtained for  $P_1$  and  $P_3$ , but not for  $P_2$ .

5. The penetration rates of five sulfonamides were compared at the same pH value. The penetration rates were in the following order: sulfisomezole > sulfamethoxy pyridazine > sulfanilamide > sulfathiazole > sulfaguanidine.

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### 192. Takeo Ueda, Shigeshi Toyoshima, Tadakazu Tsuji, and Sumiko Watanabe : Synthesis and Antiviral Effect of Hexahydro-*s*-triazines.

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The antiviral activity of several triazine derivatives on Japanese encephalitis and influenza viruses has been examined by our research group.<sup>1,2)</sup>

In connection with these studies, hexahydro-*s*-triazine derivatives were synthesized for the purpose of examining their antiviral activities.

This paper concerns with the synthesis and the antiviral properties of alkyl derivatives of 2-iminohexahydro-*s*-triazine, tetrahydro-*s*-triazine-2(1*H*)-thione and tetrahydro-*s*-triazin-2(1*H*)-one.

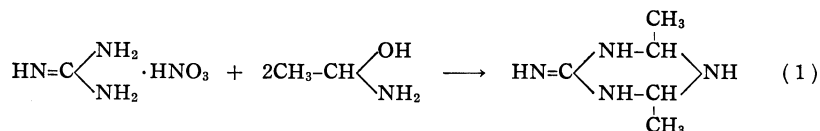
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1) T. Tsuji : This Bulletin, 2, 403 (1954).

2) I. Nakata, T. Ueda : Yakugaku Zasshi, 80, 1068 (1960).

### 1) Synthesis of 2-Iminohexahydro-*s*-triazine Nitrates

Paquin reported<sup>3)</sup> that 2-imino-4,6-dimethylhexahydro-*s*-triazine, m.p. 156~157° with decomposition, was prepared by the condensation of 1 mole of guanidine nitrate with 2 mole of acetaldehyde-ammonia at 55° according to equation (1).

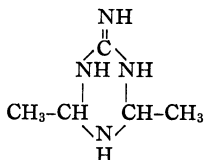


By the reexamination of this experiment, the authors obtained a compound, m.p. 156~157° with decomposition, which was considered to have the same property to that of Paquin's compound. Paquin presented the elementary analysis value for this compound as C<sub>5</sub>H<sub>12</sub>N<sub>4</sub>. However, our result was C<sub>5</sub>H<sub>13</sub>O<sub>3</sub>N<sub>5</sub>, which corresponded to nitrate salt as described in Table I. The nitric acid detecting reaction of this compound

TABLE I.

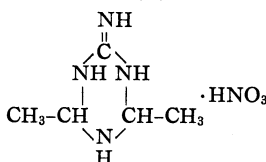
A) 2-Imino-4,6-dimethylhexahydro-*s*-triazine Nitrate

Paquin's Result

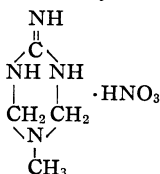


m.p. (decomp.) 156~157°, colorless prisms,  
*Anal.* Calcd. for C<sub>5</sub>H<sub>12</sub>N<sub>4</sub>: C, 46.82; H, 9.43; N, 43.75. Found: C, 46.78; H, 9.49; N, 43.66.

Present Result



m.p. (decomp.) 156~157°, colorless prisms,  
*Anal.* Calcd. for C<sub>5</sub>H<sub>13</sub>O<sub>3</sub>N<sub>5</sub>: C, 31.41; H, 6.85; O, 25.11; N, 36.63. Found: C, 31.65; H, 7.11; O, 25.05; N, 36.40.

B) 2-Imino-5-methylhexahydro-*s*-triazine Nitrate

m.p. 152~153°, colorless prisms, *Anal.*  
 Calcd. for C<sub>4</sub>H<sub>11</sub>O<sub>3</sub>N<sub>5</sub>: C, 27.12; H, 6.26; N, 39.53. Found: C, 27.40; H, 6.55; N, 39.17.

was positive and the infrared absorption spectra showed nitrate bands at 1360 cm<sup>-1</sup> and 820 cm<sup>-1</sup>. These facts suggested that this compound might be 2-imino-4,6-dimethylhexahydro-*s*-triazine nitrate.

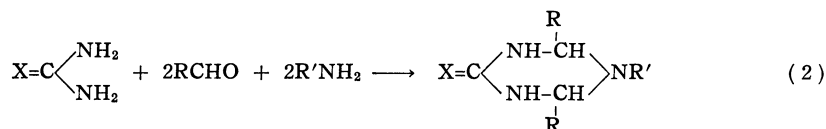
In order to obtain further confirmation, 2-imino-5-methylhexahydro-*s*-triazine nitrate was synthesized by using 1 mole of guanidine nitrate, 2 mole of formalin and 2 mole of methylamine. The results of elementary analysis, infrared spectra and positive nitric acid detection indicated that the product was nitrate, but not free base. An attempt to obtain free base of this compound was failed.

### 2) Synthesis of Tetrahydro-*s*-triazine-2(1*H*)-thione and Tetrahydro-*s*-triazin-2(1*H*)-one

The alkyl derivatives of tetrahydro-*s*-triazine-2(1*H*)-thione and tetrahydro-*s*-triazin-

3) A. M. Paquin: *Angew. Chem.*, **60**, 267 (1948).

2(1*H*)-one were synthesized by Paquin<sup>3)</sup> and Burke<sup>4)</sup> independently from 1 mole of thiourea or urea, 2 mole of aliphatic aldehyde and 2 mole of aliphatic amine as described in equation (2).



X=one member selected from a group of O and S.

R, R'=one member selected from a group of H and alkyl.

By using these methods, 4,6-disubstituted alkyl derivatives or 5-substituted alkyl derivatives of tetrahydro-*s*-triazine-2(1*H*)-thione and tetrahydro-*s*-triazine-2(1*H*)-one were obtained. The properties of these compounds were shown in Tables II and III.


TABLE II. Tetrahydro-*s*-triazine-2(1*H*)-thione Derivatives

No.	R	R'	m.p. (d.p.) (°C)	Crystal form	Formula	N %	
						Calcd.	Found
1 <sup>a)</sup>	H	CH <sub>3</sub>	(167~169) <sup>b)</sup>	needles	C <sub>4</sub> H <sub>9</sub> N <sub>3</sub> S	32.03	—
2	"	"	169.5~171.5	"	C <sub>5</sub> H <sub>11</sub> N <sub>3</sub> S	28.95	29.29
3	"	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	170~171	"	C <sub>6</sub> H <sub>13</sub> N <sub>3</sub> S	26.40	26.53
4	"	<i>n</i> -C <sub>4</sub> H <sub>9</sub>	169~169.5	"	C <sub>7</sub> H <sub>15</sub> N <sub>3</sub> S	24.26	24.11
5	"	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	168~169	"	C <sub>9</sub> H <sub>19</sub> N <sub>3</sub> S	20.88	21.01
6	"	iso-C <sub>3</sub> H <sub>7</sub>	162~163	"	C <sub>6</sub> H <sub>13</sub> N <sub>3</sub> S	26.40	26.53
7 <sup>a)</sup>	CH <sub>3</sub>	H	(180)	"	C <sub>5</sub> H <sub>11</sub> N <sub>3</sub> S	28.92	—
8	C <sub>2</sub> H <sub>5</sub>	"	148~150	plates	C <sub>7</sub> H <sub>15</sub> N <sub>3</sub> S	24.26	24.26
9	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	"	147~148	needles	C <sub>9</sub> H <sub>19</sub> N <sub>3</sub> S	20.88	20.94
10	CH <sub>3</sub>	<i>n</i> -C <sub>6</sub> H <sub>13</sub>	171~172	"	C <sub>11</sub> H <sub>23</sub> N <sub>3</sub> S	18.33	18.17

a) known compound

b) lit. m.p. 169°(decomp.),<sup>3)</sup> 180°<sup>4)</sup>

TABLE III. Tetrahydro-*s*-triazine-2(1*H*)-one Derivatives

No.	R	R'	m.p. (d.p.) (°C)	Crystal form	Formula	N %	
						Calcd.	Found
11	H	CH <sub>3</sub>	(208~209) <sup>a)</sup>	needles	C <sub>4</sub> H <sub>9</sub> ON <sub>3</sub>	36.50	36.23
12	"	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	(181.5~182)	"	C <sub>6</sub> H <sub>13</sub> ON <sub>3</sub>	29.40	—
13	"		211.5~212 <sup>b)</sup>	"	C <sub>9</sub> H <sub>17</sub> ON <sub>3</sub>	22.90	22.69
14	CH <sub>3</sub>	H	(190)	"	C <sub>5</sub> H <sub>11</sub> ON <sub>3</sub>	32.54	—

All compounds are known.

a) lit., m.p. 199°(decomp.),<sup>3)</sup> 210°<sup>4)</sup>

b) lit., m.p. 205°(decomp.)<sup>3)</sup>

The infrared absorption spectra of five compounds in this series were illustrated in Fig. 1.

### 3) Antiviral Effect of Hexahydro-*s*-triazine Derivatives on Poliomyelitis Virus

The primary screening test with the compounds shown in Tables I, II, and III was carried out using the Mahoney (type-1) strain of poliomyelitis virus.

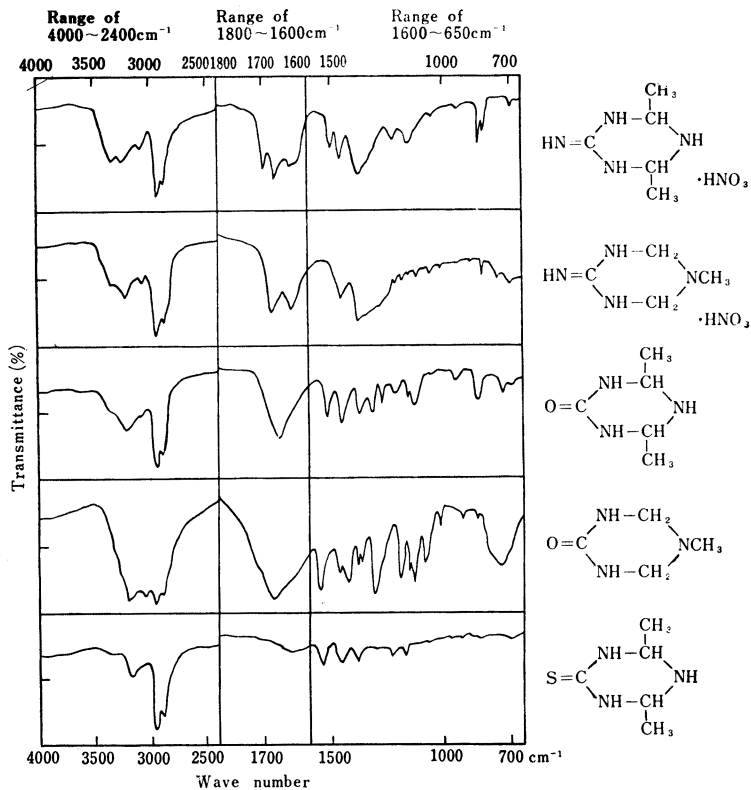


Fig. 1. Infrared Absorption Spectra of Hexahydro-*s*-triazines (in Nujol mull)

TABLE IV. Screening Test of 2-Imino-4,6-dimethylhexahydro-*s*-triazine Nitrate

a) Determination of Maximum Tolerated Concentration

<i>M</i> /50	<i>M</i> /100	<i>M</i> /200	<i>M</i> /300	<i>M</i> /500	<i>M</i> /1000
+++	++	+	-	-	-

b) First Screening Test on Mahoney Strain

Dose	Treated group CPE <sup>(2)</sup> /Total No. of tubes	Control group CPE/Total No. of tubes
10 <sup>-3</sup> <i>M</i>	0/2	1/1

a) No. of tubes which did not show any cytopathogenic effect.

c) Second Screening Test on Type-1, -2 and -3 Strains of Poliomyelitis Virus

Dose	Mahoney strain TCID <sub>50</sub> (-log)		MEF-1 strain TCID <sub>50</sub> (-log)		Saukett strain TCID <sub>50</sub> (-log)	
	Treated	Control	Treated	Control	Treated	Control
10 <sup>-3</sup> <i>M</i>	4.5	6.5	3.5	5.5	6.5	7.5

d) Inhibitory Effect on Measles Virus

Dose	TCID <sub>50</sub> (-log)	
	Treated	Control
10 <sup>-3</sup> <i>M</i>	3.0	4.0

(1) Maximum Tolerated Dose of Compounds on HeLa Cells

The maximum tolerated doses of all compounds were in the range between *M*/300~*M*/1000. In Table IV-(a), the experimental result of 2-imino-4,6-dimethylhexahydro-*s*-triazine nitrate was shown.

## (2) Primary Screening Test on Mahoney Strain

From the results of this screening test, it was found that only the 2-imino-4,6-dimethylhexahydro-*s*-triazine nitrate did show an inhibitory effect on the multiplication of the poliomyelitis virus, the result of which was shown in Table IV-(b).

## (3) Secondary Screening Test on Type-1, -2, and -3 Strain Poliomyelitis Virus

2-Imino-4,6-dimethylhexahydro-*s*-triazine nitrate, which was selected by the primary screening test was examined in more details for its effect on the type-1, -2, and -3 strain of poliomyelitis virus. The secondary screening test was proceeded by determining an effect of the compound on TCID<sub>50</sub>\*<sup>2</sup> of the virus. The experimental results were shown in Table IV-(c). In this table, it is seen that TCID<sub>50</sub> of the tested group was 1/100 of the control group on type-1 and -2 strains. On the type-3 strain, TCID<sub>50</sub> of the treated group was 1/10 of the control.

From these results, it may be said that 2-imino-4,6-dimethylhexahydro-*s*-triazine nitrate inhibited a multiplication of every type strain of poliomyelitis viruses, although its effect on the type-3 strain was weaker than those on the other strains.

## 4) Inhibitory Effect of 2-Imino-4,6-dimethylhexahydro-*s*-triazine Nitrate on Measles Virus

Since measles virus belongs to RNA virus as same as poliomyelitis virus, an inhibitory effect of the compound on measles virus was of interest to examine. Thus, the compound which showed an inhibitory effect in the secondary screening test on poliomyelitis virus, i. e. 2-imino-4,6-dimethylhexahydro-*s*-triazine nitrate, was examined as to its inhibitory effect on the Edmonstan strain of measles virus. As shown in Table IV-(d), 2-imino-4,6-dimethylhexahydro-*s*-triazine nitrate also showed an inhibitory effect on measles virus. However, the effect on the virus was not so strong and almost equal to that on type-3 strain of poliomyelitis virus.

### Experimental

**2-Imino-4,6-dimethylhexahydro-*s*-triazine Nitrate**—This compound was prepared by the method<sup>3)</sup> of Paquin's 2-imino-4,6-dimethylhexahydro-*s*-triazine.

**2-Imino-5-methylhexahydro-*s*-triazine Nitrate**—To a solution of 12.2 g. of guanidine nitrate and 15.6 g. of 40% aq. MeNH<sub>2</sub> in 10 cc. of water, 15 g. of formalin (30% of HCHO) was added dropwise at 25°. The solution was stirred for 3 hr. at this temp., and then warmed at 50~55° for 3 hr. After cooling at 5°, the solution was filtered. The filtrate was evaporated to one-fourth of the volume under reduced pressure below 40° and cooled on ice. The crystals precipitated were collected and recrystallized from dil. MeOH below 50° to colorless prisms, m.p. 152~153°, yielding 8.8 g.

**General Method for the Synthesis of Tetrahydro-*s*-triazine-2(1*H*)-thione and Tetrahydro-*s*-triazin-2(1*H*)-one Derivatives**—4,6-Disubstituted alkyl derivatives and 5-substituted alkyl derivatives were obtained according to both Paquin's<sup>3)</sup> and Burke's<sup>4)</sup> papers.

### Test of Antiviral Activity

#### a) Materials

1) **Poliomyelitis Virus**—Mahoney (type-1), MEF-1 (type-2) and Saukett (type-3) strains of poliomyelitis virus were kindly given by Dr. N. Takemori, the National Institute of Preventive Medicine.

2) **Measles Virus**—Edmonstan strain of measles virus was kindly supplied by Dr. J. Enders, the Children Medical Center, Boston.

b) **Methods**—For the test of antiviral activity on poliomyelitis and measles viruses, tissue culture method was employed using HeLa cells (wild type). 20×10<sup>4</sup> cells/ml. of the HeLa cells were added into each tube and incubated at 37° for 4 days. Then the monolayer cell sheet was established in a tube. For the growth medium the YLA supplemented with 15% beef serum was used.

1) Determination of the Maximum Tolerated Concentration of Compounds on HeLa Cells: After the monolayer cell sheet was established, the growth medium was removed from the tube and 0.9 cc. of the maintenance medium (YLA supplemented with 5% beef serum) and 0.1 cc. of the dilution of the

\*<sup>2</sup> Tissue culture infective dose fifty.

test compound were added to each tube. After an incubation at 37° for 4 days, the maximum tolerated concentration (MID) was determined by microscopic observation concerning to the state of the degeneration.

2) Effect of Test Compound on TCID<sub>50</sub> of Poliomyelitis Virus (First Screening Test): The monolayer cell sheet established tube was mixed with 0.8 cc. of the maintenance medium and 0.1 cc. of ten times MID of test compound, and then 0.1 cc. of the viral dilution (1000 TCID<sub>50</sub>) was inoculated. TCID<sub>50</sub> (tissue culture infective dose fifty) of the mahoney strain on HeLa cells was 10<sup>-7.0</sup>/0.1 cc. Two tubes thus prepared were used as the treated group tubes. The control group tube was prepared by mixing the monolayer cell sheet with 0.9 cc. of the maintenance medium and 0.1 cc. of the viral dilution (1000 TCID<sub>50</sub>). After incubating at 37° for 7 days, these tubes were observed microscopically as to the cell degeneration. When the both test group tubes did not show any cell degeneration, the test compound was selected as the compound for the second screening test.

3) Second Screening Test: Type-1, -2 and -3 strain viruses were employed. The first stage of this test was the same as the method mentioned above. 0.1 cc. of ten times diluted viral dilution was used for this test. After an incubation at 37° for 7 days, TCID<sub>50</sub> of the test group and that of the control group were determined by microscopic observation according to Reed and Muench's method.<sup>5)</sup>

4) Screening Test of Compound on Measles Virus using HeLa Cells: Edmonstan strain was used. The method was the same as that of method 2). After incubating at 37° for 2 weeks, TCID<sub>50</sub> was determined by microscopic observation.

### Summary

The condensation of guanidine nitrate with acetaldehyde-ammonia furnished 2-imino-4,6-dimethylhexahydro-*s*-triazine nitrate. 2-Imino-5-methylhexahydro-*s*-triazine nitrate was also prepared by the same way.

The alkyl derivatives of tetrahydro-*s*-triazine-2(1*H*)-thione and tetrahydro-*s*-triazin-2(1*H*)-one were synthesized by the condensation of either thiourea or urea with amine and aldehyde.

These compounds were screened as to their inhibitory effect on poliomyelitis virus by tissue culture method. Only 2-Imino-4,6-dimethylhexahydro-*s*-triazine nitrate, among the compounds screened, showed an effect, and it exerted fairly an inhibitory effect on the type-1 and -2 strains of poliomyelitis virus, but it has not so significant effect on the type-3 strain. Moreover, this compound was found to inhibit a multiplication of the Edmonstan strain of measles virus, although its effect was not so significant.

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5) L. J. Reed, H. Muench: Am. J. Hyg., 27, 493 (1938).