No significant differences were observed of the influences of the type and phase ratio of emulsion vehicle on the absorption rate.

When Carbowax 2000 was used as vehicle, phenylmercuric acetate showed a higher blood level than that of ω-ethylmercurithio-*n*-undecanoic acid or ethylmercuric chloride.

It was concluded that the rate of absorption of mercurial compounds contained in suppository vehicle through rabbit's vagina was related to the water solubility and oil-water distribution coefficient of the compounds employed.

(Received November 21, 1963)

[Chem. Pharm. Bull. 12 (5) 548 ~ 557]

UDC 615.778[547'149] : 612.387

77. Hiroshi Fukuchi, Tsutomu Takeda, Akira Kamada, Kanjiro Kobayashi, and Masaru Aoki: The Absorption of Organomercurial Compounds from the Vaginal Route of the Rabbits. II.*1

Distribution and Excretion of ω-Ethylmercurithio-*n*-undecanoic Acid and Phenylmercuric Acetate.

(*Faculty of Pharmaceutical Sciences, Osaka University**2)

Previously a comparison was made of the vaginal absorption of mercurials, especially between PMA (phenylmercuric acetate) and EMU (ω-ethylmercurithio-*n*-undecanoic acid) administered in the form of suppository prepared with cream base Rx 6 and its modified bases.*1 The comparison which was made on blood levels revealed that modifications of the cream bases had resulted in some changes of an actual absorption rate for PMA but very few for EMU.

During the present series of experiments, care had to be taken to assure lest EMU, being a new compounds, should show any possible toxic reactions. It is understood that toxic manifestations due to the absorption of organomercurial compounds are reflected on accumulation and excretion of the compounds rather than their blood level.

According to Laug and Kunze,[1] mercury deposits mostly in the kidneys following intravaginal administration; and an appreciable amount of deposit is also found in the liver when used with a jelly base or in the form of aqueous solution. A similar deposition occured when PMA was intravenously[2] or subcutaneously[3] administered.

Thus, in the present experimentation a comparison was made of EMU with PMA which has been already in practical use. And it was attempted to pursue the results of intravaginally administered EMU pertaining to tissue distribution and excretion. Rx 6, being the least irritable and synergistically antitrichomonal, was experimentally standardized as in the previous experiment.*1

It was also attempted to study the effect of detoxicants such as α-lipoic acid and BAL on the excretion of mercurials introduced.

---

*1 Part I. M. Aoki, *et al.*: This Bulletin, **12**, 540 (1964).

*2 Toneyama, Toyonaka, Osaka-fu (福地 坦, 武田 務, 鎌田 皎, 小林勘次郎, 青木 大).

1) E. P. Laug, F. M. Kunze: J. Pharmacol. Exptl. Therap., **95**, 460 (1949).
2) C. S. Prickett, E. P. Laug, F. M. Kunze: Proc. Exper. Biol. and Med., **73**, 585 (1950).
3) L. Friberg, E. Odeblad, S. Forssman: A. M. A. Arch. Indust. Health, **16**, 163 (1957).

Method and Materials

Rabbits were raised and given suppositories containing labelled mercurial compounds (Hg^{203}-PMA and Hg^{203}-EMU) as described in the previous paper.

The excretion and distribution of mercurials in the organs were followed after insertion of suppository containing 0.05% or 0.1% of labelled mercurial compounds. In the present experiment only Carbowax 2000 or Rx 6 was employed as a suppository vehicle, so that the latter, being a newly introduced vehicle, was comparatively studied with so far commonly used Carbowax 2000.

The amount of a daily excretion by fecal and urinary routes was observed every 24 hr. after an insertion. In the case of repeated administration, the insertion was made once daliy for 30 days, and the accumulation of mercurial in tissue was observed at time intervals ranging from 10 to 30 days.

In the case of single dose administration, the animal's vagina was kept clamped for 7 hr. The excretion was serially studied while the blood level was checked up. Therefore, it seems safe to consider that the animal was subjected to no washing of the suppository administered. Since it was difficult to avoid washing out in repeated administration, a loose ligature was performed as described in the previous paper.

The rabbits were kept in a metabolism cage with a steel mesh bottom. Their feces excreted in the bottom and their urine through an almite rectangular funnel attached to the mesh were collected every 24 hr. The concentration of mercurials in the urine was determined by direct counting of the aliquote volume of daily urine out-put.

In determining Hg^{203} in the feces, the feces was treated with conc. HNO_3 and direct counting was made on an aliquote amount of the solution disintegrated with HNO_3. The rabbits were killed 24 hr. after their last designated insertion with air embolism under phenobarbiturate anesthesia and their dissected tissue was squeezed to get rid of blood and weighed and treated with conc. HNO_3 for direct counting.

The concentration of mercurial (Hg^{203})-compound found in each experiment was represented as PMA or EMU respectively on the basis of the values simultaneously counted with a standard solution of each 0.2 μg. of Hg^{203}-PMA or Hg^{203}-EMU dissolved in conc. HNO_3. The counting was made for 10 min. with a well-type scintillation counter.

For injection there was employed an aliquote amount of Carbowax 2000 suppository containing PMA or EMU, being dissolved into physiological saline so as to make 100 μg. of mercurials per one ml. injection.

Results and Discussions

Urinary and Fecal Excretion following Single Dose Administration

The average daily excretion pattern with three rabbits is shown in Figs. 1~4 when a single dose administration of EMU or PMA is employed with two different suppository vehicles. Each of the three rabbits followed in those experiments showed the same trend of pattern for urinary and fecal excretion. Each figure reveals higher concentration of mercurial for fecal excretion than urinary. The cumulated amounts of mercurial excretion 7 days after an single dose insertion are presented in Table I as a mean per cent excretion for each group.

Table I. Cumulated Per Cent Excretion of Mercurials 7 Days after a Single Dose Administration of 0.2 g. Suppository containing Each 0.1% of PMA and EMU
Each Value Represents an Average of 3 Rabbits.

Routes	Excreted amount in per cent			
	PMA		EMU	
	Carbowax 2000	Rx 6	Carbowax 2000	Rx 6
Urinary	16.9	14.3	7.5	7.3
Fecal	41.0	30.0	29.5	25.2
Total excretion	57.9	44.3	37.0	32.5

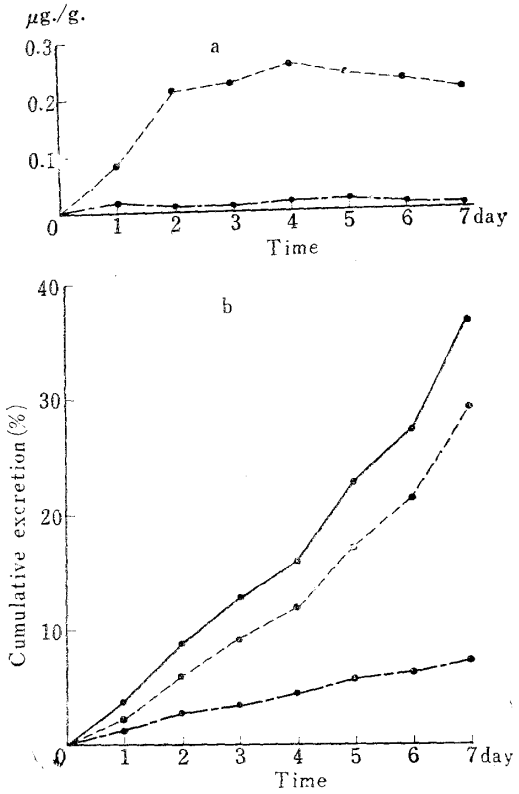

Fig. 1. Average Daily and Cumulative Excretion of EMU with 3 Rabbits Following a Single Dose Administration into Vagina

Dosage : 200 μg.
Vehicle : Carbowax 2000
a : Concentration
——— Urine
– – – – – Feces
b : Cumulative excretion
——— Total
——— Urine
– – – – – Feces

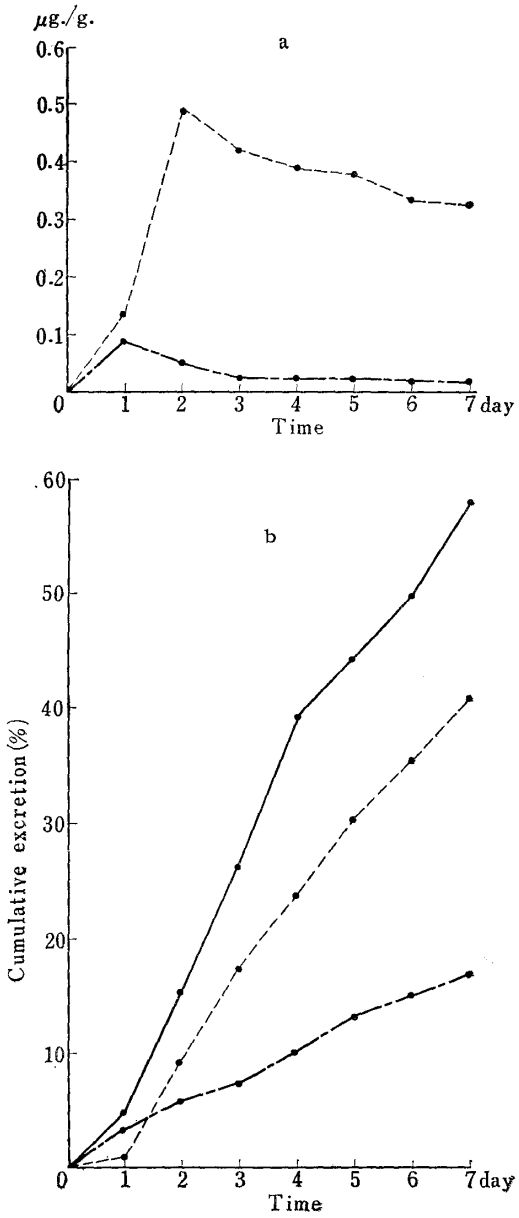

Fig. 2. Average Daily and Cumulative Excretion of PMA with 3 Rabbits Following a Single Dose Administration into Vagina

Dosage : 200 μg.
Vehicle : Carbowax 2000
a : Concentration
——— Urine
– – – – – Feces
b : Cumulative excretion
——— Total
——— Urine
– – – – – Feces

When Carbowax 2000 is used as vehicle, a total excretion of PMA shows about twice EMU. But when Rx 6 is used, only two-thirds of PMA is excreted as is the case with Carbowax. In the case of EMU no difference in excretion was observed between Carbowax 2000 and Rx 6.

This decrease in total excretion of PMA following Rx 6 suppository administration seems to reflect the result observed in the previous report*1 on the absorption of

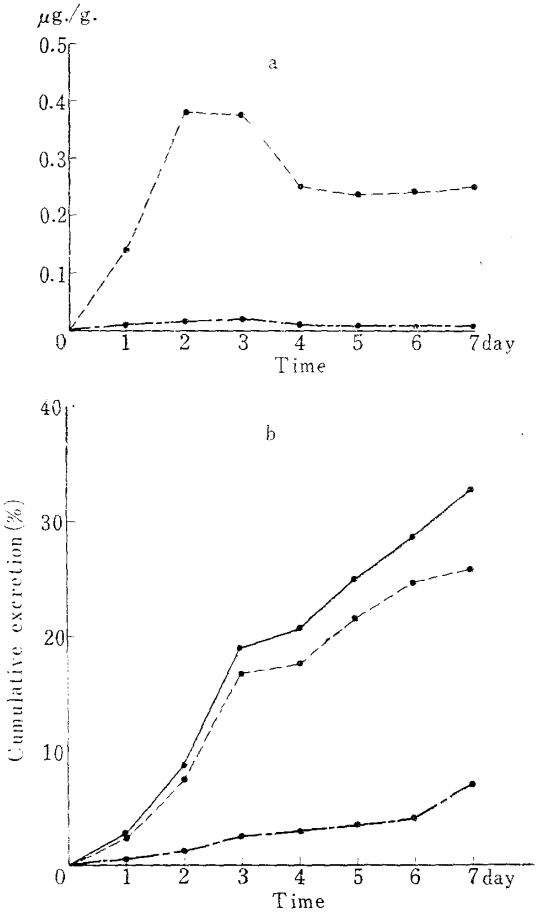

Fig. 3. Average Daily and Cumulative Excretion of EMU with 3 Rabbits Following a Single Dose Administration into Vagina

Dosage: 200 μg.
Vehicle: Rx 6
a: Concentration
——— Urine
– – – – – Feces
b: Cumulative excretion
——— Total
— — — — Urine
– – – – – Feces

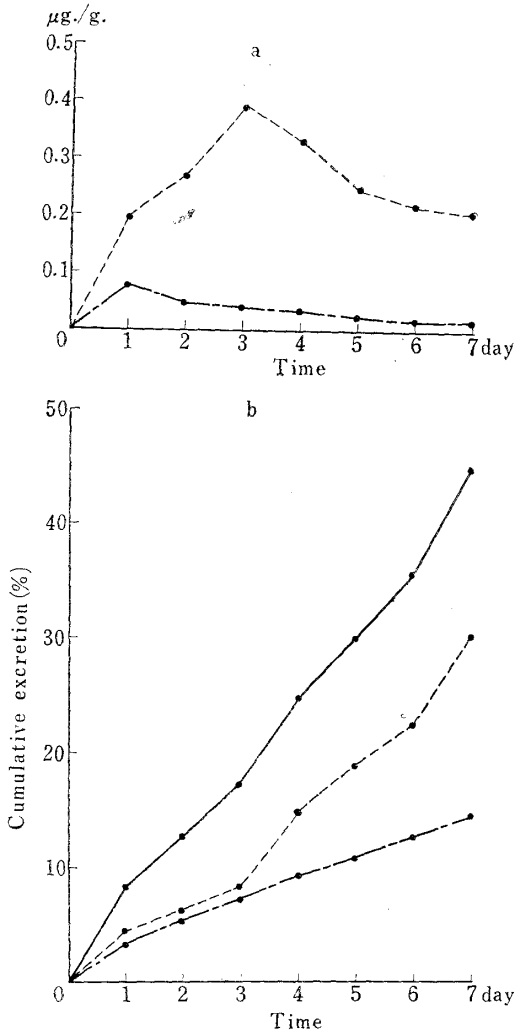

Fig. 4. Average Daily and Cumulative Excretion of PMA with 3 Rabbits Following a Single Dose Administration into Vagina

Dosage: 200 μg.
Vehicle: Rx 6
a: Concentration
——— Urine
– – – – – Feces
b: Cumulative excretion
——— Total
— — — — Urine
– – – – – Feces

mercurials from the vaginal mucosa. Then it was suggested that PMA, being converted into oil soluble phenylmercuric stearate, was distributed in the oil-phase of Rx 6 vehicle and resulting in a decrease of absorption.

Cumulative Excretion of Mercurials following Repeated Daily Administrations and their Distribution in Tissue

The cumulative amounts of excreted mercurials and their distribution in tissue were investigated with rabbits following a daily insertion of 100 μg. of mercurials which had been incorporated in Rx 6 vehicle at a concentration of 0.05%.

The daily excretion for an animal for 30 days is presented in Fig. 5 (EMU) and Fig. 6 (PMA). Although some variation is observed among rabbits of the same group, the

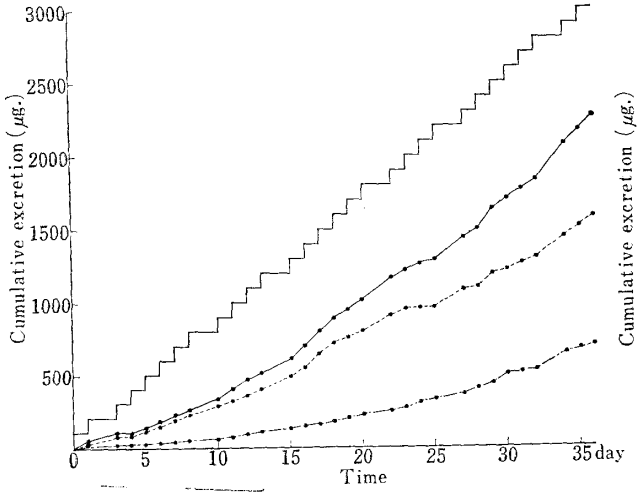

Fig. 5. Cumulative Excretion of EMU Following 30 Consecutive Days' Vaginal Administration

Dosage: 100 μg./day
Vehicle: Rx 6
——— Total
——— Urine
- - - - - Feces

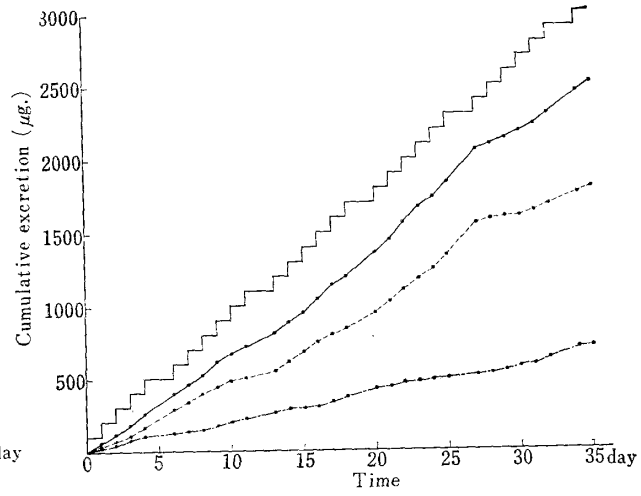

Fig. 6. Cumulative Excretion of PMA Following 30 Consecutive Days' Vaginal Administration

Dosage: 100 μg./day
Vehicle: Rx 6
——— Total
——— Urine
- - - - - Feces

curves obtained after the first 10 days' and the 20 days' observation out of a 30 day series, were simillar to those obtained in a 10 days and a 20 days series. In those figures, the cumulative amounts of mercurials administered are shown by stepwise patterns. The mean values for five rabbits for the data in above respective series of observation are presented in Table II.

Table II. Cumulative Excretion of Mercurials following 10, 20, and 30 Days' Consecutive Vaginal Administrations Studied on Each Group of Five Rabbits

Routs		Amounts of excretion (μg.)					
		PMA-10	PMA-20	PMA-30	EMU-10	EMU-20	EMU-30
Urinary	mean	131	387	644	95	213	591
	min.	90	240	500	62	124	328
	max.	190	493	855	137	306	734
Fecal	mean	456	1358	2042	396	1170	1736
	min.	310	1065	1562	181	586	1462
	max.	524	1575	2545	596	1470	2173
Total excretion	mean	587	1745	2686	491	1383	2327
	standard deviation	104	181	380	163	449	412
	(%)	58.7	87.2	89.5	49.1	69.1	77.5

Cumulative excretion curves of PMA reveal linear patterns showing a urinary excretion of ca. 200 μg. of PMA every 10 days, and 500~700 μg. for fecal excretion (Fig. 6).

EMU showed an abrupt increase of excretion rate at about 10 days' intervals (Fig. 5). The cumulative excretion of the first 10 days, showing 100 μg. in the urine and 360 μg. in the feces, is followed by a rapid increase of excretion *via* both routes, *i.e.*, 150 μg.

Table III. Distribution of Mercurials in Tissue,[a] following Repeated Daily Vaginal Administration[b]

Tissue		Amount of mercurials in tissue (μg./organ)					
		PMA-10[c]	PMA-20	PMA-30	EMU-10	EMU-20	EMU-30
Kidney	mean	49.6	73.4	111.8	51.1	94.5	107.5
	min.	41.6	54.4	94.8	32.8	50.2	78.5
	max.	55.8	124.2	140.2	58.3	138.8	146.7
Liver	mean	58.7	80.7	130.3	160.1	307.3	377.9
	min.	36.7	48.8	95.6	118.1	188.2	354.6
	max.	96.8	101.1	189.9	187.7	487.1	416.7
Spleen		0.4	0.7	0.5	0.4	0.9	0.6
Uterus		0.8	1.4	1.0	2.1	3.5	4.7
Stomach		2.6	3.3	—	15.4	—	16.9
Lungs		0.9	2.3	2.8	5.1	5.5	7.9
Heart		0.8	0.5	0.9	1.7	1.4	1.9
Brain		0.1	0.9	1.1	1.3	1.8	1.5
Gall		0.3	0.5	0.1	0.2	0.6	0.4
Bladder		1.0	1.1	1.1	0.7	1.5	1.0

a) Each value represents a mean amount of mercurials (μg./organ) for five rabbits correspondingly measured by the unit of PMA or EMU.
b) Daily dose: 0.2 g. of Rx 6 suppository containing 0.05% Hg-compound.
c) PMA-10 denotes the result of 10 consecutive days' administration.

Table IV. The Distribution of Mercurials in Tissue in Micrograms per Gram Tissue

Tissues	Distribution of mercurials (μg./g. tissue)					
	PMA-10	PMA-20	PMA-30	EMU-10	EMU-20	EMU-30
Kidney	7.11	7.46	12.94	7.28	12.30	13.82
Liver	1.13	1.29	1.63	2.98	4.57	4.91
Spleen	0.42	0.29	0.29	0.37	0.59	0.42
Uterus	0.16	0.17	0.19	0.34	0.38	0.66
Smallintestine	0.17	0.28	0.20	0.58	0.37	0.56
Rectum	0.26	0.14	0.23	0.36	0.33	0.46
Stomach	0.11	0.12	0.27	0.62	—	0.44
Lung	0.19	0.11	—	0.47	0.55	0.63
Heart	0.11	0.04	0.12	0.32	0.27	0.30
Brain	—	0.06	0.13	0.23	0.24	0.24
Gall	0.13	0.07	0.26	0.24	0.32	0.41
Bladder	0.24	0.25	0.27	0.25	0.47	0.31

Each value represents the mean of determination of PMA and EMU in the organ of five rabbits.

Table V. Gross Findings of Organs Dissected 10, 20, and 30 Days after Initial Administration which was followed by Consecutive Administration

Days of consecutive daily administration / Findings	PMA			EMU		
	10	20	30	10	20	30
Gallbladder whitened	1/5	3/5	2/5	2/5	4/5	4/5
Bile brown-yellow	—	—	1/5	—	2/5	1/5
Gallbladder shrank	—	—	—	—	1/5	2/5
Liver white spotted and white stripped	—	—	—	—	—	2/5
Kidney	the findings were not remarkable					

1/5 denotes one out of five rabbits, 2/5 two out of five rabbits etc.

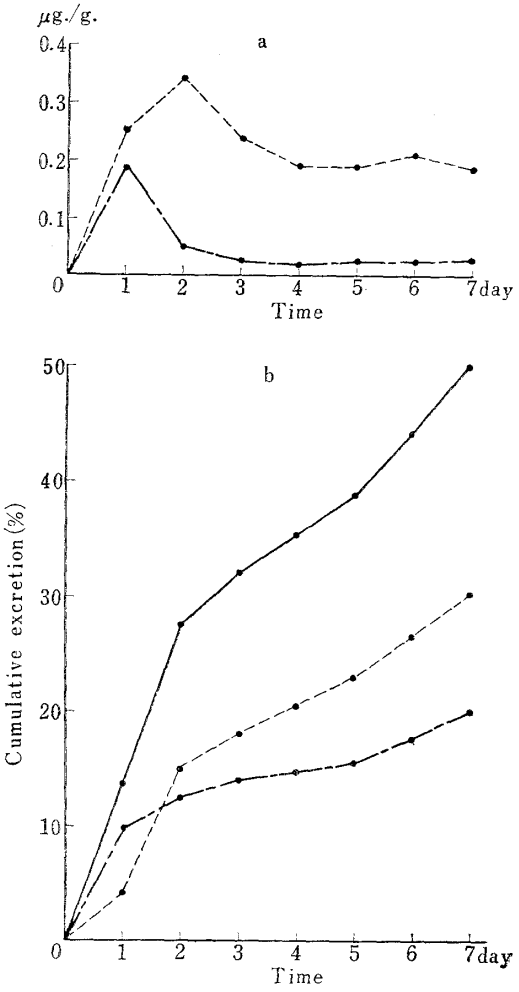

Fig. 7. Average Daily and Cumulative Excretion of PMA with 3 Rabbits Following a Single Dose Administration into Vagina and an Intramuscular Administration of BAL (100 mg.)

Dosage : 200 μg.
Vehicle : Rx 6
a : Concentration
——— Urine
– – – – – Feces
b : Cumulative excretion
——— Total
——— Urine
– – – – – Feces

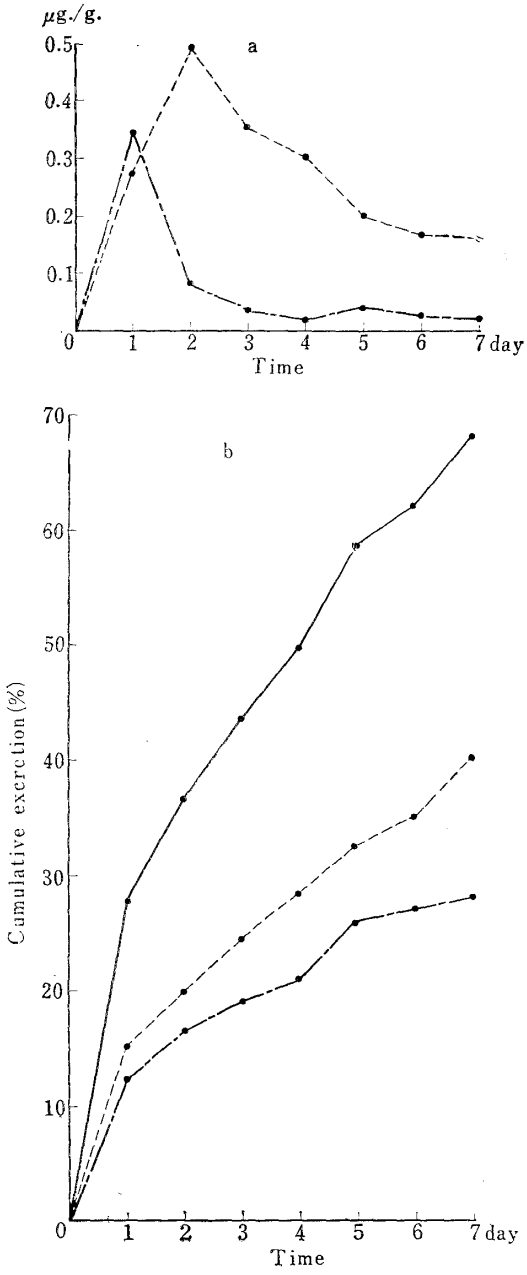

Fig. 8. Average Daily and Cumulative Excretion of PMA with 3 Rabbits Following a Week's Oral Administration of α-Lipoic Acid (10 mg./day) before Experiment

Dosage : 200 μg.
Vehicle : Carbowax 2000
a : Concentration
——— Urine
– – – – – Feces
b : Cumulative excretion
——— Total
——— Urine
– – – – – Feces

from a urinary and 500 μg. from a fecal route for the next 10 days are followed by 280 μg. from a urinary and 800 μg. from a fecal route for the last 10 days.

Those excretion patterns seem to reflect the distribution of mercurials in tissue especially in the kidney and liver. The mean amounts of mercurials distributed in tissue with five rabbits following repeated administrations are tabulated in Table Ⅲ regarding the organs dissected at an intervals of 10, 20, and 30 days after being treated with PMA or EMU daily. A marked deposition of mercurials took place mainly in the kidney and liver.

The amounts of PMA deposited in the kidney and liver increase steadily as the administration of the mercurial increases during the experimental period. But EMU reveals a different trend of deposition and its amount is found to be larger than PMA in both organs especially in the liver. Here it must be admitted that the mercury content of EMU is lower than PMA. Therefore, as far as the mercury deposit is concerned, EMU reveals less amount in the kidney but more amount in the liver than PMA. A large amount of EMU deposits within 20 days from the initial administration, and then a less increase of the deposition is observed. Referring to Figs. 5 and 6,

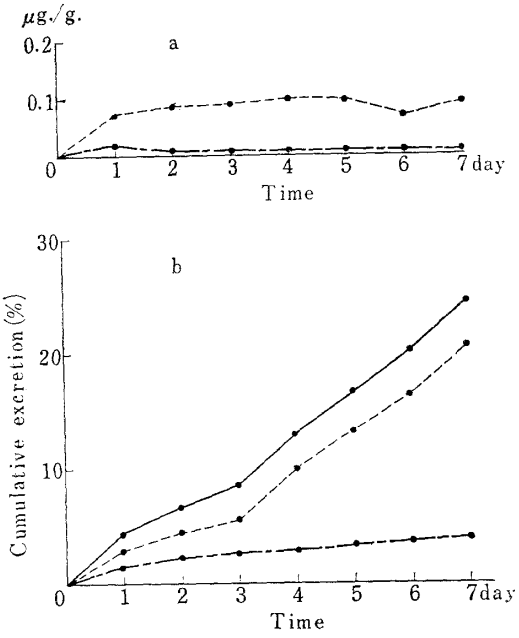

Fig. 9. Average Daily and Cumulative Excretion of EMU with 3 Rabbits Following a Single Administration into Vagina

Dosage : 100 μg.
Vehicle : Carbowax 2000
a : Concentration
——— Urine
– – – – – Feces
b : Cumulative excretion
——— Total
——— Urine
– – – – – Feces

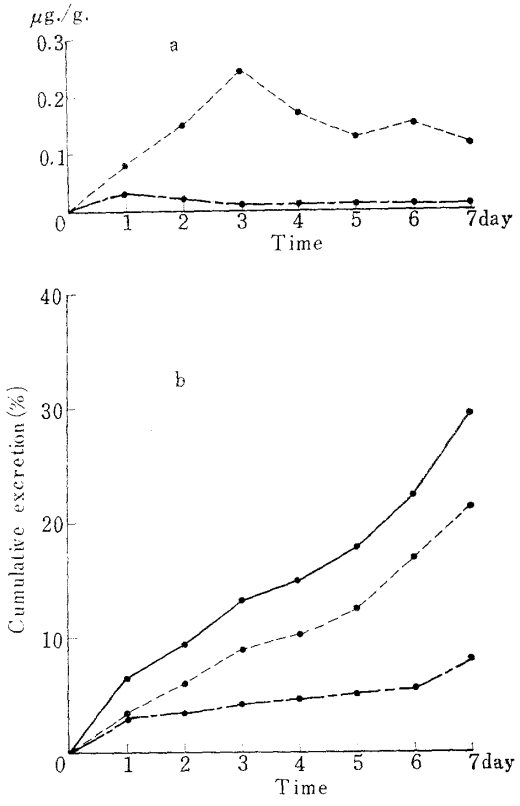

Fig. 10. Average Daily and Cumulative Excretion of EMU with 3 Rabbits Following a Week's Oral Administration of α-Lipoic Acid (10 mg./day) before Experiment

Dosage : 100 μg.
Vehicle : Carbowax 2000
a : Concentration
——— Urine
– – – – – Feces
b : Cumulative excretion
——— Total
——— Urine
– – – – – Feces

a suggestion is made that the saturation of EMU in those organs tends to be obtained during the earlier period of experiments. This assumption can be fortified by the amounts of deposition of mercurials per gram of tissue (Table Ⅳ). Table Ⅳ evidences that the concentration of mercurials in the liver or kidney is more than ten times that in other organs which possess a low and almost an equal affinity with mercurials. The gross findings of organs dissected 10, 20, and 30 days after initial administration which was followed by consecutive daily administration (Table Ⅴ) will be added in another paper with further observation on a pathological bases.

Influences of Additions of BAL or α-Lipoic Acid on the Excretion of Mercurials

An attempt was made to study effects of BAL and α-lipoic acid on the excretion of mercurial compounds after single dose administration (Figs. 7 and 8). One hundred mg. of BAL was intramusculary administered to rabbits immediately after a single insertion of PMA suppository. Ten mg. of α-lipoic acid was orally administered for seven consecutive days prior to a single insertion of a suppository containing either PMA or EMU. The animal's excreta were treated and subjected to the estimation of the rate of mercurial excretion with the method already described.

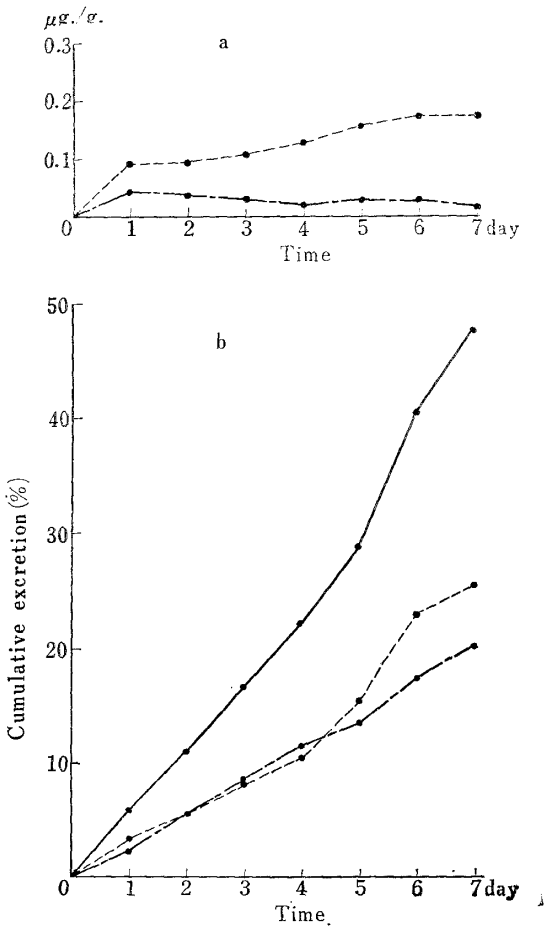

Fig. 11. Averge Daily and Cumulative Excretion of EMU with 3 Rabbits Following a Single Intravenous Administration

Dosage : 100 μg. of EMU in 1 ml. of saline

a : Concentration
——— Urine
– – – – – Feces

b : Cumulative excretion
——— Total
— — — — Urine
– – – – – Feces

When the excretion curves in Fig. 4 and Fig. 7 were compared, it was revealed that addition of BAL resulted in an increase of urinary excretion of PMA, but no influence was observed with fecal excretion.

The addition of α-lipoic acid showed a two fold increase in the urine and no increase or decrease in the feces of PMA (Fig. 2 and Fig. 8). A simillar result was also observed for EMU following the addition of α-lipoic acid (Fig. 9 and Fig. 10).

The present data obtained support to assume that α-lipoic acid may be analogous to BAL so far as the acceleration of mercurial excretion is concerned. This assumption seems to be worthy of further study.

A comparative study was made of the amounts and rates of mercurials excreted into the urine following a single intravenous administration of 100 μg. of EMU dissolved in 1 ml. of saline. The excretion of mercurial into the urine following intravenous administration of EMU was three times that following vaginal administration, while the excretion with fecal specimens was the same for the two routes of administration (Fig. 9 and Fig. 11). A simillar result was also observed for PMA.

It was demonstrated that the excretion of mercurial into the urine following intravenous administration of the mercurial compounds was higher or not less than following vaginal application. This confirms that the vaginal application is free from a risk of the intravaginally retained amount of PMA or EMU being washed out into the urine.

The authors wish to express their heartfelt thanks to Dr. W. M. Cox, Jr., former director of Scientific Relation and Dr. W. T. Sumerford, director of Pharmaceutical Chemistry, Mead Johnson & Company for their valuable assistance and co-operation in carrying out this work.

Summary

The excretion and tissue distribution of ω-ethylmercuri(Hg^{203})-thio-*n*-undecanoic acid (EMU) after vaginal administration were pursued in comparison with phenylmercuric (Hg^{203})-acetate (PMA).

PMA was excreted into feces and urine twice as much as EMU when a Carbowax 2000 suppository was employed.

The excretion of EMU was almost equal irrespective of the use of Carbowax 2000 or Rx 6 (emulsion vehicle containing stearic acid). A decrease in total excretion of PMA by the use of the emulsion vehicle was observed over a Carbowax 2000 vehicle. The decrease would indicate a decrease in absorption due to a change of PMA into more lipophilic stearate.

Cumulative excretion of PMA after repeated daily administrations showed a linear relationship, but in the case of EMU, a more increase of excretion was observed, showing a correspondence with the accumulation in tissue. Both PMA and EMU showed a high mercury storage in the liver and kidneys.

A comparative study was made of the amounts and rates of mercurials excreted following a single intravenous administration. The excretion of mercurial into the urine following intravenous administration of the mercurials was higher or not less than vaginal application. Acceleration of mercurial excretion was observed when BAL or α-lipoic acid was given simultaneously with insertion of a suppository containing mercurials.

(Received November 21, 1963)