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CHANGE OF NUCLEIC ACID FRACTION of BONE MARROW IN BENZENE POISONED RABBIT

Midori OKA and Masami KIMURA

The haematological changes of benzene poisoning may be explained by mitotic inhibition in the bone marrow[1]. However, very few informations are available on changes of biochemical substances in the benzene poisoned bone marrow[2,3]. Since the nucleic acid plays an important role in the cell division, it is reasonable to assume that the nucleic acid may be effected qualitatively or quantitatively in benzene poisoning. The present study was undertaken to provide mainly quantitative data on the nucleic acid fraction of rabbit bone marrow treated with benzene.

Experimental Methods

Treatment of Rabbit Bone Marrow

Thirty-two female rabbits were subjected to the injection of a mixture of equal parts of benzene and pure seasame oil. The animals were received daily one milliliter of benzene per kilogram of body weight for seven days, and killed on the 8th day. As the control, 2 female rabbits were given only the seasame oil.

Shortly after the rabbit was killed by injection of air into the blood vessel, the bone marrow tissue was carefully taken out from the humerus, femur and tibia in a room of low temperature (2～3°C). The procedure of chemical fractionation of the tissue was immediately carried out on each five animals. At the same time the cell nuclei were prepared from the bone marrow tissue of 27 rabbits. Control animals were used only for the chemical fractionation of the tissue.

The cell nuclei were isolated from the bone marrow tissue with Mirsky's method[4]. Fifty grams of the frozen tissue were placed in a blendor vessel together with 50 ml. of 0.5 M sucrose and 400 ml. of 0.25 M sucrose-0.0033 M $CaCl_2$. The mixture was gently homogenized by running the blendor at a low speed for 4 minutes, followed by a high speed for one minute. The homogenate was filtered through nine layers of gauze. The filtrate was centrifuged at 1,000 g for 7 minutes at 2°C and the supernatant was discarded. The sediment was resuspended in 100 ml. of 0.25 M sucrose-0.0030 M $CaCl_2$ and the suspension was again homogenized at low speed and was washed in the centrifuge at 1,000 g for 7 minutes. Further washings with the same solution in the centrifuge were repeated two or three times.

The number of nucleated cells for the wet weight of the bone marrow tissue was counted with Gerades method[5], and expressed as the ratio to the wet weight of isolated nuclei. In the case of chemical fractionation, the number of nucleated

cells was counted in advance of the chemical procedure, and expressed in the same way.

Chemical Fractionation of Bone Marrow Cells and Nuclei

The acid soluble fraction, lipid fraction, RNA fraction, DNA fraction and the residue were separated from the bone marrow cells as well as from the isolated nuclei by the modified Schmidt-Thannhauser's method[5]. The wet tissue or the isolated nuclei were homogenized in a blendor vessel with 0.6 N perchloric acid at the high speed. The homogenate was centrifuged at 12,000 rpm for 20 minutes at 2°C. In some cases the centrifuging duration required one hour as to clear the supernatant. The supernatant fluid was taken off with a pippete and the precipitate was washed twice with 0.6 N perchloric acid by the same procedure. The collected supernatants were called the acid soluble fraction. The final precipitate was washed with 95% alcohol, 95% alcohol-ether (1 : 1v/v) and ether in the centrifuge at 12,000 rpm for 15 minutes. Each organic solvents containing lipid substances were collected and were designated as lipid fraction. To the precipitate 0.3 N KOH solution was added and the mixture was incubated at 37°C for 24 hours. The solution was neutralized with 6 N HCl and was added with 60% perchloric acid to 5% of final concentration. The suspension was centrifuged at 12,000 rpm for 15-60 minutes at 1°C and the supernatant was discarded. Such extraction was repeated two or three times. 5% perchloric acid was added to the remained precipitate and this mixture was centrifuged. Supernatants containing RNA fraction were collected after each washing, and 5% perchloric acid was added to it as giving a definite volume. The sediment which remained in the cenrifuging tube was hydrolysated with 0.6 N perchloric acid at 100°C for 3 minutes, and the hydrolysate was centrifuged at 12,000 rpm for 15-60 minutes at 2°C. The precipitate was washed repeatedly with 0.6 N perchloric acid. The supernatants (DNA fraction), after hydrolysis and washing, were collected and added with 0.6 N perchloric acid to a definite volume. The residue was dried in vacuum.

Analytical Method

Sugar: The orcine reaction and diphenylamine reaction were applied for the determination of pentose and deoxypentose in RNA and DNA fraction, respectively[6,7,8]. The amounts of RNA and DNA in the bone marrow tissue were calculated by these sugar contents.

Phosphorus: The amounts of phosphorus in each fraction were measured according to Allens method[9].

Nitrogen: The amounts of nitrogen in some fractions were determined with micro Kjehldal method using H_gSO_4 as the catalyst of the digestion[10].

Ultraviolet Absorption: Measurements of ultraviolet absorption for RNA and DNA fraction were carried out with spectrophotometer. The ratio of the optical density

at 260 mμ to that at 230 mμ in a fraction was calculated from the absorption curve. The value of a molar absorptivity at 260 mμ, e (P), based one gram-atom of phosphorus per liter was obtained from the optical density and the amounts of phosphorus.

Experimental Results

The body weights, numbers of white cells of the rabbits which were treated or untreated with benzene, and the counts of nucleated cells or nuclei of the bone marrow are illustrated in Table 1. The loss of body weight of benzene treated

Table 1. Body weight, number of white cell and bone marrow of rabbit.

Rabbit No.	Administration	Body Weight (g)		Number of white cell		Bone marrow tissue (g)	Number of nucleated cells or nuclei	
		(B)	(A)	(B)	(A)		$\times 10^9$/Tissue	$\times 10^6$/mg of Tissue
1	non		2375		5070	5.04	6.15	1.22
2	non		1765		9133	5.31	7.86	1.48
3	oil	1945	2150	8133	6666	5.65	7.23	1.28
4	oil	1825	2140	6200	5500	4.13	6.90	1.67
5	benzene	1605	1270	8033	570	4.38	0.40	0.09
6	benzene	1625	1310	9360	3000	4.88	0.83	0.17
7	benzene	1655	1340	6933	533	4.21	0.40	0.09
8	benzene	1630	1465	7066	1233	4.31	0.72	0.17
9	benzene	1265	1320	7566	1131	5.87	0.48	0.08
10	non		1720*		8765*	137**	1.31″	1.75
11	non				9083*	6.3**	2.14″	1.75
12	benzene	1740*	1510*	10583*	1986*	138**	0.38″	0.87

Column (B) shows the value before the treatment with benzene and column (A) shows the value after the administration for a week.

* Sign presents the mean value. No.10: 27 rabbits, 11: 2 rabbits, 12: 27 rabbits.

** Sign presents the total weight of rabbit's bone marrows which are treated with benzene.

″ Sign shows the value for the wet weight of the isolated nuclei. No. 10: 0.749 g, 11: 1.220 g, 12: 0.432 g.

animals was observed except one animal, and the number of white cells showed a noticeable decrease after administration of benzene. However, wet weight of bone marrow in treated animal did not show a significant decrease compared to that of untreated. The number of nucleated cells or nuclei fell off remarkably in the bone marrow of the rabbit which was injected with benzene. The body weight, the number of white cells in the bone marrow of seasame oil injected rabbit, which was retained as control was the same as their values of the rabbit which was not injected with reagents.

As shown in Table 2, the phosphorus content in RNA and DNA fraction were expressed as phosphorus per 100 mg. of the tissue as well as phosphorus per nucleated cell or nucleus. In both fractions the amounts of phosphorus per tissue decreased

Table 2. Amounts of phosphorus in RNA and DNA fraction

Rabbit No.	RNA fraction			DNA fraction		
	10g of P 100mg of tissue	10g of P a nucleated cell or a nucleus	N/P	10g of P 100mg of tissue	10g of P a nucleated cell or a nucleus	N/P
1	24.8	0.21		47.6	0.38	
2	33.6	0.23		55.6	0.38	
3*	31.8	0.215		55.3	0.39	
4*	35.8	0.25		65.5	0.43	
8**	14.5	0.87		3.0	0.27	
9**	9.5	1.215		4.2	0.49	
10	88.6	0.51	3.95	168	0.96	2.45
11	83.6	0.48	3.97	145	0.735	2.45
12**	65.6	0.765	7.45	35	0.35	9.23

* Sign shows control rabbit and ** sign shows the benzene treated rabbit.

in the benzene treated animals but the amounts of phosphorus per nucleated cell or nucleus were different in RNA and DNA fraction. The value in the former increased, while the value in the latter remained unchanged or decreased in the benzene treated animals. The ratio of P/N in both fractions increased significantly in the benzene treated animals as shown in Table 2.

Characterizations of the absorption curves of RNA and DNA fraction, the ratio of E_{260} and E_{230} and e(P) at 260 mμ were shown in Table 3.

Table 3. Data obtained from the absorption curves of RNA- and DNA- fractions

Fraction	RNA				DNA			
Rabbit No.	Wave length		E_{260}/E_{230}	e_{260} (P)	Wave length		E_{260}/E_{230}	e_{260} (P)
	min.	max.			min.	max.		
1	237	260	1.4	16,300	235	267	1.5	11.300
2	236	261	1.45	14,300	235	266	1.7	10,000
3//	237	261	1.4	15,900	236	267	1.7	9,600
4//	237	261	1.4	15,700	236	267	1.5	9,200
8*	241	261	0.8	15,100	240	257	0.7	25,600
9*	241	261	0.8	15,600	240	254	0.7	20,600
10	235	258	1.5	11,500	233	266	1.6	10,900
11	234	259	1.8	12,100	235	267	2.1	10,300
12*	238	262	1.5	13,300	236	267	1.7	28,200

// Control, * Treated with benzene.

Some examples of the curve were presented in Fig. 1, 2, 3 and 4. In RNA and DNA fraction, slight transfer of the wave length of min. and max. absorbance was recognized in the benzene poisoning. There was a considerable increase in e(P) of DNA fraction but not in e(P) of RNA fraction. In both fractions which were

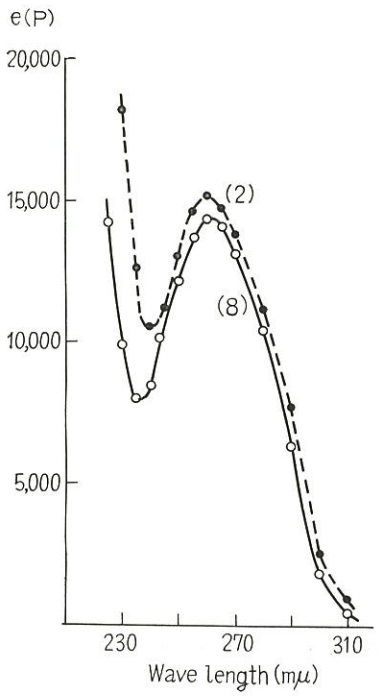

Fig. 1. Absorption curves of cell-RNA fraction

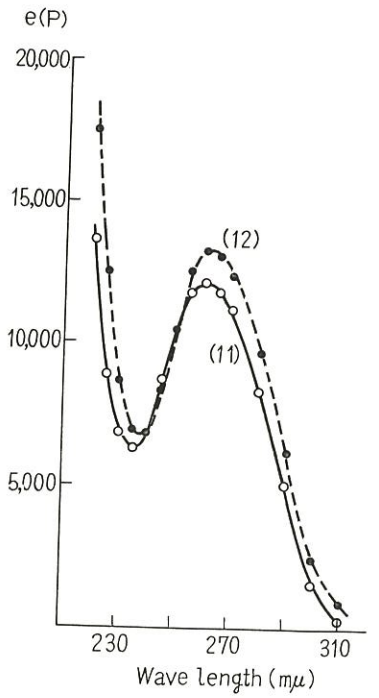

Fig. 2. Absorption curves of nuclei-RNA fraction

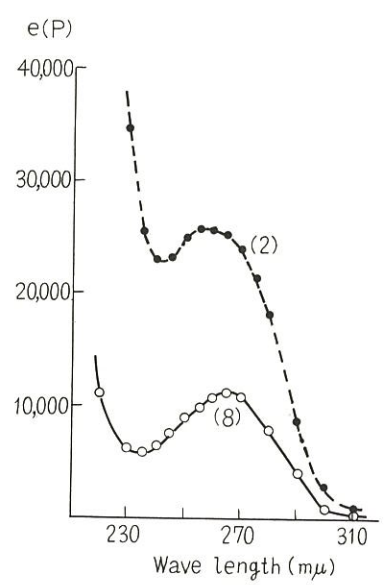

Fig. 3. Absorption curves of cell-DNA fraction

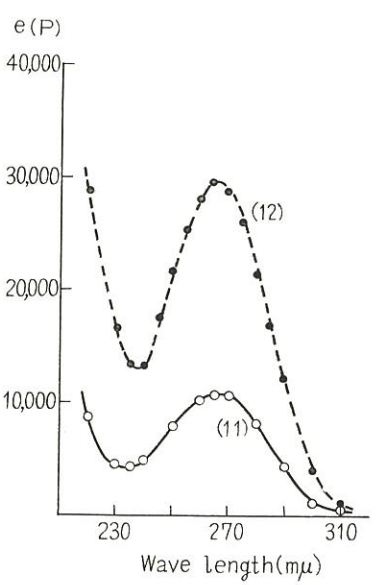

Fig. 4. Absorption curves of nuclei-DNA fraction

Table 4. RNA and DNA contents of rabbit bone marrow

Rabbit No.	Fraction	RNA measured with orcine reaction mg.	DNA measured with diphenyl-amine reaction mg.	Corrected RNA mg.	Corrected DNA mg.	Content for 100mg. of tissue# RNA mg.	Content for 100mg. of tissue# DNA mg.
1	RNA-	25.0	5.0	24.4		0.48	
	DNA-	4.1	44.55		49.5		0.98
2	RNA-	30.1	6.4	31.65		0.60	
	DNA-	11.3	71.9		78.3		1.47
3〃	RNA-	27.3	8.9	26.55		0.64	
	DNA-	7.85	59.6		68.5		1.16
4〃	RNA-	32.5	8.4	31.45		0.58	
	DNA-	8.9	72.0		80.4		1.47
8*	RNA-	11.1	2.05	10.9		0.25	
	DNA-	1.1	8.3		10.4		0.24
9*	RNA-	11.6	1.7	11.4		0.19	
	DNA-	1.2	9.1		10.8		0.20
10	RNA-	5.9	3.7	5.4		0.73	
	DNA-	2.5	23.7		27.4		3.66
11	RNA-	12.25	3.9	11.8		0.97	
	DNA-	1.9	51.1		55.0		4.51
12*	RNA-	1.9	2.9	3.4		0.79	
	DNA-	3.3	11.1		14.0		3.24

〃 Control, * treated with benzene # No. 10, 11, 12 : content for the nuclei

Table 5. RNA and DNA per nucleated cell or nucleus of rabbit bone marrow.

Rabbit No.	RNA estimated from 10^{-9}g			DNA estmated from 10^{-9}g		
	P′	base′	sugar	P″	base″	sugar
1	2.5	3.6	3.8	4.2	5.4	8.1
2	2.8	3.6	4.0	4.2	9.9	10.0
3*	2.6	3.7	3.9	4.3	9.2	10.0
4*	3.0	4.3	4.4	4.8	6.5	11.1
8**	10.5	14.1	15.2	3.0	8.7	14.5
9**	14.6	19.5	23.6	5.4	6.3	22.4
10	6.1	6.4	4.2	10.6	13.1	20.9
11	5.8	5.9	5.5	8.2	9.0	25.7
12**	9.2	9.9	9.1	3.9	10.8	37.0

* Sign shows control rabbit and ** sign shows the benzene treated rabbit.

′ Supposed that RNA-P was 8.3% and e(P) was 11,000, RNA was calculated by each measured value.

″ Supposed that DNA-P was 9.1% and e(P) was 8,800, DNA was calculated by each measured value.

obtained from tissue, E_{260}/E_{230} decreased in the benzene poisoned animals. These results might be caused not only by the changes in the nucleic acid but also by the contaminants which were produced on metabolic pathway in the abnormal cell condition.

Since orcine reaction is not only specific for the pentose but also for the deoxy-

pentose, calculation of the amounts of RNA and DNA from sugar contents of RNA and DNA fraction must by very careful. If the diphenylamine should not react perfectly with pentose and the orcine reacts with the deoxyribonucleic acid at the color yield of 1/8 for the ribonucleic acid*, the amounts of RNA and DNA could be estimated by both methods and thus obtained data are indicated in Table 4. RNA and DNA content in 100 mg. of tissue decreased in benzene treated animal, but these in the isolated nuclei did not show any decrease.

Discussion

It is generally accepted that the benzene attacks the haematogenic organs, and in this experiment the nucleated cell counts of bone marrow in the rabbit which was treated with benzene decreased remarkably. In spite of the decrease of cell counts, the bone marrow tissue did not show any noticeble weight loss. The bone marrow of treated animals seemed to be different from the normal one when it was taken out, that is, it was rather slushy. From these, it seems the bone marrow of the treated animal takes a different composition of intercellular substances in addition to the cell damage.

In the poisoned animals, the amounts of RNA-P and DNA-P which were estimated as the value for the weight of tissue decreased as presented in Table 2. But the detailed discussion on the change of nucleic acid in the benzene poisoning seems to be difficult from these values, as the number of cells fluctuated widely. So, the amounts of RNA and DNA per nucleated cell or nucleus were calculated from the assumption that RNA and DNA can be estimated with the contents of phosphorus and the extinction at 260 mμ by using a suitable correction factor. Obtained values are shown in Table 5. As the nucleotide is composed of one mole of phosphate, base and sugar, the values of RNA and DNA calculated from each element may be expected to take the same figure theoretically. But, as shown in Table 5, three values of RNA or DNA did not coincide. The reason for this is that, as shown in Table 4, RNA fraction contains a small amounts of DNA, while DNA fraction contains a tiny amounts of RNA. Therefore, if the DNA or RNA values calculated from phosphorus or base is corrected concerning the contaminated RNA or DNA respectively from the data obtained by sugar method, a good agreement will be expected between three values calculated from each element. But, as experimental results show this agreement was not obtained among these corrected values, especially in DNA.

It seems to be unreasonable that the total RNA in a cell is less than the nuclear RNA and the amounts of DNA in a cell are not in agreement with the amounts of it in a nucleus. Perhaps it would be caused by the counting procedure of nuclei as

* The value was confirmed in the determination of RNA (Daiichi Chemicals Co.) and DNA (Nutritional Biochemicals Co.) by the orcine and diphenylamine reaction at the same condition as the chemical fraction.

the denuded nuclei adhered each other.

The changes of nucleic acids in the bone marrow cells of benzene treated animals are clearly seen in the summarized Table 5. The amounts of RNA in a cell or nucleus increase considerably in benzene treated animals. Concerning DNA in benzene poisoning, DNA calculated from base showed no noticeble change, DNA from phosphorus showed no change in a cell but some decrease in a nucleus, and DNA from sugar increased in both cell and nucleus. Furthermore, it must be noticed that the increase of P/N of DNA fraction, abnormality of its absorption curve and its e (P) shows a great value. These results suggest that there may be a biochemical change in DNA fraction, especially with regard to the structure of DNA in the bone marrow of benzene poisoned rabbits.

Summary

1. Female rabbits were exposed to benzene for a week, and change of nucleic acid fraction in the bone marrow were studied.

2. The change of RNA and DNA content were estimated from the analysis of phosphorus, base and sugar in the bone marrow of the treated and untreated rabbits.

3. In benzene treated animal the total RNA in a nucleated cell and the nuclear RNA increased, while DNA calculated from base showed no noticeble change, DNA from phosphorus showed no change in a cell but some in a nucleus, and DNA from sugar increased in both cell and nucleus.

Acknowledgement

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NUCLEIC ACID OF BONE MARROW IN BENZENE POISONING

要　　旨

ベンゼン中毒時における家兎骨髄の核酸区分について

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ベンゼン中毒が放射線障害と同様造血臓器をおかし，白血球の減少などの症状を与えることはよく知られている事実であるが，近年，この中毒作用は細胞分裂を阻害するものであると考えられるようになつた。この点について生化学的な面から解明するためにベンゼン中毒時の骨髄の核酸区分について研究した。

家兎（メス）に体重 1kg 当り 1mℓ のベンゼンを皮下注射により１週間投与し，次の日屠殺した。直ちに humerus, femur, tibia の骨髄をとり出し分析に供した。一方，十数匹の家兎から得られた骨髄より Mirsky 法により細胞核を単離し，同様分析に供した。一部修正した Schmidt-Thannhauser 法により RNA および DNA 区分に分別し，各区分の燐含量を Allen 法により，糖含量をオルシンおよびヂフェニルアミン反応により，また塩基量を紫外部吸収により求めた。各測定値より有核細胞あるいは細胞核１個あたりの RNA および DNA 量を算出した。その結果，細胞中の RNA および細胞核中の RNA はベンゼン中毒時において増加した。DNA については，細胞として分析した場合と細胞核として分析した場合，塩基量から計算した DNA には変化がなかつた。しかし，燐含量からの計算では前者では変らず，後者では減少した。また，糖含量からの計算ではどちらの場合も増加した。これらの結果に加えて，DNA 区分の N/P 値の増加，吸収曲線の異常性，e (P) 値の増加などが認められ，ベンゼン中毒時の家兎骨髄の DNA 区分において何らかの生化学的変化が起つていると推論できよう。

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PRELIMINARY REPORT ON NUCLEOTIDES OF RABBIT BONE MARROW IN BENZENE POISONING

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It has been well known that the Xray-irradiation brings about the damage of the haematogenetic tissues and affects the biosynthesis of deoxyribonucleic acid. Recently, there has been reported that the nucleotides accumulate in some organs following the Xray-irradiation[1,2]. In the benzene poisoning, a similar damage of the bone marrow is recognized pathologically. The present paper reports how much nucleotides accumulate in the rabbit bone marrow in the benzene poisoning.

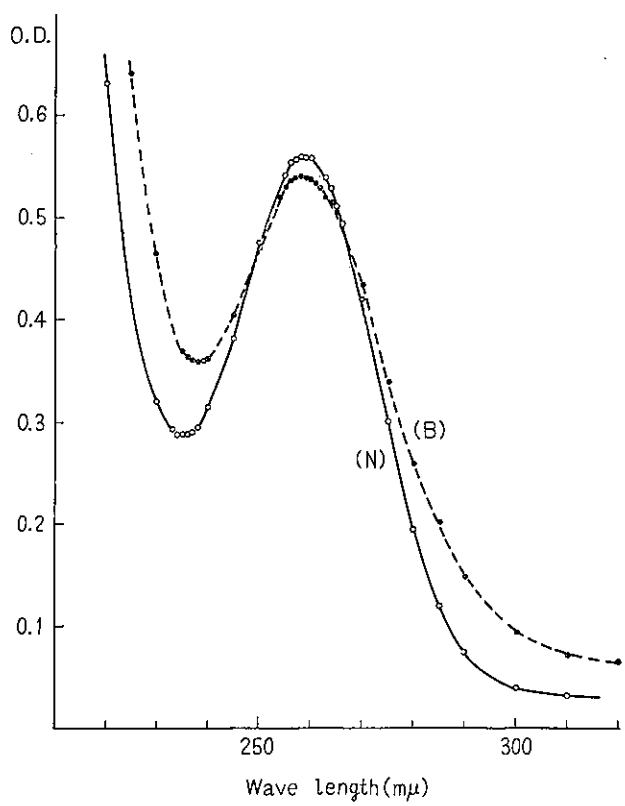

Fig. 1. Absorption curves of acid soluble fraction.
curve (N): untreated, diluted with three times
curve (B): treated, diluted with three times

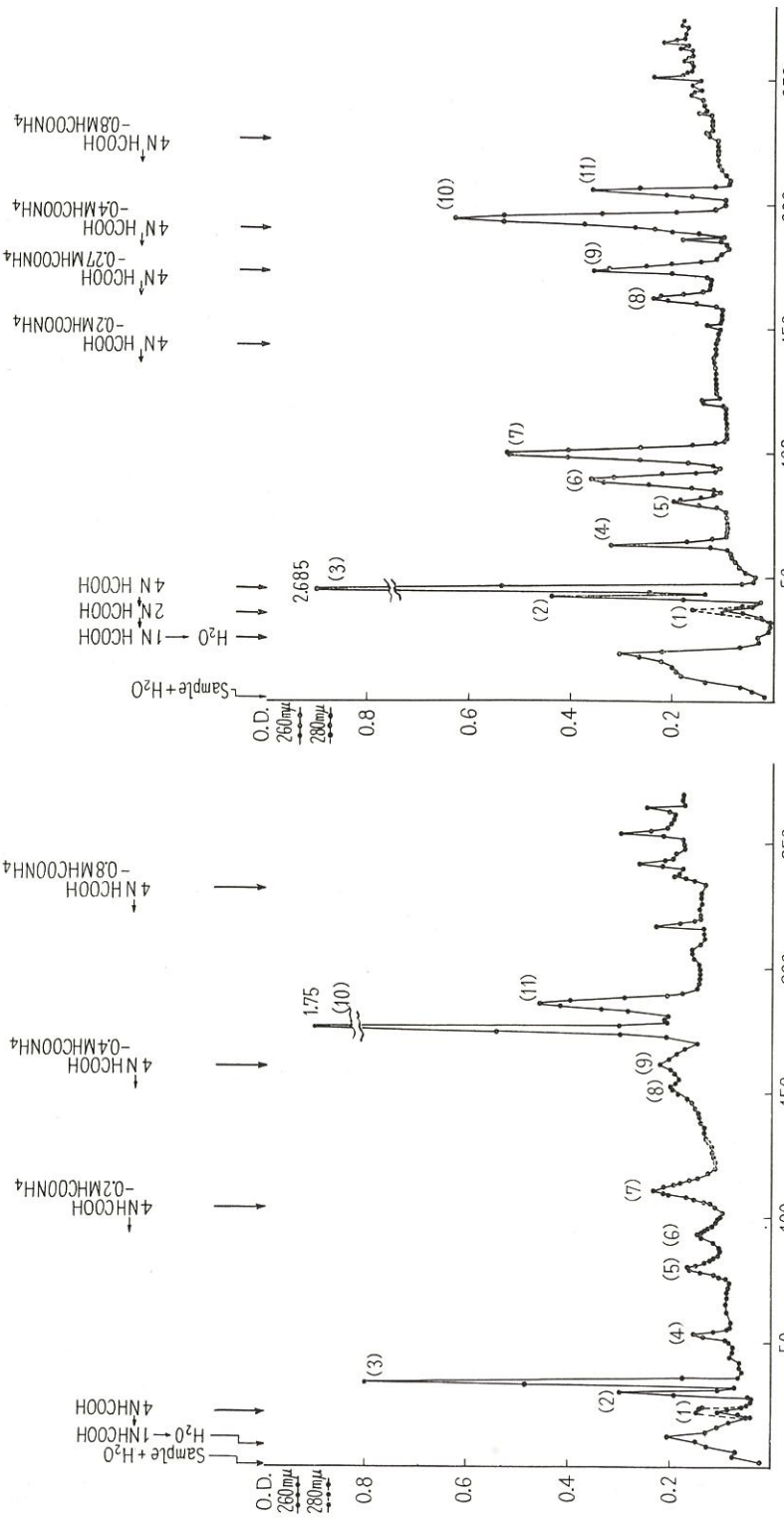

Fig. 2. Chromatography of the acid soluble fraction from the bone marrow of the benzene untreated rabbit on column of Dowex-1-formate (26×0.9 cm)

Fig. 3. Chromatography of the acid soluble fraction from the bone marrow of the benzene treated rabbit on column of Dowex-1 formate (26×0.9 cm)

In Fig. 2 and 3, The elution position of peak (1), (2), (3), (4), (5), (6), (7), (8), (9), (10) and (11) may perphaps correspond to CMP, DPN, AMP, TPN, GDP, UDP (CDP), ADP, UDP ?, GDP ?, ATP and GTP (UTP).

EXPERIMENTAL

Female rabbits were injected with benzene (1ml./kg. of body weight) for a week. They were killed on the 8th day and the bone marrow tissue was immediately taken up from humeri, femur and tibia in a room with low temperature. The tissue was homogenized with 0.6 N perchloric acid in a blendor at a high speed for 5 minutes. The mixture, without passing through the layers of gauze, was centrifuged (30 min. at 12,000 rpm) refrigatorly. There were some fat-like materials floating in the supernatant fluid. The supernatant was sucked up gently by a pippete and the remained precipitate was washed twice with 0.6 perchloric acid in a centrifuge tube. The collected supernatants were not perfectly clear. The solution was neutralized with 6 N KOH at low temperature until its pH became about 7. The precipitate which occured in the neutralization was taken off by centrifuging (15 min. at 12,000 rpm at 1°C) and it was washed with water in a centrifuge. These supernatants were collected and were called the acid soluble fraction. Its absorption curve was shown in Fig. 1.

In benzene treated rabbit 50 ml. of the acid soluble fraction was chromatographed on column of Dowex-1[8], 0.9 cm. in diameter and 26 cm. in height, and in untreated rabbit 20 ml. of the acid soluble fraction was chromatographed on the same column. A continual change in the eluent was achieved by adding 1 N formic acid to a mixing chamber initially filled with water and then by adding successively 4 N formic acid, 4 N formic acid containing 0.2 M ammonium formate, 4 N formic acid containing 0.4 M ammonium formate and 4 N formic acid containing 0.8 M ammonium formate. The column was operated at room temperature. 3.7 ml. and 3.2 ml. of effluent were collected with the fraction collector, respectively. The optical density of the effluent was measured at 260 mμ and 280 mμ with ultraviolet spectrophotometer. The elution curves of the both chromatography are shown in Fig. 2 and Fig. 3. The nucleotide contained in each fraction was judged by the order of elution. The mole of nucleotide was calculated with the following assumption, E_{260}/mmole : 14.2, 11.8 and 9.9 for adenylic, guanylic and uridylic, E_{280}/mmole : 13.0 for cytidylic, respectively. In Table 1, content of nucleotides in the acid soluble fraction in the bone marrow of benzene treated and untreated rabbit is expressed as the value per gram of the bone marrow tissue and per nucleated cell.

DISCUSSION

It is dominated by the expression of the amounts of nucleotides whether they accumulate in the bone marrow cell of the benzene treated rabbit or not. As shown in Table 1, when the amounts of nucleotide are expressed per gram weight of the tissue, free nucleotides contained in the bone marrow cells are shown to decrease in the benzene poisoning. However, when the amounts of nucleotides are calculated

Table 1. Nucleotides of rabbit bone marrow which is untreated or treated with benzene.

Condition	Untreated		Treated		Ratio**
Nucleotide	$\frac{\mu mole}{g}$	$\frac{\mu mole \times 10^{-9}}{cell}$	$\frac{\mu mole}{g}$	$\frac{\mu mole \times 10^{-9}}{cell}$	
Cytidylmonophosphate	0.050	0.025	0.037	0.30	8
Adenylmonophosphate	0.284	0.186	0.194	2.28	12
Guanylmonophosphate	0.085	0.022	0.058	0.27	5
Uridylmonophosphate	0.093	0.080	0.064	1.13	21
Adenyldiphosphate	0.242	0.107	0.165	1.29	8
Guanyldiphosphate	0.121	0.036	0.082	0.46	6
Uridyldiphosphate	0.206	0.074	0.141	0.90	6
Adenyltriphosphate	0.600	0.155	0.409	1.87	5
Guanyltriphosphate*	0.298	0.051	0.170	0.53	3

The wet weight of the untreated and treated rabbit bone marrow is 2.75 g and 5.87 g, respectively. The number of nucleated cells is 4.033×10^9, and 0.484×10^9, respectively.

** Ratio shows the value of (mole of nucleotide at untreated/cell)/(mole of nucleotide at treated/cell).

* The fraction may contained uridyltriphosphate.

per nucleated cell, it is demonstrated that free nucleotides increase in that state. This result may be elucidated by the fact that the weight of tissue remains unchanged but the number of nucleated cell decreases notably in benzene poisoning. As RNA in the bone marrow cell increases in the benzene poisoning, the accumulation of nucleotides in the bone marrow cell may be caused by the inhibition of the biosynthesis of DNA. It is not known whether this inhibition is brought into the metabolic pathways of nucleotides or it is brought into the process of polymerization, but the accumulation of nucleotides indicates that any change in the biosynthesis of DNA occurs in the bone marrow cell of benzene treated rabbit.

Summary

The acid soluble fraction was prepared from the bone marrow in benzene treated and untreated rabbit. Nucleotides contained in the fraction were fractionated with column chromatography of Dowex-1-formate. Nucleotide per nucleated cell shows that each free nucleotide is accumulated in the bone marrow cell in benzene treated animals as compared with that in untreated ones.

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M. KIMURA

要　　旨

ベンゼン中毒時の家兎骨髄に於けるヌクレオチドに関する知見

木　村　正　己

核酸合成に対する放射線の作用についてはかなり広く研究されているが，放射線障害に類似した血液所見を呈するベンゼン中毒の場合に，核酸合成が如何に影響されるかという報告は殆んどない。ベンゼン中毒の病理学的所見として，骨髄細胞に異常が見られ，殊に核分裂異常像が認められた事は放射線障害の場合と対比して非常に興味深い。近年，X線照射後，胸腺とか脾臓のような臓器においてかなり多量のヌクレオチドの蓄積があることが見出され，放射線の影響の一つとして，ヌクレオチドの重合反応が阻止されるのではないかという知見が呈出されている。そこで，ベンゼン中毒の場合の骨髄においても同様な蓄積が起っているのではなかろうかという点について実験したので，その結果を茲に報告する。

体重 1kg 当り 1 m*l* のベンゼンを7日間投与した家兎の骨髄（上腕骨，脛骨及び大腿骨）をとり出し，0.6N 過塩素酸にて処理した。この酸可溶性区分中に存在するヌクレオチドをイオン交換樹脂 Dowex-1 (0.9×26cm) のカラムを用い，4N 蟻酸および (0.2～0.8M) ギ酸アンモニウムの溶離液で漸増的溶出によるクロマトグラフを行い，正常な家兎の骨髄から得られたものと比較した。少くともこのクロマトグラフでは異った溶出ピークを見出すことはできなかったが骨髄細胞1個あたりのヌクレオチドモル数を比較してみると，ベンゼン中毒時の方が高い値を示すことが見出された。そこで，X線照射の場合と同様にベンゼン中毒によっても骨髄にヌクレオチドの蓄積が起ると考えられる。

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EXPERIMENTAL STUDIES ON CADMIUM STEARATE POISONING

II. DISTRIBUTION AND EXCRETION OF CADMIUM IN RATS

Hiroshi YOSHIKAWA, Noboru HARA and Kiyoyuki KAWAI

In the first reports[1], authors reported on the dissociation and the intraperitoneal LD_{50} of cadmium stearate. We have further studied on the excretion and the distribution of administered cadmium stearate and have attempted to illustrate the chemical nature of cadmium in feces of cadmium stearate.

Methods

Male rats (Wister-King Strain) were injected subcutaneously and intraperitonealy as 3 mg. of cadmium per kilogram of body weight for cadmium stearate. The same dose of cadmium chloride was also injcted to other rats similarly to compare it with cadmium stearate.

After the administration of cadmium stearate and chloride, the animals were observed daily for their body weight changes, and their urine and feces were collected

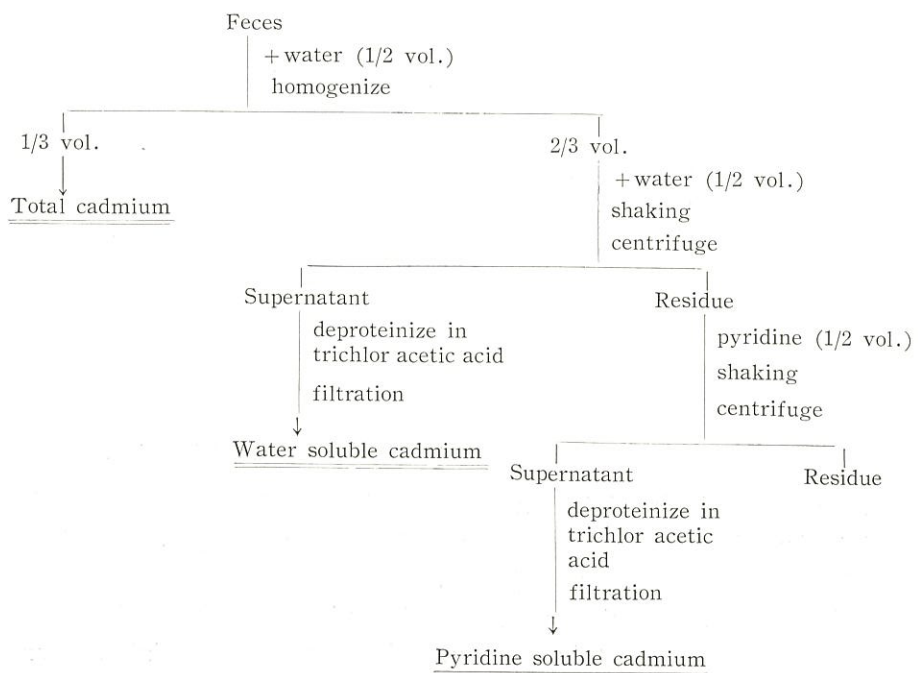

Fig. 1. Method of separation of cadmium compounds in feces.

daily for 7 days. On the eighth day animals were killed, and the various organs were resected. The cadmium contents of all subjects were determined by the Dithizone method[2] after wet ashing with nitric, sulfric and perchloric acid.

For the observation of fraction of cadmium excreted in feces, two groups of each five male rats received intraperitoneally single injection of 3 mg. of cadmium per kilogram of body weight as cadmium stearate and chloride. On the first and second day after injection the feces in each of the five rats were collected together. The water soluble cadmium and the pyridine soluble cadmium in feces were separated by methods as shown in Figure 1.

Results

1. *The Effect of injected Cadmium on the Changes of Body Weight in Rats*

The fluctuation of body weight in each rats following injection of cadmium stearate and chloride showed the same type of changes for the same route of injection. The average of three rat's weight are shown in Figure 2.

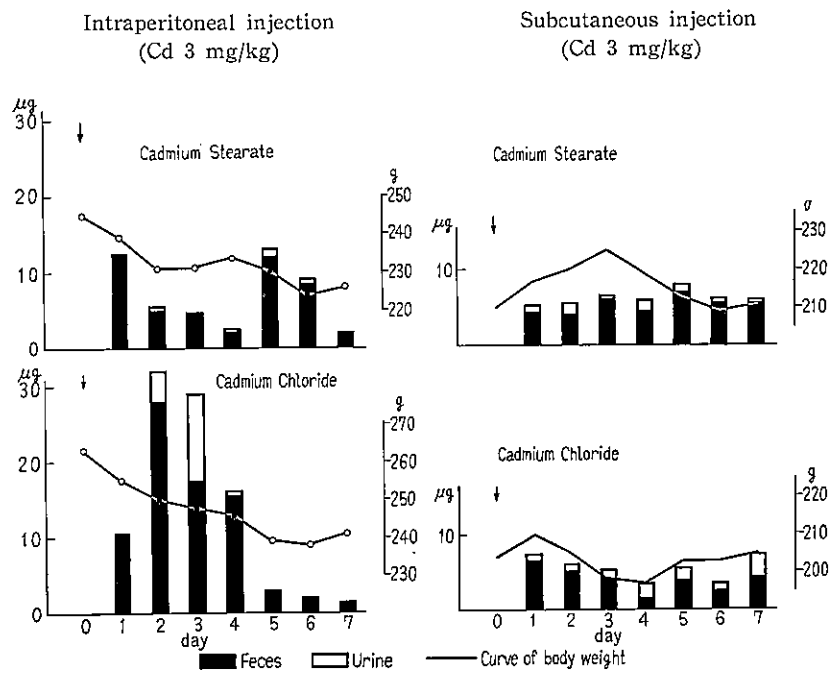

Fig. 2. Excretion of cadmium after single administration.

In the intraperitoneal injection groups, body weight decreased from the first day after injection with cadmium stearate and chloride respectively, and tended to recover from about the sixth day after injection.

In the subcutaneous injection groups, the body weight of cadmium stearate injected rats showed tendency to increase until the third day after injection and thereafter decreased, and again tended to increase from the seventh day. On the other

hand, the cadmium chloride injected group showed to increase on the first day after injection, then body weight progresively decreased, and again showed the process of recovery from the fifth day.

2. *Excretion of Cadmium after single Injection*

Excretion experiments of cadmium in urine and feces after intraperitoneal or subcutaneous injections were performed with cadmium stearate and chloride. The daily excretion of cadmium after injection is shown in Table 1 (A, B) and summarized in Figure 2.

Table 1. The urinary and fecal excretion of cadmium after single injection as 3mg. of cadmium per kilogram of body weight. (μg. of cadmium per day)

(A) Intraperitoneal injection

		Cadmium Stearate								Cadmium Chloride							
Rat No.		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	Rat No.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
1	urine	0	0	0	0.8	1.8	0	0	2	0	0	0	0	0	—	—
	feces	18.7	8.4	9.3	1.1	22.6	14.9	1.4		30.6	0	19.8	17.2	7.8	3.5	—
6	urine	0	0.8	—	0	—	0.6	0	4	0	0.5	0	0.5	0	0	0
	feces	6.4	1.7	0.4	2.1	1.4	2.2	2.0		0.8	6.0	5.4	0.8	0	3.6	3.9
11	urine	0	0	0	0	0	0	0	7	0	11.8	34.5	0	0	0	0
	feces	0	0	0	0.3	0	0	0		0	79.0	28.0	29.0	1.3	0	0

(B) Subcutaneous injection

		Cadmium Stearate								Cadmium Chloride							
Rat No.		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	Rat No.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
2	urine	0.5	1.7	0.4	1.2	1.0	0.6	0.5	6	1.8	0.6	1.0	1.5	1.8	1.0	5.0
	feces	3.2	1.9	3.0	1.3	4.3	4.1	4.1		4.3	1.0	1.5	1.2	1.6	2.2	—
3	urine	0.6	1.0	—	2.6	—	0.5	—	7	—	—	—	—	—	—	—
	feces	6.0	8.4	8.1	9.2	6.3	10.7	6.8		6.6	3.2	9.1	2.2	4.8	0.1	4.9
5	urine	—	—	—	—	—	—	—	8	1.0	0.8	1.6	2.0	1.0	1.1	0.9
	feces	5.7	1.8	6.5	2.7	10.3	2.0	6.5		5.5	11.6	8.9	0.9	3.2	4.4	3.6

In both groups of rats injected with cadmium stearate and chloride intraperitoneally, only small quantities of cadmium was found occasionally in the urine, and a the greater part was found in feces. This suggests the normal route of excretion of cadmium was via the gastrointestinal tract. From the observation of daily changes of cadmium excretion it was noted that cadminm stearate injected rats showed a slow excretion throughout all observation periods. On the contrary the group of cadmium chloride injected rats showed the maximum excretion at the second day,

thereafter the amount of excreted cadmium showed the progressive decreases.

In rats with the subcutaneous injection the values of cadmium excretion was about half of that of intraperitoneal injection. But the route of excretion of cadmium was mainly the gastrointestinal tract as in the intraperitoneal injection. However, small quantity of cadmium was excreted in urine every day unlike the intraperitoneally injected group. The daily amount of excreta did not show any difference between cadmium stearate and chloride administration groups, and the slow rate of excretion of cadmium was observed throughout experimental periods. However, the individual difference in cadmium excretion was remarkable in each rat.

3. *Distribution of Cadmium in the Body after single Injection*

The distribution of cadmium in the rat body at the eighth day after single injection are shown in Table 2 and data are summarized in Figure 3.

Table 2. Distribution of cadmium in various organs (μg. per gram of dry weight) at the eighth day after single injection. (Cd 3 mg/kg)

	Intraperitoneal Injection				Subcutaneous Injection	
	Cadmium Stearate		Cadmium Chloride		Cadmium Stearate	Cadmium Chloride
	Rat No. 1	Rat No. 6	Rat No. 2	Rat No. 4	Rat No. 2	Rat No. 8
Liver	52.5	40.9	64.4	66.7	107.6	42.8
Kidney	29.7	10.9	5.2	8.8	95.5	44.9
Stomach	17.3	8.4	2.0	27.5	17.3	0
Intestine	7.8	1.6	4.3	12.7	17.0	7.2
Mesosteniale	2.6	1.2	1.0	8.6	—	—
Spleen	10.9	23.5	0.9	19.5	26.8	6.5
Lung	12.7	3.5	3.5	13.5	3.1	2.3
Heart	15.4	18.7	2.6	10.6	9.3	5.8
Brain	7.0	—	—	—	—	—
Testicle	1.3	6.0	0.5	7.9	6.9	4.2
Blood	1.3	0	1.2	1.0	—	—

In rats which were administred single intraperitoneal injection with cadmium stearate and chloride (3 mg. of cadmium per kilogram of body weight), highest cadmium contents were recognized in the liver and relatively large amounts were found in kidney, spleen and stomach. After subcutaneous administration of same doses in rats, cadmium was chiefly deposited in liver and kidneys. Relatively large cadmium contents were demonstrable in spleen, stomach and intestine.

No difference in the distribution of cadmium in the rats body could be detected between cadmium stearate and chloride by intraperitoneal or subcutaneous administration of a single dose. However, significant difference in the distribution of cadmium in animals body was found concerning method of administration (intraperitoneal and

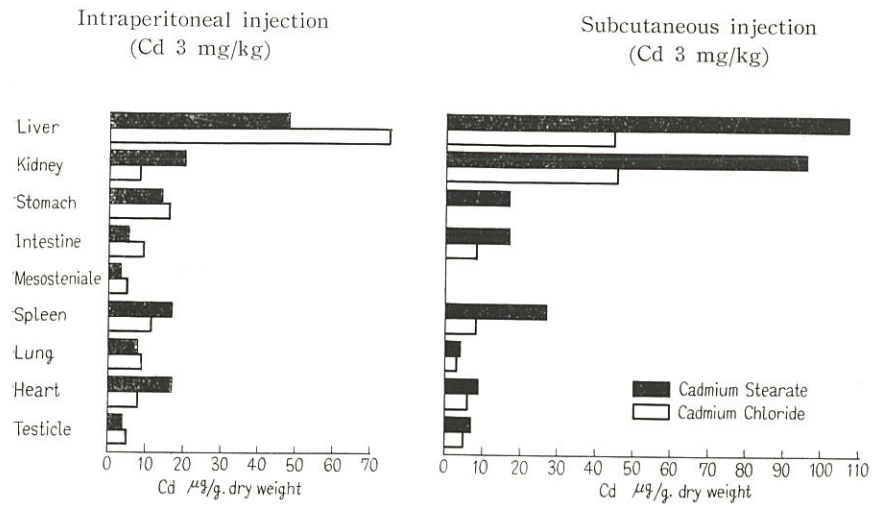

Fig. 3. Distribution of cadmium in various organs at the eighth day after single administration.

subcutaneous injection).

4. *Fraction of Cadmium in Feces*

To clarify the chemical nature of cadmium in feces, water soluble and pyridine soluble cadmium was separated by the extraction method as shown in Figure 1, and cadmium contents were determined in each extraction. Data are summarized in Table 3. As shown in this Table, the mean value of the water soluble cadmium con-

Table 3. Fraction of water soluble and pyridine soluble cadmium in feces.

		Total Cd. (γ)	Water Soluble Cd. (γ)	Pyridine Soluble Cd. (γ)	Total Cd-(Water Soluble Cd+Pyridine Soluble Cd) (γ)
Cadmium Chloride	I*	22.2	12.0	4.5	5.7
	II+	44.2	30.2	6.9	7.1
	%	100.0	61.2	17.9	20.9
Cadmium Stearate	I*	17.7	9.5	3.6	4.6
	II+	18.6	11.1	5.4	2.1
	%	100.0	56.7	24.7	18.7

* cadmium contents in feces in the first day after intraperitoneal injetion (Cd 3 mg/kg), and these values were represented together with five male rats.

\+ cadmium contents in feces in the second day after intraperitoneal injection (Cd 3 mg/kg), and these values were represented together with five male rats.

tents in cadmium stearate injected rats was 56.7% of total cadmium in feces, while it was 61.2% in cadmium chloride injected rats. This indicats that the water soluble cadmium content in feces was not remarkably different between both subjects injected. However, the mean value of the pyridine soluble cadmium was 24.7% of total cadmium in feces in the cadmium stearate injected group, and that of 17.9% with the cadmium chloride injected. That is, greater amount of pyridine soluble cadmium was excreted in cadmium stearate injected rats than cadmium chloride injected. It seems reasonable from the data to assume that some part of cadmium stearate injected intraperitonealy in rats was excreted as intact form i.e., cadmium stearate.

Discussion

It is an interesting fact that the clinical picture presented in the case of organometalic poisoning varies widely from that of inorganic metal poisoning. And organometalic compound, together with the advances in complex compound, have a tendency of increasing use in modern industry. Accordingly the investigation on the mechanism of action of organo-metal in body is an important problem in the study of occupational disease.

Authors investigated the toxicity of cadmium stearate, and have already reported in the previous paper[1] on the dissociation and toxicity by means of intraperitoneal LD_{50} of cadmium stearate. The present paper still refers to the excretion and distribution of cadmium after single injection of cadmium stearate and reports whether the cadmium stearate has the same toxic effect as cadmium chloride.

Following single intraperitoneal injection, body weight changed similarly in the cadmium stearate and chloride injected groups, but following the subcutaneous injection, the fall of body weight began later and recovered slowlier in cadmjum stearate injected group than cadmium chloride injected rats. It is a well known fact that the absorption of various subjects from peritoneal cavity is definitely rapid, and cadmium stearate was dissociated in the peritoneal cavity as reported in the previous paper[1]. Therefore, it seems reasonable from these data to assume that the absorption of cadmium into intraperitoneal cavity shows the same speed in both subjects. On the contrary, cadmium injected subcutaneously absorpted in the different velocity between both subjects, that is, it seems that the absorpotion of cadmium stearate from subcutaneous tissue is slower than that of cadmium chloride. Accordingly, the toxicity of cadmium stearate revealed more slowly than that of cadmium chloride.

Only small quantities of cadmium is eliminated renally and the normal route of excretion of cadmium is in feces via the gastrointestinal tract. Similar fact was discribed by Potts et al[3], and the experiments by Friberg[4,5], in which cadmium sulfate was injected subcutaneously or intravenously, had shown that the excretion of cadmium in urine is in small quantities. This phenomenon was observed in either cadmium stearate or cadmium chloride, and either subcutaneous of intraperitoneal

injection. However, following intraperitoneal injection cadmium was not recognized in urine every day, and following subcutaneous injection small amounts of cadmium were found every day. These facts showed the difference existed in transport of absorbed cadmium between the intraperitoneal and subcutaneous injection. This corresponds with distribution of cadmium in various organs as shown in Figure 3. That is, the cadmium contents of kidneys were large in subcutaneous injected rats compared with those of intraperitoneal injected ones. From these results, it seems reasonable that the majority of the cadmium absorbed peritonesally accumulated in the liver, and the remaining were sent on the general circulation, on the other hand the cadmium absorbed subcutaneously was first of all on general circulation, thereafter accumnlated mainly in the liver and kidney. Observation on rabbits[4], rats[5], and dogs[9] showed that injected cadmium is demonstrable in substantial amounts mainly in liver and kidney.

Moreover, the relationship between the content of cadmium and the remarkable injury in testes was noticeable. The destructive effect of cadmium on tetsicular tissue was already recognized by the authors[1,6] and reported also by Părizek[7,8], and cadmium eventually leads to the total destruction of the testes, which in turn rapidly evokes castration phenomena. However as shown in Figure 3, cadmium contents in testes was demonstrated relatively small compared with those of other organs, and similar results was illustrated by Burch et al[10] after oral administration in dogs. Therefore, action of cadmium on testes may be specific.

From above described results together with the results reported in the first report[1], it may come to the conclusion that the poisoning of cadmium stearate is not essentially different from that of cadmium chloride. Then, in order to prove this fact, authors investigated the chemical nature of cadmium in feces after single peritoneal injection of cadmium stearate as well as cadmium chloride. The feces were used because of the fact that they contain relatively large amounts of cadmium. And the authors found out that cadmium stearate is soluble in pyridine but not insoluble in water. Therefore, cadmium in feces is separated by means of extraction as shown Figure 1. into water soluble and pyridine soluble cadmium. The water soluble cadmium contained mainly inorganic cadmium, probably as the form of cadmium chloride and phosphide etc, and water soluble protein-cadmium complexes. On the other hand the pyridine soluble cadmium is chiefly the lipid-cadmium complexes containing cadmium stearate. It will be seen, from the results of analysis in Table 3, that the no remarkable difference was shown between both cadmium compounds. But the pyridine soluble cadmium was demonstrated relatively large amount in feces with cadmium stearate injected rats compared with that of cadmium chloride injected, and it shows that the administrated cadmium stearate is excreted in its original form. It should be noted, moreover, that the pyridine soluble cadmium was about 18% of total cadmium excreted in feces of rats injected with cadmium chloride intraperi-

tonealy. This pyridine soluble cadmium does not include the cadmium stearate. The biochemical action of heavy metals on protein metabolism has been studied extensively up to now. However, studies should be carried out also in connection with the lipid-cadmium combination. It must be considered, hereupon, that cadmium is possibly bound to the bile acid in bile duct during the route from liver in intestine. But this poses interesting problems concerning the physico-pathology of cadmium poisoning.

SUMMARY

To clarify the toxic effect of cadmium stearate, 3 mg. of cadmium per kilogram of body weight for cadmium stearate was injected subcutaneously or intraperitoneally in rats, and amounts of excretion, the distribution in various organs as well as the chemical nature of cadmium in feces were investigated. Similar doses of cadmium chloride was administered to compare the toxicity with that of cadmium stearate, and the following results were obtained.

1) The body weight decreased from the first day after intraperitoneal injection and tended to recover from the sixth day in both groups of rats which were administered cadmium stearate and chloride. However, in subcutaneous injection groups it increased until the third day after cadmium stearate injection and thereafter tended to decrease and again began to increase from the seventh day. On the other hand, body weight of cadmium chloride injected rats showed the increase in the first day, then progressively decreased, and again showed the process of recovery from the fifth day.

2) The main route of excretion of both cadmium compounds is in feces via the gastrointestinal tract and only small quantities of cadmium was excreted in urine.

The daily amounts of cadmium excretion in the cadmium stearate intraperitoneally injected rats changed slightly throughout observation periods. On the contrary cadmium chloride injected group showed the maximum excretion at the second day, then decreased progressively. On the other hand, the subcutaneous injection groups took similar attitude either cadmium stearate or chloride, that is, a slow excretion was observed throughout observation period.

The renal elimination of cadmium was not always recognized every day in intraperitoneal injected rats, while small amounts was found every day in subcutaneous injected group.

3) In rats, which were injected cadmium stearate and chloride intraperitoneally, the maximum content of cadmium was found in liver and relatively large amount was observed in kidney, spleen and stomach. After subcutaneous injection, cadmium was chiefiy deposited in liver and kidney, comparatively large cadmium contents were recognized in spleen, stomach, and intestine. The distribution of cadmium in organs of cadmium stearate and chloride administrated rats failed to show any essential difference. But cadmium content in kidney varied in each rat administered with different

method.

4) The chemical nature of cadmium in feces after single injection was investigated by means of separation on the water soluble and pyridine soluble cadmium. The average value of the water soluble cadmium was 56.7% of total cadmium in feces of cadmium stearate injected rats and the pyridine soluble cadmium was 24.7% of total cadmium. In cadmium chloride injected rats, the former was 61.2% and the latter was 17.9%, respectively. The amount of pyridine soluble cadmium excreted in feces of cadmium stearate administered rats was larger than that of cadmtum chloride injected ones.

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要　　旨

ステアリン酸カドミウム中毒の実験的研究

第 2 報　カドミウムの排泄，並びに体内分布について

吉 川　博　　原　登　　河 合　清 之

前報に於いて，ステアリン酸カドミウムの解離性並びに腹腔内注射による LD_{50} について報告した。今回は更に，注入したステアリン酸カドミウムの体内分布と排泄，並びに体内吸収後に排泄された糞中カドミウムの化学的性状について検討した。

実験は雄性ラッテを用い，ステアリン酸カドミウムと塩化カドミウムを，カドミウムとして 3 mg/kg を，皮下並びに腹腔内注射し，7 日間の体重測定，毎日の糞尿を採集した。更に 8 日目に殺し，各臓器を剔出した。これらの試料についてカドミウム含有量をヂチゾン法で定量した。なお糞中に排泄されたカドミウムの化学的性状を検討するために，各 5 匹宛の雄性ラッテの 2 群

に，ステアリン酸カドミウムと塩化カドミウムをカドミウムとして 3 mg/kg を腹腔内注射した。注射後第 1，2 日目の糞を，各群 5 匹一緒にして採取し，本文中に示した方法で，カドミウムを水溶性とピリジン溶性カドミウムに分離し，定量した。

体重の変動は，腹腔内注射群では両化合物ともに，注射第 1 日目から減少し，6 日目から増加の傾向を示した。皮下注射群では，ステアリン酸カドミウム注射ラッテは 3 日目まで増加し，その後減少し，7 日目から再び増加の傾向がみられる。塩化カドミウム注射ラッテでは第 1 日目は増加し，それ以後漸次減少し，5 日目から再び増加の傾向を示した。

カドミウム排泄の主径路は，両化合物共に消化管を経由して，糞中にみられる。尿中排泄は極めて少量である。腹腔内注射によるカドミウムの排泄は，ステアリン酸カドミウムでは 7 日間を通じて緩慢な排泄を示すが，塩化カドミウムでは 2 日目に最高の排泄を示し，それ以後漸次減少した。他方，皮下注射では，両化合物共に，観察期間を通じて緩慢な排泄を示した。

カドミウムの体内分布は，ステアリン酸カドミウムと塩化カドミウムの注射で本質的な差異は認められない。腹腔内注射では肝臓に著明に蓄積され，腎臓・脾臓・胃にも比較的多く含有される。皮下注射では主として肝臓・腎臓に蓄積され，脾臓・胃・腸にも比較的多い。

糞中に排泄されたカドミウムの化学的性状を，水溶性カドミウムとピリジン溶性カドミウムに分けて検討した。ステアリン酸カドミウム腹腔内注射ラッテ群では，前者が排泄されたカドミウム量の 56.7%，後者は 24.7% であり，塩化カドミウム投与群では，それぞれ 61.2%，17.9% であった。ピリジン溶性カドミウムが，ステアリン酸カドミウム投与群で，多少排泄量が多いが，併し塩化カドミウム投与群でも，ピリジン溶性カドミウムがかなり排泄されることは興味ある事実と考えられる。

前報並びに今回の実験から，ステアリン酸カドミウムによる中毒は，解離したカドミウムによる毒性によるものと考える。

Bull. Nat. Inst. Indust. Health, 5, 25~31, (1961).

EXPERIMENTAL STUDIES ON THE TOXICITY OF ALKYL-TIN COMPOUNDS

REPORT I. CHANGES OF ORGAN WEIGHT IN MICE TREATED WITH DI-, TRI-, AND TETRA-BUTYLTIN SALTS

Hiroshi YOSHIKAWA and Michiko ISHII

Alkyltin compounds have been used as stabilizer of polyvinyl chloride in industry, and as fungicide or insecticide for the reserve of seeds or wood as they have powerful fungicidal or insecticidal properties. Therefore alkyltin compounds are being increasingly used in the industry.

Many works on the toxicity[1-5] of alkyltin compounds have been reported in recent years. It seems to be accepted that the dust and fume of inorganic tin are less harmful, and it produces only a benign pnumoconiosis[6-8] among workers in industries. But alkyltin compounds have a powerful toxicity. It is very interesting that the toxicity and the mechanism of action are different according to their chemical constituent especially to the number of alkyl groups they combine with.

In the present series of experiments, we have attempted to investigate the biological action of alkyltin compounds, especially butyltin salts, and further more the elucidate the difference of toxicity and mechanism of action among di-, tri-, and tetra-alkyltin compounds. This paper describes the changes of organ weight in animals treated with dibutyltin dichloride (Bu_2SnCl_2), tributyltin chloride (Bu_3SnCl), and tetrabutyltin (Bu_4Sn).

Method

The dibutyltin dichloride, tributyltin chloride, and tetrabutyltin were prepared and supplied for use by the Members of Laboratory or Sankyo Chemicals Company.

Each group of about 10 healthy male mice was given single intraperitoneal injection of 1 to 3.7 mg. per kilogram of body weight of each butyltin salt in Tween 80 respectively, as shown in Figure 2. The change of body weight and symptoms were observed daily for 7 days. On the 8th days, all of the survived mice were killed and the resected various organs were weighed. The weight of organs was represented as ratio per body weight (%). But the brain weight was represented as the actual value (g.), because the brain of adult male mice had a constant weight unrelated to the body weight. Normal level of weight of various organ was determined from those of 20 healthy male mice, and the reliance limits of 95% are adopted as shown in Figure 2.

RESULTS

I) *Effects of Butyltin Salts on Growth Rates of Mice*

The change of body weight of mice treated of butyltin salts for 7 days after single intraperitoneal injection are presented in Figure 1. The body weight of mice treated with Bu_2SnCl_2 decreased progressively for 4 to 6 days and thereafter increased, but at the 7th day they did not return to the preexperimental level. The weight of mice treated with Bu_3SnCl decreased slowly till the third day except 2 groups in which slight increase was observed on the first day, but increased gradually thereafter, and reached to the former level at the 7th day. The weight of mice treated with Bu_4Sn decreased on the first day and thereafter recovered to the previous level. These results show that the inhibitory effect of these tin compounds on the gain of body weight in mice decreased in the following order, $Bu_2SnCl_2 > Bu_3SnCl > Bu_4Sn$.

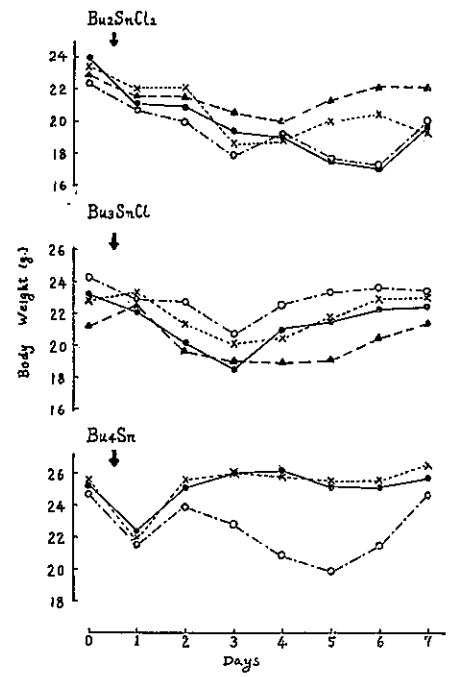

Fig. 1. Effect of single intraperitoneal injection on growth rates in mice.

II) *Symptoms*

In Bu_2SnCl_2 treated animals, the complain of thirst was observed immediately after the injection, and intense diarrhea was noticed in the early stage. However, the mice treated with Bu_3SnCl and Bu_4Sn did not show such symptoms. Any symptom which suggests the impairment of the central nervous system was not observed in any group.

Autopsy of the mice treated with Bu_2SnCl_2 and Bu_4Sn revealed the enlargement of liver with yellow color, but the animals with Bu_3SnCl did not show such change. Furthermore, the remarkable jaundice was observed in some animals poisoned with Bu_2SnCl_2, while only one was attacked in the case of Bu_4Sn.

III) *Organ Weight*

The rate of organ weight to body weight at the 8th day following the single intraperitoneal injection in mice are illustrated in Figure 2. As shown in this Figure,

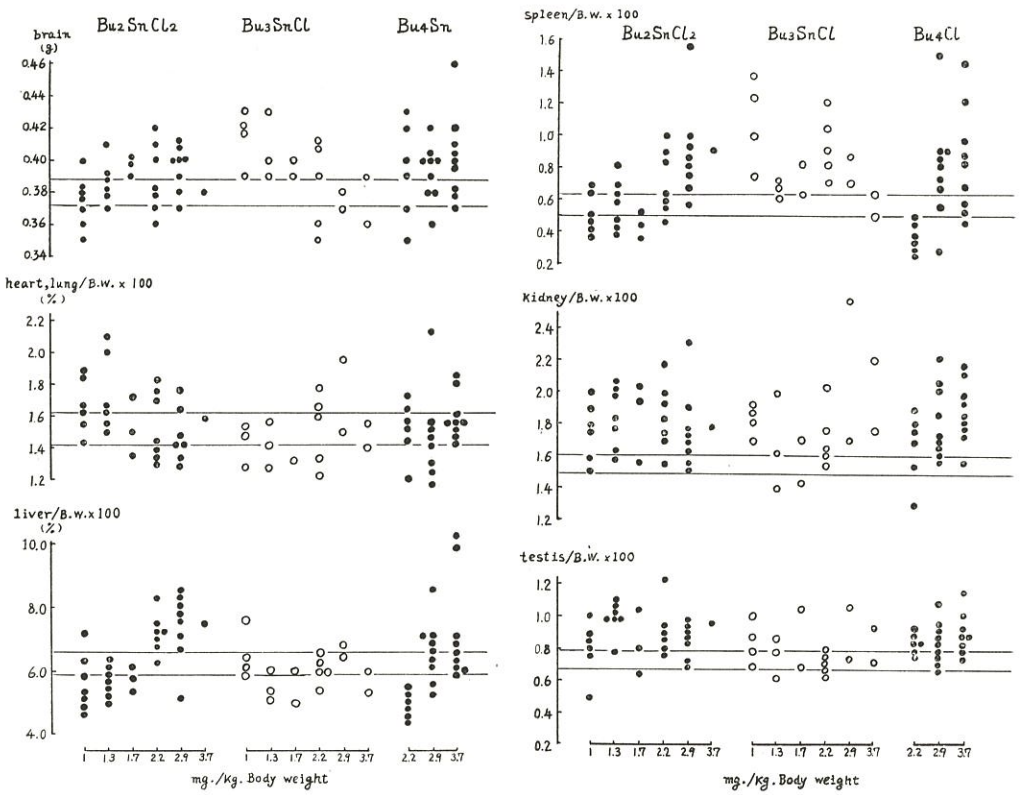

Fig. 2. Changes of organ weight of butyltin compounds-poisoned mice.

a remarkable difference in organ weight is observed among animals treated with Bu_2SnCl_2, Bu_3SnCl, and Bu_4Sn. The increase of brain weight was noticed in mice treated with Bu_3SnCl and Bu_4Sn compared with that of normal mice, but, that of mice treated with Bu_2SnCl_2 was remained at normal level. The weight of liver in mice treated with Bu_2SnCl_2 and Bu_4Sn gained progressively with increasing doses, and it is interesting that the liver weight per body weight of the mice treated with small amount of these compounds showed the value below normal. On the contrary, the liver weight of animals treated with Bu_3SnCl retained normal level and was not affected by the injected doses. In the mice treated with Bu_2SnCl_2 and Bu_4Sn spleen

weight per body weight showed the same tendency in case of liver weight, that is, increased progressively with increasing doses, but in mice treated with Bu_3SnCl the spleen weight per body weight increased compared with that of normal mice and had no relation with injected doses. Further more the kidney weight per body weight in mice treated with butyltin chloride increased generally compared with that of normal mice.

These results suggested that Bu_2SnCl_2, Bu_3SnCl, and Bu_4Sn show the remarkable difference in biological action. Moreover, it should be noted that the animals treated with Bu_4Sn showed similar attitude in case of Bu_2SnCl_2.

Discussion

Ethyltin compound have most severe toxicity among the alkyltin compounds, and di- and tri-alkyltin were the most noxious substances among the alkyltin compounds, while mono- and tetra-alkyltin have a low toxicity[2]. The trialkyltin salts were extremely effective against several fungi when tested in vitro[10], while mono- and tetra-alkyltin were ineffective and the diethyltin was less active as a fungicide. On the other hand, the trialkyltin was proved to be more toxic compared with dialkyltin, when LD_{50} was adopted[5]. Experimental results obtained from rat brain and liver mitochondria[11,12] have shown that there was a clear distinction between the biochemical actions of the di- and tri-alkyltin compounds, that is, the main action of dialkyltin was an inhibition of α-keto acid oxidase and that of trialkyltin was an interfere of oxidative phosphorylation. On the one hand, the pathological change of the former was the damage of liver and biliary tract and that of the latter was represented by oedema of brain.

We have attempted to elucidate the distinction of the biological action among the di-, tri-, and tetra-butyltin salts.

From the changes of body weight following single intraperitoneal injection with these three butyltin salts, the Bu_2SnCl_2 is most hazardous, and the inhibitory effect of these compounds on the gaining of body weight in mice decreased in the following order, $Bu_2SnCl_2 > Bu_3SnCl > Bu_4Sn$.

The observation on symptoms and change of organ weight revealed that the mice treated with Bu_2SnCl_2 showed the remarkable diarrhoea and jaundice, as well as the enlargement and the increase of weight of liver tinted yellow. The mice treated with Bu_3SnCl did not show such symptoms and showed the increase in weight of brain and spleen. The mice treated with Bu_4Sn showed a intermediate attitude between Bu_2SnCl_2 and Bu_3SnCl. There was recognized a clear distinction between the biological actions of Bu_2SnCl_2 and those of Bu_3SnCl, and these results coincided with the results of previous papers that dibutyltin salts[5] produced the biliary and hepatic lesion and triethyltin salts[4] caused oedema in the central nervous system. But it is interesting that the biological action of mice treated with Bu_4Sn show a

intermediate attitude between Bu_2SnCl_2 and Bu_3SnCl. Stoner et al[1] noted that when tetraethyltin was given to rabbits the symptoms exhibited after an initial latent period closely resembled to those of triethyltin. Cremer[18] showed that the tetraethyltin is converted into triethyltin in the body of rats and of rabbits. We recognized, however, the mice poisoned with Bu_4Sn show similar response to Bu_2SnCl_2. Of course, the gain of brain weight following Bu_4Sn injection is corresponded with that of Bu_3SnCl.

The interesting data obtained from this experiment is the reaction of spleen due to these butyltin salts. The spleen weight of mice treated with Bu_2SnCl_2 and Bu_4Sn increased gradually with the increase of doses. It is evident that this fact indicates general phenomenon, that is, in general, toxic action increases gradually as nonspecific reaction with the administration of increasing doses of poison. However, the spleen weight of mice treated with Bu_3SnCl remarkably increased regardless of injectected doses, this is, following the injection of Bu_3SnCl even in small dosages, which attained a normal level in weight of animals treated with Bu_2SnCl_2 and Bu_4Sn, effected to increase the weight. Therefore, the same result[9] was obtained in rabbits, which were administered subcutaneously in succession with Bu_2SnCl_2 and Bu_3SnCl, that is, the spleen weight remarkably increased in the case of animal treated with Bu_3SnCl, but not increased in the case of Bu_2SnCl_2. This fact indicates the necessity of investigation on the role of Bu_2SnCl_2 and Bu_3SnCl in the change of blood cell, because, one of the function of spleen is the formation or destruction of blood cell, and the extent of anaemia in rabbits treated with single injection of Bu_3SnCl is more pronounced than that of Bu_2SnCl_2[14]. These facts suggests that the Bu_2SnCl_2 and Bu_3SnCl have the different haemolytic action.

SUMMARY

To elucidate the biological action of di-, tri-, and tetra-butyltin salts, the changes of body weight and of organ weight as well as symptoms of mice were observed following the intraperitoneal injection of these compounds. The results obtained were as follows:

1) The decreasing order of body weight in mice treated with these compounds is as follows: $Bu_2SnCl_2 > Bu_3SnCl > Bu_4Sn$.

2) The mice treated with Bu_2SnCl_2 showed the complain of thirst and intense diarrhoea in the early stages. However, the mice treated with Bu_3SnCl and Bu_4Sn did not show such symptoms.

Autopsy of animals revealed that Bu_2SnCl_2 and Bu_4Sn caused the enlargement of liver tinted with yellow, but Bu_3SnCl did not show such change. The remarkable jaundice was recognized in some mice poisoned with Bu_2SnCl_2, however, such symptom developed in only one of Bu_4Sn poisoned mice.

3) the change of the organ weight in mice treated with butyltin salts were as

follows:

Brain weight of the mice treated with Bu_3SnCl and Bu_4Sn increased, while that of mice treated with Bu_2SnCl_2 remained in nomal level.

Liver weight per body weight of the mice treated with Bu_2SnCl_2 and Bu_4Sn increased progressively with increasing dosages, but that of animals treated with Bu_3SnCl retained normal level regardless of injected dosages.

Spleen weight per body weight of the mice treated with Bu_2SnCl_2 and Bu_4Sn increased progressively with increasing dosages, but that of animals administered with Bu_3SnCl increased independently to injected dosages.

Kidney weight per body weight of the mice treated with butyltin salts increased in the majority cases.

ACKNOWLEDGEMENT

The authors wish to express thanks to the Members of Sankyo Chemicals and Company Limited for supplying butyltin compounds, and to Mrs. Honma in our Laboratory for help with our work.

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要　　旨

アルキル錫化合物中毒の実験的研究

第1報　Di-, Tri-, Tetra-butyltin Compounds の腹腔内注射による マウスの臓器重量の変化

吉　川　　博　　石　井　道　子

アルキル錫化合物は，ポリ塩化ビニールの安定剤，並びに殺菌剤・殺虫剤として使用されてい

る。

このアルキル錫化合物の毒性や作用機序については，最近かなり研究されて来た。無機錫は毒性の低いものであり，産業上では良性の塵肺発生の報告例があるにすぎない。併し，アルキル錫化合物は非常に強い毒性を持ち，更に興味あることは，錫と結合しているアルキル基の種類によって毒性が異り，又同一のアルキル基であっても，錫と結合しているアルキル基の数によって，その作用機序の異ることが知られて来た。

著者らはアルキル錫化合物，特にブチル錫塩化物について，生体反応の面から有害作用を検討し，併せて di-, tri-, tetra-butyltin の毒性並びに作用機序の差異を追求した。本論文では dibutyltin dichloride (Bu_2SnCl_2), tributyltin chloride (Bu_3SnCl), tetrabutyltin (Bu_4Sn) をマウスの腹腔内に注入した場合の態度を，臓器重量の変化から検討し，3化合物の生体に対する作用の差異を追求した。

実験方法としては，成熟雄性マウスに，1 mg/kg. body weight から 3.7 mg/kg. body weight までの色々の濃度のブチル錫化合物を腹腔内に注射した。7日間，毎日の体重と症状を観察し，8日目に生存していたマウスの各臓器の重量を測定した。

体重に及ぼす影響は，Bu_2SnCl_2 が最も強く，次いで Bu_3SnCl, Bu_4Sn の順序であった（第1図）。

腹腔内注射による症状では，Bu_2SnCl_2 投与群は注射直後に渇を訴え，初期に著明な下痢を起した。併し，Bu_3SnCl, Bu_4Sn 投与群では，これらの症状を示さず，又中枢神経系の障害を思わせる症状も認められなかった。

解剖時の肉眼的所見としては，Bu_2SnCl_2 と Bu_4Sn 投与群は肝臓が著明に肥大し，黄色を呈していたが，Bu_3SnCl 投与群はこの様な変化を認めなかった。又 Bu_2SnCl_2 投与群では数例に顕著な黄疸を認め，Bu_4Sn 投与群では1例に黄疸を認めた。

臓器重量の測定結果は次の如くであった（第2図）。

脳の重量は，Bu_3SnCl と Bu_4Sn 投与群は正常値より増加を示し，Bu_2SnCl_2 投与群では正常値を示した。

肝重量（体重比）は，Bu_2SnCl_2 と Bu_4Sn 投与群では，投与量の増加に従って比率は上昇するが，Bu_3SnCl 投与群では投与量が増加しても上昇することなく，正常値を示した。

脾重量（体重比）は，Bu_2SnCl_2 と Bu_4Sn 投与群では，投与量の増加に伴って比率は上昇するが，Bu_3SnCl 投与群では投与量に関係なく，即ち Bu_2SnCl_2 では正常値を示す様な少量でもすでに上昇を示す。

腎重量（体重比）は，3 butyltin compounds ともに上昇を示す。

以上の結果から Bu_2SnCl_2, Bu_3SnCl, Bu_4Sn の生体反応にはかなり著明な差異が認められた。

Bull. Nat. Inst. Indust. Health, 5, 32～47, (1961).

INTERACTION BETWEEN SURFACE OF QUARTZ PARTICLE AND SOME ORGANIC COMPOUNDS RELATED WITH STRUCTURE OF PROTEIN

ADSORPTION OF 2-AMINOPYRIDINE, 2-PYRIDONE AND PEPTIDES ON GROUND AND LEACHED QUARTZ PARTICLES

Reisuke SODA

A toxic effect of quartz particle on monocyte depended upon the procedure of particle preparation[1]. A leached quartz particle exhibited higher toxicity than unleached (ground) particles. From the studies by X-ray diffraction, electron diffraction, infrared spectrum, solubility in phosphoric acid and in sodium hydroxide aqueous solution of those particles, it was suggested that the surface properties of the particles changed by treatment of the particle and the surface structure was deformed into other structure than original crystal structure by grinding[1-4].

These results can be explained by the assumption that some biological materials interact with the surface of the quartz particle and the toxicity of quartz particle is related to surface properties or structures of the particles. From the results obtained with some studies on silica and silicosis it is infered that the silicosis is caused from an interaction between the surface of solid quartz particle and some biological substances in the monocyte.

Present paper is concerned with an interaction between some quartz particles and some substances, structure of which is in relation to protein.

Protein is one representative of biological materials and exists in cell and others widely. This substance is an important constituent of biological body. The other substances are also important but at present these structures and properties are not well clarified. Protein is well investigated and its structure is determined by many investigators[5]. The structural elements and molecular structures of protein are finely determined. Accordingly it may be reasonable to take up protein-like substance in the first step of the work.

Protein has peptide chain as the skeletal, and has amide group, amine group, carboxylic group and others as structural elements. In peptide structure of protein, NH bond is nearly trans position against CO bond[6], but in collagen some peptide has cis form about those bonds. The latter configuration of amide bond is reproduced by 2-pyridone. The materials which are studied in the present work, are selected from above consideration.

Interaction of such a system as mentioned above is considered as adsorption, catalytic reaction, chemical reaction (combination) and others. These reactions can be detected by various method. They are spectrometry (ultraviolet, Raman, and infrared spectra), adsorption of gas and electric conductivity, etc. Those methods for detections are supplementary each other, but one of those is also powerful weapon to attack the above stated reactions. Ultraviolet spectrum tells us an electronic configuration of compound, particularly conjugated system and infrared spectrum reflects molecular structure, particularly atom position in molecule. They are concerned with bond properties of molecule. Therefore if there are some difference between the structure in an independent state and that in an interaction state, the spectrum should show any difference between these two states.

Experimental

Material and samples.——2-Aminopyridine obtained from Tokyo Kasei Co. Ltd., was recrystallized twice from hot ligroin and then distilled in vacuo. White colored crystal was obtained (m. p. 57°C). This substance was kept in colored bottle held by desiccator. 2-Pyridone was synthesized from 2-aminopyridine by diazotization[7], and purified from hot benzene (m. p. 106°C, white needle crystal).

Peptides, *l*-alanyl-glycine, *l*-alanyl-*l*-leucyl-glycine and *l*-leucyl-*l*-alanyl-glycine were kindly provided from Sakakibara who synthesized these peptides from amino acids and purified.

Potassium bromide was powdered in 200 to 300 mesh heated to dry, which was stocked in desiccator.

Quartz particle was supplied from Hamada. He prepared various kinds of quartz powder by grinding, selected their particle sizes by suspension of particle in water and centrifugal precipitation, and leached by NaOH aq. This procedure was previously reported in more detail[1].

A fraction of centrifugal precipitate of monocyte treated with or without quartz particle were supplied from (Mrs.) Koshi and (Miss) Yasukawa. These samples were freeze dried and stocked in freezing room.

Procedures——(1) About 1 mg of quartz particle was suspended in water of about 10 cc. Ten cc. of about 0.00001 M aqueous solution of pyridines was mixed with the above suspension and shaked violently. Then the mixture was kept in an ice box for about one day. A part of this mixture solution was diluted appropriately and absorption spectra were observed at room temperature in the region of wavelength from 200 to 400 mμ.

(2) A water suspension of quartz particle of about 2 mg per 10 cc. and aqueous solution of peptides of about 10 mg per 5 cc. was mixed and shaked violently and then kept in ice box for about one day. After one day, the particles were separated by centrifugal precipitation. The precipitated particles were washed with water and

separated by centrifugal method from water twice. On the final precipitate separated from the supernatant water, about 100 mg of potassium bromide powder and then about 10 cc. of water was added. The suspended solution was freeze dried. The freeze dried powder pressed by suitable die and press into pellet and an infrared absorption spectrum was observed in the wavelength region from 4000 to 400 cm^{-1}. at room temperature.

(3) Centrifugal fraction of monocyte in freeze dried state was again dissolved in 10 cc. of water and about 100 mg of potassium bromide powder was added. Then the mixture solution was freeze dried and pressed into potassium bromide pellet. An infrared absorption spectrum of this pellet was observed in the wavelength region from 4000 to 400 cm^{-1}. at room temperature.

Spectrophotometer——Ultraviolet and visible absorption spectra were recorded with a Cary model 14 spectrophotometer. Wavelength accuracy of this instrument in this experiment was about ± 2 mμ. Infrared absorption spectrum was recorded with a Perkin—Elmer model 221 spectrophotometer. Region of wavelength 2 to 15 μ was measured by NaCl prism and 11 to 24 μ by KBr prism. Resolution of this instrument was set to be 0.02 μ at 12 μ, and reproducibility was 0.005 μ in any wavelength region observed.

Results

(1) *Ultraviolet absorption spectra of pyridines with quartz particle.*——2-Aminopyridine is a strong base which has pk value of 6.86[8]. 2-Pyridone has a structure of cis form of amide bond and this refers to collagen skeletal bond (peptide). Absorption peaks occur at 227 and 288 mμ for 2-aminopyridine, and at 223 and 293 mμ for 2-pyridone both in water solutions. Absorption coefficients of these bands are the order of 10^4. These bands of both compounds show no difference when the substances are mixed with leached or short time ground quartz particle in water. But only 2-aminopyridine mixed with quartz particle ground for 6 hrs. shows a little shift at 293 mμ. No shift is observed for 2-pyridone with quartz. These aspects are clearly shown in Fig. 1. The transmittance of sample in these wavelength regions is poor and the absorbance of spectral curve is over than 1.0 in this study for the sake of scattering of quartz particle as illustrated by blank curve in Fig. 1. Spectra of 2-pyridone and 2-aminopyridine mixed with quartz particle are the same curves as those obtained by adding the quartz spectrum (due to scattering) on spectra of the compounds. There appears no change in the band width and shape on the spectra of both compounds. Only a shift at 290 mμ peak of 2-aminopyridine is a change observed in this experiment. The shift is about 5 mμ and this value is larger than the accuracy of the instrument. The other change is less than 2 mμ, therefore the shift at 290 mμ is only a detectable change. These results corresponds with the fact that basic material is generally adsorbed on silica surface. That is, 2-aminopyridine

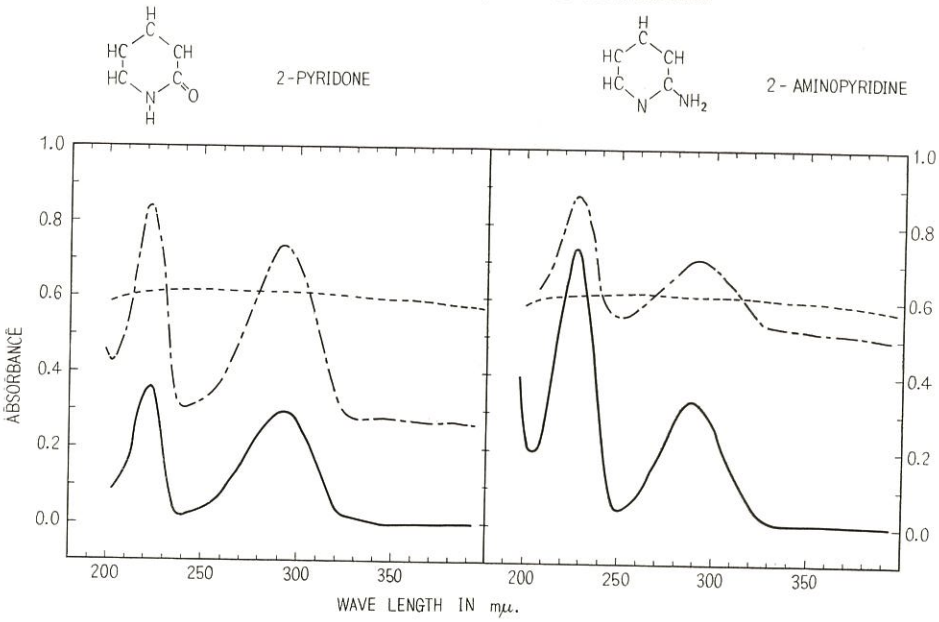

Fig. 1. Absorption spectra for adsorption of pyridines by quartz particle Q-B-6H. Left figure is obtained with 2-pyridone and right with 2-aminopyridine.
····· Q-B-6H suspension for blank.
—— Pure pyridines in water.
—·— Pyridines with Q-B-6H in water.

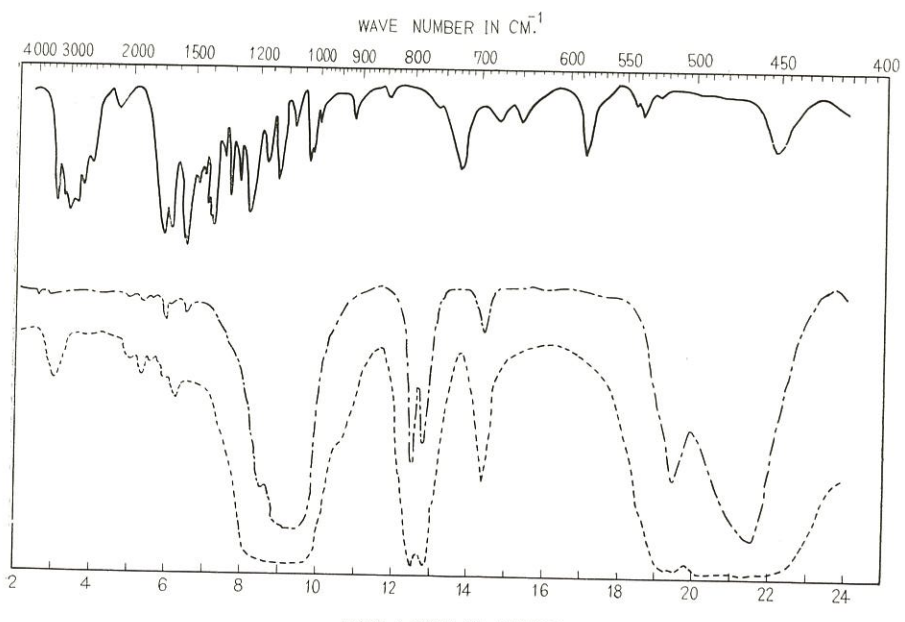

Fig. 2. Infrared absorption spectra of *l*-alanyl-glycine with and without quartz particles, Q-I-L and Q-I-100H.
—— *l*-Alanyl-glycine alone.
····· *l*-Alanyl-glycine with Q-I-L.
—·— *l*-Alanyl-glycine with Q-I-100H.

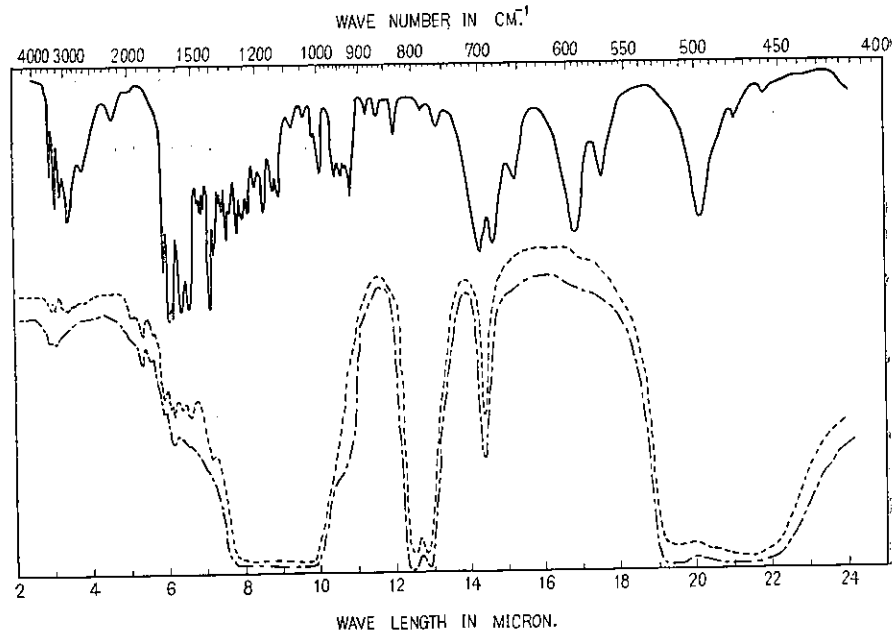

Fig. 3. Infrared absorption spectra of *l*-leucyl-l-alanyl-glycine with and without quartz particles, Q-I-L and Q-I-100H.

—— *l*-Leucyl-l-alanyl-glycine alone.
..... *l*-Leucyl-l-alanyl-glycine with Q-I-L.
—·— *l*-Leucyl-l-alanyl-glycine with Q-I-100H.

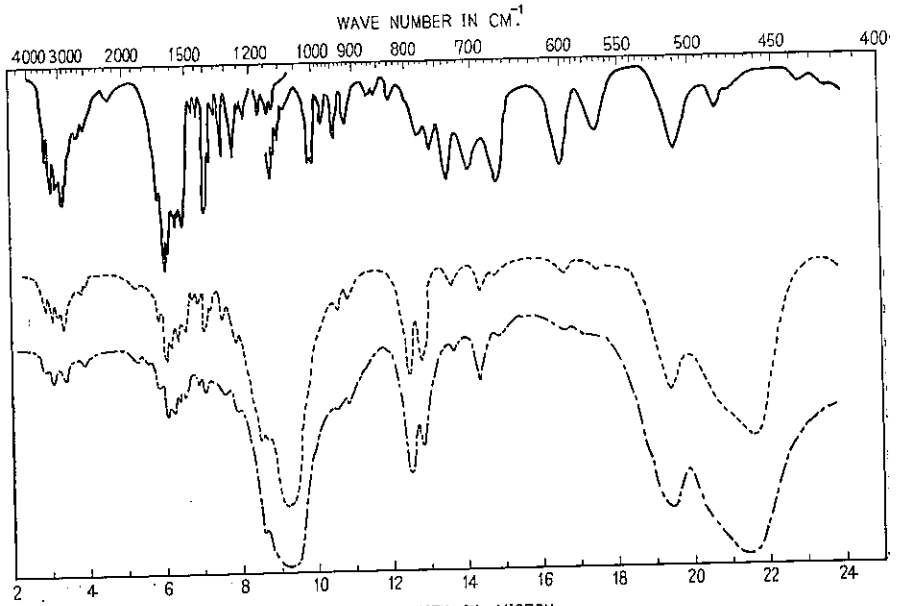

Fig. 4. Infrared absorption spectra of *l*-alanyl-l-leucyl-glycine with and without quartz particles, Q-I-L and Q-I-100H.

—— *l*-Alanyl-l-leucyl-glycine alone.
..... *l*-Alanyl-l-leucyl-glycine with Q-I-L.
—·— *l*-Alanyl-l-leucyl-glycine with Q-I-100H.

may be partly adsorbed on quartz particle but 2-pyridone is not absorbed or is adsorbed by so small amount that the spectral change due to the adsorption is not detectable. Absorption band at 290 mμ is considered to be caused by the transition which is concerned in the state of N atom. Therefore present result means that the adsorption occurs through the system of N atom group.

(2) *Infrared absorption spectra of peptides with quartz powders.* ——*l*-Alanyl-glycine has an unit of peptide linkage, and *l*-leucyl-*l*-alanyl-glycine and *l*-alanyl-*l*-leucyl-glycine have two units. These compounds have structures of amino group ion $-NH_3^+$, carboxylic ion $-COO^-$, and conjugated -NH-CO-bond (peptide). Infrared absorption bands due to peptide are designated as amide band I, II and so on. These peptide compounds on quartz particle give rise to infrared bands of the compounds and to those of quartz as shown in Figs. 2, 3 and 4. Therefore the spectral bands due to peptide compounds are obtained substracting the bands due to quartz from observed spectra bands. The results are summarized in Table I. Quartz gives rise to bands at 1180, 1090, 515and 460 cm^{-1}. with very strong intensities as illustrated in Fig. 5. It gives rise also to bands at 800, 780 and 695 cm^{-1}. with medium intensities. Amounts of materials adsorbed on the surface of the particle are considered to be very small and so spectra due to the adsorbed materials can be scarecely observed. If materials are adsorbed on surface in multilayer, spectral band due to the adsorbate will be detectable in some spectral regions. From Figs. 2, 3 and 4, and Table I, it is clear that *l*-alanyl-glycine is not adsorbed on quartz particles with appreciable amount. If monolayer

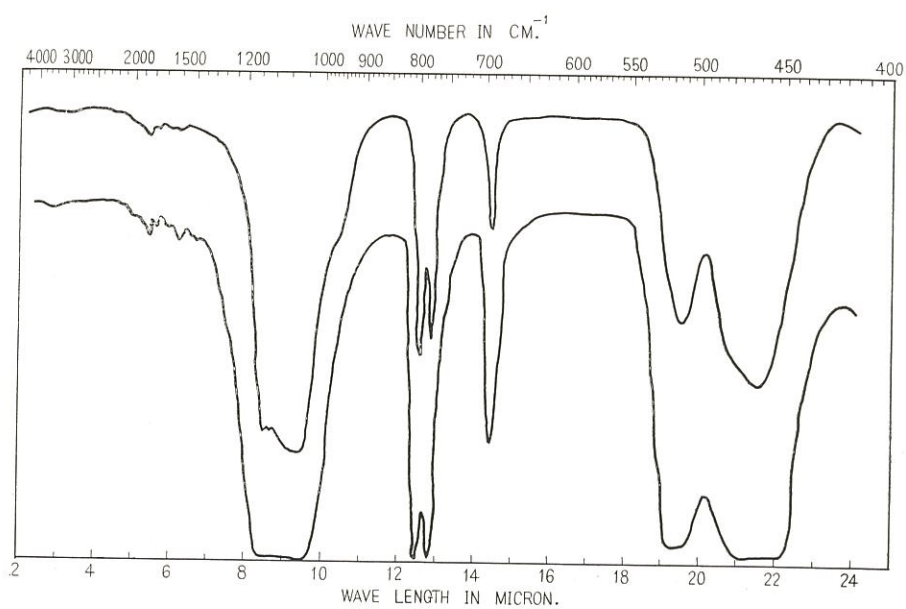

Fig. 5. Infrared absorption spectra of quartz, Q-I-L and Q-I-100H for blank. The upper spectrum is obtained by Q-I-100H and the lower by Q-I-L.

Table 1. Infrared absorption band positions of peptides with and without quartz particles.

l-Ahanyl-glycine			*l*-Leucyl-*l*-alanyl-glycine			*l*-Alanyl-*l*-leucyl-glycine		
free	with Q-I-L	with Q-I-100H	free	with Q-I-L	with Q-I-100H	free	with Q-I-L	with Q-I-100H
3290	3370	?	3400	3380	3380	3380	3370	(3370)
			3260	3260	(broad)	3240	3240	3240
3050			3070	3060		3080	3060	
2970			2960	2950		2960	2940	2950
2840			2930	2770		2870		
2650			2600			2630	2600	
2530				2560		2520	2510	2520
2130			2180			2170		
1686		1689	1689	(1689)	(1689)	1689	1689	1689
1639		1640	1645	1645		1642	1645	1642
		1620						
1548			1623	1618	(1610)	1618	1613	1610
1541		(1520)	1563	1563		1563	1560	1558
			1515	1520	(1517)	1520	1517	1517
1462			1462			1471	1466	
1443			1453			1443	1439	1441
			1437					
1416								
1403			1399	1397	1393	1403	1401	1403
1389			1376			1379	1377	
						1372		
			1351			1346		
1337			1339					
1311			1316			1318	1318	1312
			1304					
						1266	1263	1261
1271						1241		
1235						1221		
						1002		
1001						970		
			955			941	941	941
	940		943					
			932			918	919	918
917			924			868		
849						730	730	731
726						705		
			698			671	671	671
675			680					
650			655					
587			695	595	598	604	602	598
			571			574	573	
						571		
545								
540								
			497					

film established around the surface of quarts particle which has mean diameter of 1μ, then the amount of a functional group which is adsorbed in one position on the particle surface could be calculated as follows. A surface area of a quartz particle of 1μ in diameter is $3.1415\times10^{-8}\,cm^2$., and as a density of quartz is 2.65, a mass of

the quartz particle is 1.3875×10^{-12}g, then the total area of 1 mg or the quartz particle is estimated as 22.6cm^2. The group adsorbed on the surface of quartz particle is estimated to occupy the area of about 10Å^2,[4], then the quartz particles of 1 mg in total mass could adsorb the functional group of about 3.75×10^{-8} moles in monolayer. The absorption coefficient of infrared band of a chemical functional group is about 200 *l*/mole cm.[9] and this value is obtained by a strongest band. From this value a minimum detectable amount of the group is estimated as 6.3×10^{-7} mole. Therefore in this case monolayer adsorption is not detectable because the amount of the adsorbed group is less than the infrared spectral sensitivity. From above stated argument it may be concluded that the adsorption may occur in multilayer structure.

Generally an irregular structure of surface has larger area than a regular one. *l*-Alanyl-glycine is scarecely adsorbed on quartz, but a little adsorption is found on nonleached quartz particle. *l*-Leucyl-*l*-alanyl-glycine and *l*-alanyl-*l*-leucyl-glycine are clearly adsorbed on quartz particle and the latter is more adsorbed on leached quartz

Table 2. Characteristic band positions and assignments of peptides.

Band Positions			Assignments
AG*	LAG*	ALG*	
3300	3380	3400	ν(OH), ν(NH).
	3260	3240	
3050	3060	3070	(CH).
2970	2930	2950	
2840	2800	2870	
(2130)	(2180)	(2170)	ν(NH) of NH_3^+ ion.
1686	1690	1690	ν(C=O).
1640	1645	1642	Amide I band.
	1620	1615	$\nu(NH_2)$ of amine.
1548	1560	1560	Amide II band or $\nu(COO^-)$.
	1520	1520	Amide II band.
1462	1462	1470	δ(CH).
1443	1450	1440	ν(C–O) and δ(OH).
1403	1397	1402	$\nu(COO^-)$.
1390	1380	1380	
1311	1316	1318	ν(C–O) and δ(OH).
1271		1263	
		1221	ν(C–N) ?
940	943	940	δ(OH) of quartz ?
920	924	918	δ(CH) ?
726		730	δ(CH).
675	680	671	Skeletal vibration
587	595	602	
	571	573	

* AG, *l*-Alanyl-glycine; LAG, *l*-Alanyl-*l*-leucyl-glycine;
ALG, *l*-Leucyl-*l*-alanyl-glycine.

than on non-leached quartz as shown in Figs. 3 and 4. The shift of band position is not found clearly between non-adsorbed and adsorbed peptide compounds on quartz. The main bands observed for these samples are assigned and summarized in Table 2. The assignment is not valid because each band property is not determined precisely, for example by means of deuteration of NH bond, dichroism of the band, and so on. But tentatively these bands are assigned, particularly considering the other results obtained with peptides, amino acids and other organic compounds[10]. The change caused by property of surface structure of quartz and adsorbed compounds are summarized in Table3.

Table 3. Effect of surface properties and peptide structure on the adsorption.

Peptide bonds \ Surface properties / Compounds		Regular structure Q-I-L	Irregular structure Q-I-100H
One	*l*-Alanyl-glycine	No evidence for adsorption.	Slightly adsorbed but doubtful.
Two	*l*-Leucyl-*l*-alanyl-glycine	Clearly adsorbed (but no structural distortion of peptide).	More clearly adsorbed than on the regular quartz.
	l-Alanyl-*l*-leucyl-glycine	Clearly adsorbed more than on Q-I-100H.	Adsorbed but less than on Q-I-L.

(3) *Infrared spectra af centrifugal fraction of monocyte treated with and without quartz particle*——In the present experiment, fraction of monocyte is not purified by other treatment than centrifugal precipitation of component of cell. Therefore various components such as amino acid, protein lipid, and nucleic acid, etc., are all condensed in this fraction. Main components can only be detected with infrared spectrum and minor components can scarecely be detected. As crystallization water and water adsorbed in biological material are not negligible in the cell, infrared spectrum of the cell component obtained in the present experiment is largely interfered by the water bands. Futhermore spectra obtained are very complicated because the sample is a mixture of every biological substances in the cell. Accordingly aoy clearcut conclusion can not be drawn from the present result by infrared spectrum, but it would be allowed to deduce some speculation about the reaction between the biological material in the cell and quartz particle. Spectra of the components of monocytes treated with and without quartz particle are shown in Fig. 6. In the wavelength region under 11μ no definite bands due to components occur. This is perhaps caused from the interference of water in the observed region. The difference between the two sorts of quartz particle Q-I-L and Q-I-100H is not found. The peak positions of main bands are summarized and the bands are tentatively assigned as shown in Table 4. Two clear differences are observed between monocyte components treated with and without quartz particle, and between the components treated with Q-I-100H and others. The former is seen in the band at 1565 cm^{-1}. and the latter is seen in the band at 1163 cm^{-1}. The band 1540 cm^{-1}. is reasonably assigned to amide II band[11] of secondary amide which occurs

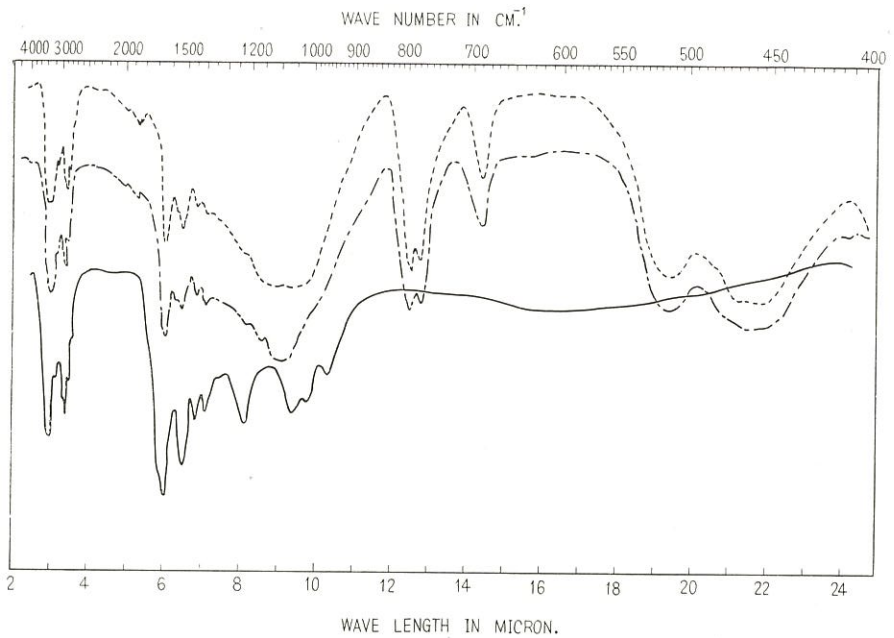

Fig. 6. Infrared absorption spectra of some fractions of monocytes by centrifugal separation.
..... Fraction of monocyte treated with Q-I-L.
—·— Fraction of monocyte treated with Q-I-100H.
—— Fraction of monocyte untreated with quartz particle.

Table 4. Infrared absorption band positions and assignments of some fractions of monocytes

Band positions			Assignments
Monocyte with Q-I-L	Monocyte with Q-I-100H	Monocyte without quartz	
3320	3320	3330	ν (O-H) and ν (N-H), hydrogen bonded
3090	3100	3080	ν (C-H) of aromatic and aliphatic hydrocarbon groups
2960	2960	2960	
2930	2920	2920	
2860	2870	2860	
1970	1980	—	May be attributable to quartz
1860	1870	—	
1653	1650	1650	ν (C=O) of peptide (amide I band)
1563	1565	—	New band
1538	1538	1536	Original δ (N-H) of -CO-NH-R (amide II band)
1451	1449	1453	δ (C-H), bending etc.
1391	1403	1403	δ (O-H), ν (C=S)
1227	1225	1232	P-O bond vibration of nucleic acid ?
—	1163	—	ν (P-O)
		1068	ν (C-N), ν (C-O)
		1028	ν (P-O-C-) etc.
		966	$\delta\perp$ (C-H) etc.

generally at 1500 to 1550 cm^{-1}. This band is considered to shift to 1560 cm^{-1}. as a result of interaction with quartz particle, as mentioned later. The new band 1560 cm^{-1}. is very weak but exists as a shoulder of 1538 cm^{-1}. band in both cases. Amide II band has mainly a character of NH deformation vibration[11] and this result means that new properties is formed in amide NH bond through the interaction of quartz particles (Q-I-L and Q-I-100H) with cell materials.

The band at 1163 cm^{-1}. can not be exactly assigned but a stretching vibration of P-O bond gives rise to the band in this region[11], and so the 1163 cm^{-1}. band is tentatively assigned to P-O stretching vibration. Assignment of the bands in 3 μ region is considered to be reasonable, but in the other region some assignments are dubious. It is unnecessary to get a decisive assignment of bands for the speculation and it is sufficient for the present purpose to obtain only the difference between spectra of monocyte components with and without quartz. The other details than the above mentioned bands are not yet investigated as no further experiment is carried out, for example, separation of each components.

Discussion

Amorphous silica such as silica gel and aerosil, adsorbs several substances at its surface and pore[12]. Charcoal, alumina and titania etc., adsorb also these substances at their surfaces[12]. Sometimes these surfaces act as catalysts or reactants[13]. Generally there are O-H group, C=O group or metal oxygen bond on their surfaces and they play important roles by adsorption through hydrogen bond etc., and reaction (electronic energy transfer).

As mentioned already, infrared spectra of peptides on quartz or some fractions of monocytes treated with quartz account for the adsorption of some organic compounds on the surface of quartz particle. *l*-Alanyl-glycine is not adsorbed in appreciable amount, but other two peptides are adsorbed in multilayer forms on the particles. In this case it is reasonably considered that higher polymer is adsorbed in more extent than the lower and the configuration of higher order is important for structural depending adsorption. By the adsorption of peptides on quartz particle there are no shift found in the region of observed spectra. Therefore the structural change does not occur or is not detectable in the adsorption. This may mean that the adsorption occurs at the some points of surface and the other molecules are adsorbed on the molecules existed in monolayer. The multilayer of adsorbed molecules would be in the simiar structure as that of solid state of pure peptide. Probably the some layers are strongly combined with monolayer and are not easily removed out by washing with water except in the case of *l*-alanyl-glycine. The number of the layer is assumed to be more than ten, from the experimental results of spectra obtained with *l*-alanyl-*l*-leucyl-glycine and *l*-leucyl-*l*-alanyl-glycine.

The further speculation gives following possibility of adsorption mechanism about

the structure of the system. *l*-Alanyl-*l*-leucyl-glycine is adsorbed more largely on leached quartz Q-I-L than on unleached Q-I-100H, but other peptides are adsorbed more largely on unleached quartz particle. The latter case is generally seen in adsorption phenomena, because the surface area of amorphous silica is larger than that of crystalline silica and the amount of adsorption parallel with the surface area. The former should require the other explanation. The number of layer of the peptide adsorbed on quartz may be determined by the adsorption energy which is evolved from the system by interaction and makes the system stable, i.e., the system is put in the minimum position of potential energy.

Then it is possibly considered that the structure of *l*-alanyl-*l*-leucyl-glycine and the regular surface structure of crystalline silica interact suitably into monolayer adsorption and the more peptide molecules are easily combined further on the arranged monolayer. Final structure is perhaps very stable and therefore is not easily removed out by washing with water. Peptide bond structures of *l*-alanyl-*l*-leucyl-glycine and *l*-leucyl-*l*-alanyl-glycine are similar but these two compounds are different in the order of combination of amino acid adjacent to glycine residue. Side chain is aliphatic hydrocarbon in both cases, and the energy of interaction between hydrocarbon and quartz is lower than that between polar group and quartz which establishes generally hydrogen bonding. The other factors determining energy of the state are sterric effect, higher order effect such as dispersion, dipole interaction, and others. In this case the sterric effect may be the most important factor because it has close relation to spatial structure of molecule. If it were the case, there should be a stable form in the state of multilayer adsorption of *l*-alanyl-*l*-leucyl-glycine on the quartz particle.

In the case of peptides on quartz particles it is not clear whether the NH bond or COOH bond makes hydrogen bonding with the quartz surface group. As infrared spectrum show no significant change in band position nor appearance of new band, the structure in adsorption state can not be discussed in detail.

From the change in spectra of some fraction of monocyte it may be inferred that new properties appears in NH bond when quartz particle interacts with some components in monocyte and perhaps P-O bond nature does also. The latter can not be taken into account for alternative assignment of this band (1163 cm^{-1}.). Generally the stronger the hydrogen bond is, the deformation vibration of NH bond shifts toward higher wave number. The author assumes that amide II band at 1538 cm^{-1}. shifts to 1565 cm^{-1}. and this band has mainly NH deformation vibration character. Accordingly it is possibly considered that NH bond of protein and other substances in the cell interact with quartz particle and is adsorbed in other state than the original or changes their structures through reaction with the particles.

Ultraviolet and visible absorption spectra of 2-aminopyridine on quartz tells us that NH bond of this compound interacts with quartz surface group and establishes hydrogen bonding. Therefore from the results of infrared and absorption spectra in

the present work, it could be concluded that NH bond interacts with quartz surface group and establishes hydrogen bonding N-H·····.

On the other hand, in order to establish the hydrogen bonding in the form N-H ·····N or N-H·····O etc., there should exist polar group on the surface. Quartz is constituted of Si and O atoms and it is considered that silanol group Si-O-H exists on the surface. Then N-H·····O-Si hydrogen bonding is the most probable form in this case. There are two probable positions attacked by NH bond, $\begin{matrix}Si\\Si\end{matrix}\!\!>\!O\cdots\cdots$ and $\begin{matrix}Si\\H\end{matrix}\!\!>\!O$ ·····. Regular surface of crystalline silica has regular form of Si-O-H and irregular amorphous silica has arbitrary distribution of Si-O-H structure. The number of silanol group on unit surface area may be not so different between regular and irregular surfaces, but total number may be larger in amorphous structure than in crystalline surface. Furthermore structure $\begin{matrix}Si\\Si\end{matrix}\!\!>\!O$ also is not regular with amorphous silica. But the interaction energy may be larger with Si-O-H than with Si-O-Si. Tsuchiya[14] stated that some gas molecules were probably adsorbed at the position of O atom of Si-O-H group on the surface of amorphous silica. Several references provide similar results[15-19]. Anomalous phenomena of silica in the biological or biochemical step of silicosis may be partly attributable to silanol group structure on the particle surface. In spite of a little data of this work and literatures, it could be concluded that silanol group on the surface of particle silica is one factor which has important effect on silicosis.

Chemical functional group is detectable by spectrophotometrical methods such as infrared spectrum and absorption (electronic) spectrum, as mentioned already. These methods tell us the electronic structure, molecular structure and several informations about the states of the compound. Furthermore they provide other informations about their qualitative aspects in the reaction of compounds, for example, differences between reactants and products in band intensity or band position. Properties of surfaces of quartz particle and other silica particles are further investigated by reflection spectrum or micro spectrometry. McDonald reported the surface property of silica particle by infrared spectrophotometry[15,16]. Seratosa and Bradley[20] were in correspondence with the reflection infrared spectrum of clay surface and discussed the O-H orientation on the surface. Pickering and Eckstrom[21] deviced the cell of the multiple reflection system and investigated the adsorption of gas on some metal film.

Though experimental technique is very poor in the present work, several improtant informations are obtained about the interaction of quartz particle with some organic and biological materials. The spectrophotometrical method can be applied without chemical reaction in substances. Therefore it is very useful to know their native and original aspects, but at present as the sensitivity is limited for the technical problem, the applicability of the method is not satisfactory. In future, spectrum

would tell us several important and key solutions about our problems.

Summary

Adsorption of some organic substances with relation of the protein structure on quartz particle and their interaction were studied by means of spectroscopy. It was concluded by absorption spectrum that 2-aminopyridine was adsorbed but 2-pyridone was not adsorbed on ground quartz particles in water.

l-Alanyl-glycinc was scarecely adsorbed but *l*-alanyl-*l*-leucyl-glycine and *l*-leucyl-*l*-alanyl-glycine were adsorbed on quartz particle and the former was adsorbed more strongly on leached quartz than on non-leached quartz particles. These reactions were carried out in water. The precipitated fraction of monocyte interacted with quartz particles had some different components from that of the cell treated without quartz particles. Those two qualitative results were obtained by means of an infrared spectroscopy.

It is reasonably concluded that N-H group interacts with silanol group and hydrogen bonding is established between these two groups. A model is drawn about the mechanism of the adsorption of N-H group on surface of the quartz particle.

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要　　旨

石英粒子表面と蛋白質に関係のある二三の有機化合物との間の相互作用

2-アミノピリジン，2-ピリドン及びペプチドの磨砕及びリーチした石英粒子への吸着

左　右　田　礼　典

珪肺病因論に関する既報の論文[1]に於て，単核細胞内の生物化学的物質とシリカとの間に何らかの相互作用が示唆された。そこで蛋白質のような構造単位をもつ有機化合物と石英粒子の間に反応が起るか否か，また起るならばどのような相互作用かを知る事を目的として実験を行った。測定は分光法によって行い，有機化合物水溶液と石英粒子と混合した懸濁液の紫外線吸収スペクトル及びこの溶液の遠沈分の赤外線吸収スペクトルから相互作用を検討した。

2-アミノピリジンは塩基性が強く，石英粒子に吸着されることが第一図のスペクトルから推定出来る。即ち 290 mμ 附近の吸収帯が吸着反応によって約 5mμ 程長波長に移動している。2-ピリドンではこのような結果が得られなかった。この結果はアミノ基を通して石英表面との間に相互作用のあったことを推定させる。

二三のペプチドについての赤外線吸収スペクトルの結果からペプチド構造の大きい分子の方が

吸着され易いこと，立体構造等分子の高次構造が吸着され易さに影響を及ぼしていることなどが考えられる。第二，三，四図から第五図に示す石英粒子そのもののスペクトルを差引いたスペクトルの吸収帯の位置と，純ペプチド分子のそれを第一表に示した。第二表は主な吸収帯とその振動型の帰属を，第三表は測定結果の総括を示した。*l*-アラニルグリシンではリーチしていない石英粒子に僅か吸着しているかに見えるが，むしろ吸着されないと考えた方が妥当である。二つのペプチド結合を有する *l*-アラニル-*l*-ロイシル-グリシン及び *l*-ロイシル-*l*-アラニル-グリシンは明かに石英粒子に吸着される。この事は石英粒子のスペクトルに重なってペプチドの吸収帯が可成りの強度で見られることから結論される。更に *l*-アラニル-*l*-ロイシル-グリシンは表面をリーチした石英にリーチしていない石英粒子よりも多く吸着されていること示された。これは立体的障害に基くものではないかと考えられた。即ち単なる水素結合であれば表面積の大きいと考えられるリーチしてない石英粒子の方が多く吸着すべきであるから，規則的な表面の基とペプチドの基との間の相互作用に立体効果によるエネルギーが大きく影響していると推定される。

単核細胞と石英粒子を反応させ，その遠沈区分を凍結乾燥法で臭化カリ錠剤として赤外線吸収スペクトルを測定した。第六図はそのスペクトルである。石英に基く吸収を除いた吸収帯の位置とその帰属を第四表にまとめた。アミドⅡ吸収帯と帰属される 1540 cm^{-1} の吸収帯の高波数側に石英と反応させた細胞では 1563 cm^{-1} に弱いがはっきりした吸収帯が現われる。これはアミド結合特に $\rangle$N-H の構造が何か変化を受けていることを推定させる。元来の $\diagdown$C-N$\diagup_{H}$ 等の結合に変化があることを意味する。

以上の結合をもとに考察すると，アミノ基アミド基等の N-H 結合を有する化合物と石英粒子との間には粒子表面のシラノール基 Si-O-H を通して強い水素結合が形成され，シラノール基の空間配位と化合物分子全体の形，大きさとの関係も吸着エネルギーに寄与する大きな要素であると推定された。この場合の水素結合の形は $\rangle$N-H$\cdots\cdots$O-Si$\diagup\!\!\diagdown$（H） であろう。

現在測定技術の限界のため多くの成果は期待出来ないが，しかし測定法の改良により更に精密に且直接的に表面の性質，構造をスペクトルによって知る可能性が大きいから，分光的方法による表面反応，表面構造の研究を通して石英粒子の細胞外における作用が明かにされるものと期待される。

Bull. Nat. Inst. Indust. Health, 5, 48~54, (1961).

LOUDNESS LEVEL OF PERIODICALLY INTERMITTENT SINE WAVES.

Toshisuke MIWA

There are many sorts of works producing noises that are intermittent periodically in industry. These are rivet, air hammer, rock-drill, coal-pick, and large or small hammer works. One way to determine the level of intermittent noises is to make use of the sound level meter. However, meter circuits of it have time constant about 0.2 sec, so that the level with shorter duration than 0.2 sec can not be measured correctly by it. Another ways[1] to decide the level of intermittent noises having shorter duration are to employ the peak-meter, or to compare the peaks between noises and sine wave by the syncroscope. But we have only a few basic experiments to determine the correlation between the sound pressure level and the loudness level of these sounds in the past. Namely, Pollack[2] proposed from the experiment the method of calculating the loudness level of intermittent noises and Garner[3] measured the loudness level of intemittent sine waves by another way. However, these experiments are still not enough to solve above problems. This paper represents an attempt to measure the loudness level of intermittent sine waves.

Apparatus and Procedure

The block diagram of this apparatus is shown in Fig 1. The sine wave that is intermittent in time and constant in the level is fed into one ear and the sine wave that is continuous in time into the other ear. Then the continuous sound is varied to the level that is judged to be equal loudness and the level of it is recorded automatically with in certain width of variance on the recording paper (by subjects). In this case, intermittent sound is made by interrupting the sine wave with the linear bidirectional gate. Peak to peak level of this intermittent one is kept always constant at level of 90, 100, or 110 dB in one course of the experiment and the duty cycle of this wave is controlled by square waves (Fig 1). The duty cycle of square waves is changed by relays combined with Decatron. The following five kinds of time durations were chosen, t_1/t_2, =65/265, 25/100, 15/100, 10/55, 5/25, msec, where t_1 is burst period and t_2 rest period. The reason of this choice in the level and the duty cycle is that industrial intermittent noises have commonly pulse width about 10-250 msec and these peak to peak levels are usually more than 90 dB. The frequency of the sine wave included in the intermittent sound was chosen 0.5, 1, 4 and 8 kc.

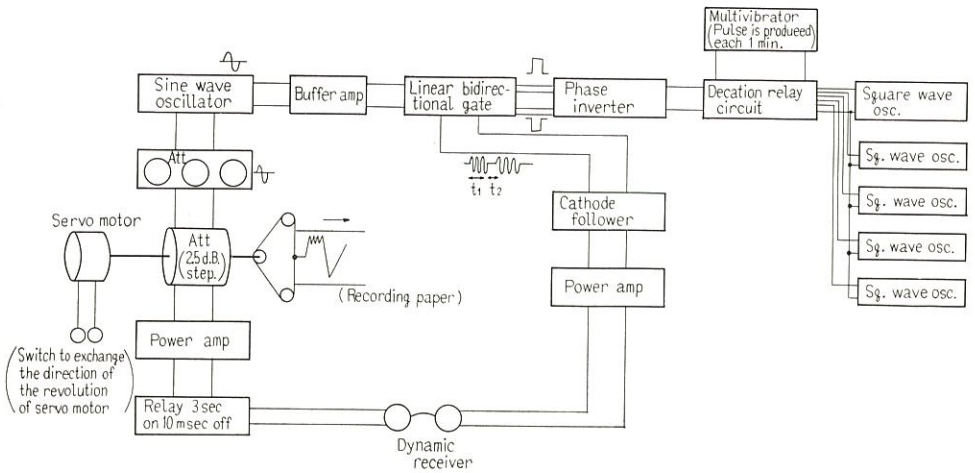

Fig. 1 The block diagram of the apparatus.

On the other hand, the continuons sine wave is attenuated to the proper level and is fed into the next attenuator (2.5 dB steps and all over 50 dB) wich is driven by servo meter (4 rpm). The subject compares the continuous sound with the intermittent one and when he feels the former larger or smaller than the latter, he can adjust the attenuator to the equal level by exchanging the direction of the servo motor. The motion of this attenuator is recorded automatically on the paper which moves with the velocity of 8 cm/min. The recocded line means the equal loudness level with some variances. To avoid the perceptional accomodation, the standard sound is interrupted every 3 sec periodically for 10 msec by the relay which is driven by the free running multivibrator and is fed into the dynamic receiver. Subjects were 3 men and 2 ladies under thirty years of age to avoid presbycusis.

CALIBRATION[4,5]

The sound pressure level of the dynamic receiver was calibrated by the 103 type condenser microphone and the coupler of the audiometer standardized in JIS. The pres-

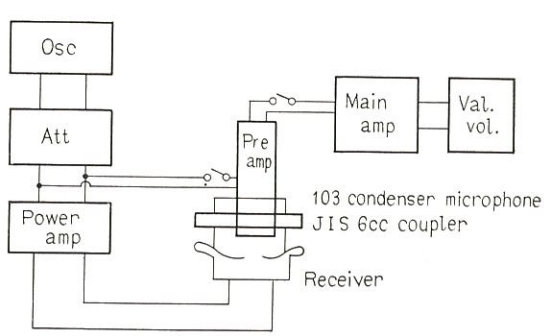

Fig. 2 The block diagram of the calibration.

sure level of the continuous sound is measured by the resistance substitution method. When the input voltage of attenuator is A volts and its frequency is f c/s, the following equation is used to calculate the sound pressure level (Fig 2).

$$p_f = 74 - (\text{sensitivity of microphone})_f + 20 \log A - (\text{attenuator})_{dB}.$$

The peak to peak level of intermittent sine wave was compared with the continuous one by using the syncroscope.

Result and Discussion

When the frequency of the sine wave included in the intermittent sound equals to that of the continuous sine wave, the following facts can be known as are illustrated in Fig 3.

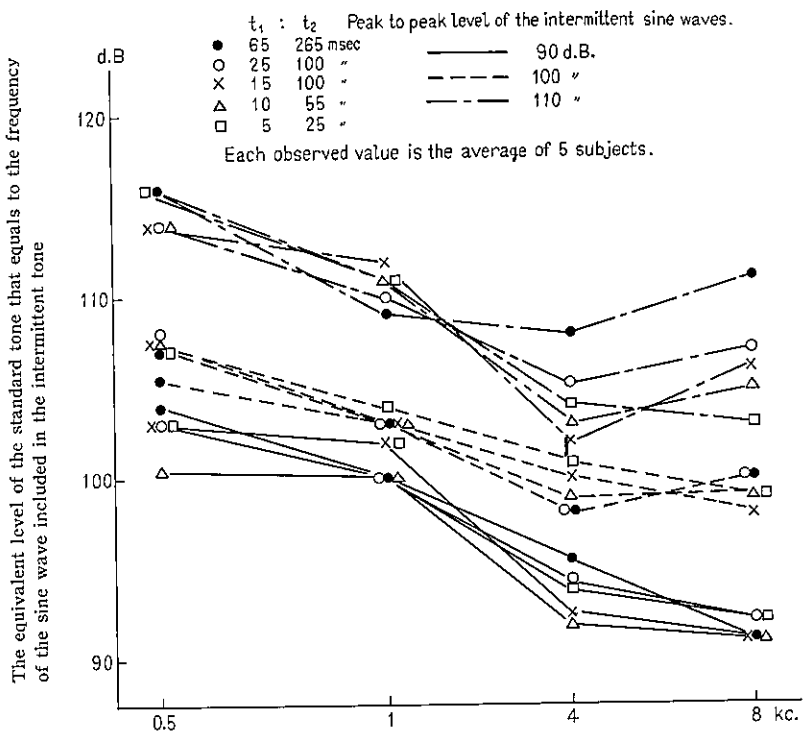

Fig. 3, Experimental result of loudness level measurement. In this case, the standard tone which is continuous in time equals to the frequency of the sine wave included in the intermittent tone.

(1) The higher the frequency is, the lower the equivalent level of the intermitt ent sound becomes.

(2) The intermittent sound of different time duration did not show the same loudness level.

(3) The intermittent sine wave was louder than the continuous one in lower frequency, but was not always true in higher frequency.

(4) The human auditory sensation feels approximately the peak to peak level

of the intermittent sound.

In this experiments, variance of the individual loudness level was ±2.5 dB. The variance of it by day was ±3 dB and the variance of the group average of it by day was ±1 dB.

When the continuous tone is emitted always 1 kc as the standard, the following result is obtained as shown in Fig 4. That is, the higher the frequency is, the higher

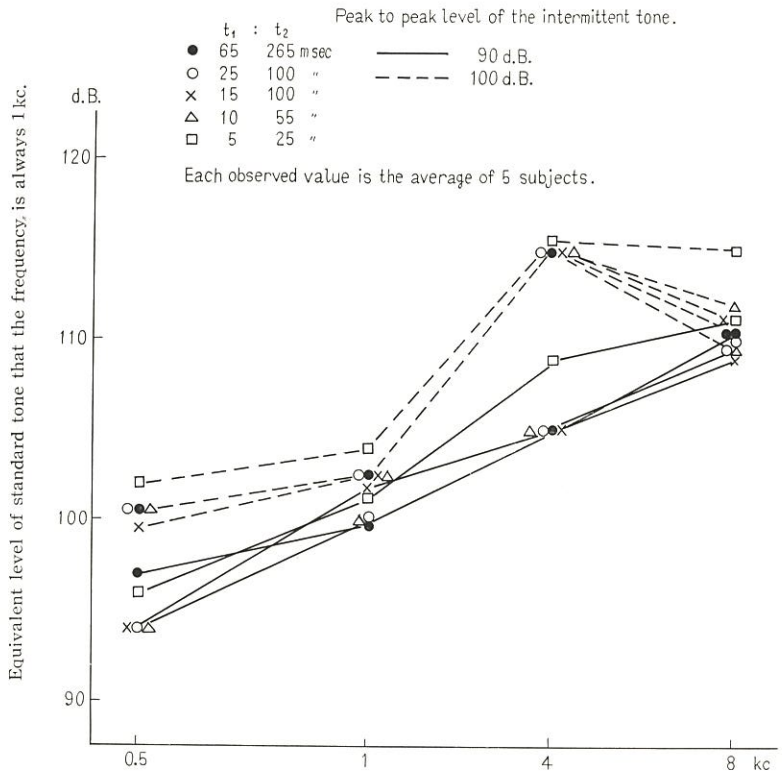

Fig. 4. Experimental result of loudness level measurement. In this case, the standard tone is always I kc.

the loudness becomes and this result was contradictory to Fig 3. In this case, the intermittent sound was always louder than the continuous one. Moreover, the intermittent sound of each different time duration in the same frequency showed almost the same loudness level. Further, when the peak to peak level of the intermittent sound was 110 dB at 4 and 8 kc, the equivalent level of the standard tone became more than 120 dB and the experiment was difficult because of pain due to louder sound. Therefore, it seemed that the comparison of the intermittent sound with the one being always I kc would produce unknown factors in the results.

Then, there are many methods[6,7,8] to determine the loudness level of the inter-

mittent sound, but the automatic method described above has the following advantage. That is, the stimulation time to the subject can be unified. When the subject heard the sound for sometime at the level judged as the equal loudness, the variance of the loudness level in time can be measured. Further, various kinds of errors depended upon the tester can be eliminated and the time required for the measurement can be shortened and even the testor can also become the subject. Therefore, this automatic method was chosen in this measurement.

Conclusion

We knew that, in the experiment which the frequency of the sine wave included in the intermittent sound equals to that of the continuous sine wave, the human auditory perception showed approximately the peak meter response. However, when the standard sound was always I kc, it was difficult to decide the equal loudness owing to compare the different frequency.

Appendix

(I) The circuit of the square wave[9]: This is shown in Fig 5. The duty cycle of the square wave was changed by the condenser and resistance. The C. and R. of the each time duration were selected by the relay combined with Decatron.

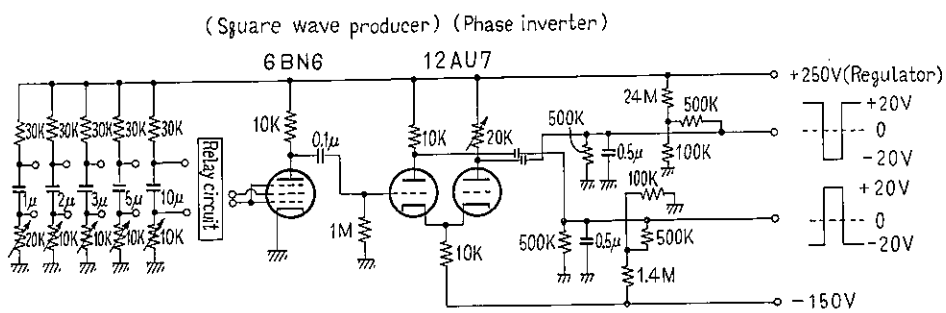

Fig. 5. Circuit of the square waves.

(II) Linear bidirectional gate[10]: This gate is stable and clickless. Circuit is shown in Fig 6.

(III) The distribution circuit of the pulse using Decatron: This circuit is used to change five kinds of the time durations. The Decatron which moves the relay is driven by the pulse of the free running multivibrator with repeated frequency of 60 sec and when one measurement finished, it is driven by the pulse with repeated frequency of I sec to return to the first state. The reset can not be used in this case because it will drive the relays simultaneously and may break down the vacuum tube that is used to amplify the current to work them as shown in Fig 7.

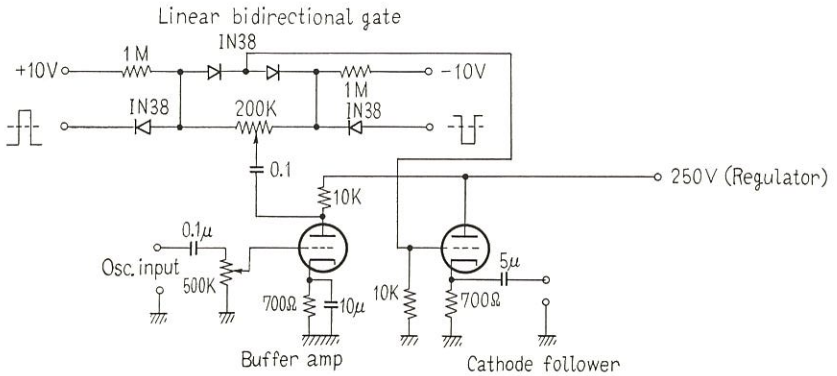

Fig. 6. Linear bidirectional gate.

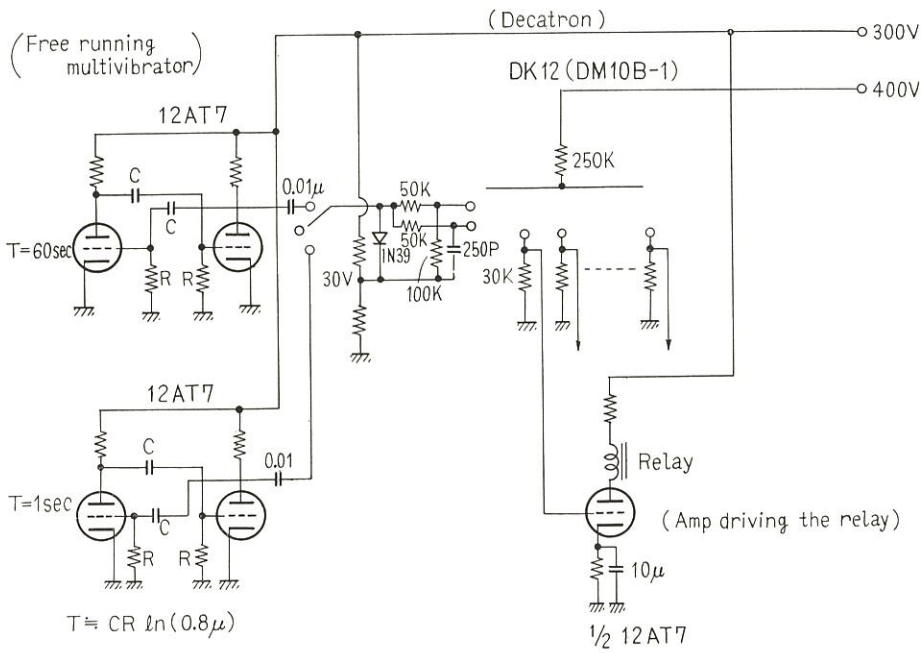

Fig. 7. Pulse distribution circuit used with Decatron.

Acknowledgement

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要　　旨

パルス化正弦波のラウドネスレベルの測定

三　輪　俊　輔

リベット，エヤーハンマー，サクガン機等より発生する音は 10～250 m. sec のパルス幅をもつ繰返し音である。現在騒音計が音響測定に利用されているが，之は 0.2 秒の時定数がメーター回路にあるので，之等のパルス音の計測には誤動作を伴ないがちである。他方ピークメーターやシンクロスコープを用いれば，パルス音の物理的レベルは求められるが，聴覚との対応をきめるラウドネスレベルとの相関は測定が困難な為に余り求められていない。ゆえにこの問題を究明せんとして新しい装置を試作して実験を行なった。

測定方法は一耳に連続音を，他耳に間歇音を聞かせ等価点を求めるのであるが，之をすべて自動的に行い，求めたラウドネスレベルも自動記録される様にしてある（第1図参照）。

連続音の周波数はパルス化正弦波の成分正弦波のそれと常に同じにした。そしてこのレベルは間歇音に等しくなる様に被検者がかえられる。次にパルス音のデューティ・サイクルは次の5種をえらんだ。

$$\frac{t_1\ (\text{発音時})}{t_2\ (\text{休止時})} = \frac{65}{265},\ \frac{25}{100},\ \frac{15}{100},\ \frac{10}{85},\ \frac{5}{25}\ \text{各 msec.}$$

又パルス音のピークピークの音のレベルは 90, 100, 110 dB をえらんだ。

第3図に示す如く次の結果をえた。

（i）間歇正弦波の成分正弦波の周波数が高くなる程，等価連続音のレベルは低くなっている。

（ii）低周波では連続音よりも間歇音の方が大きく感じられている事がわかるが，高周波では異なって来ている。

(iii) 人間の聴覚はほゞ間歇音のピークピークレベルを，感じている。

以上を要するに，

パルス性騒音の測定にには，ピークメーターの利用を考慮する必要がある様に思われた。

労 衛 研 報 第5号, 55～60, (1961).

某自動車車体塗装工場作業者の血液所見

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左右田礼典 松下 秀鶴 松村 芳美 本間 光子

HAEMATOLOGICAL STUDIES ON THE WORKERS IN A MOTORCAR SPRAY PLANT

Hiroyuki SAKABE, Hiroshi YOSHIKAWA, Michiko ISHII, Hiromichi HASEGAWA, Mitsuo SATO, Reisuke SODA, Hidetsuru MATSUSHITA, Yoshimi MATSUMURA and Mitsuko HOMMA

昭和 34 年春のビニールサンダル糊着作業者のベンゼン中毒禍以来，有機溶剤中毒予防規則の施行をみ，ベンゼンを含む溶剤の使用は一般的には極めて少なくなり，トルエンその他の代用溶剤に代わりつつあるが，この溶剤の転換が労働者の健康に及ぼす影響は重要な問題である。

この影響を検討するために，われわれは，某自動車車体塗装工場の環境調査及び塗装作業者の血液検査を行なった。この工場においては，塗装用溶剤として従来はベンゼンを含むものを使用していたが，昭和 34 年8月より「ベンゼンを含有せず」との証書の添付してある，トルエンを主体とする溶剤の使用にきりかえている。

なお本調査は昭和 35 年 11 月に行なった。

I. 作業の概要

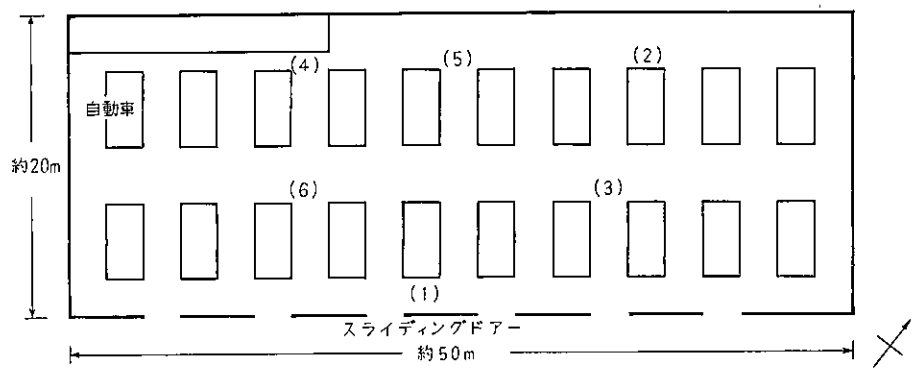
本工場では，主として自動車車体の塗装作業が行なわれているが，その前段階としてサンドブラッシング及び錆止めのためのプライマーの吹きつけが行なわれ，次に下地塗料であるオイルパテ，オイルサフェイサーが塗装され，ついでラッカーの上塗りがスプレーガンを用いて行なわれている。この上塗りは，耐水性サンドペーパーを水にぬらして磨く中とぎを間にはさんで計4回行なわれ，最後にコンパウンドという粉末で磨かれた後，自然乾燥されて塗装作業は終了する。

サンドブラッシング，プライマーの吹きつけは各々隔離した工場で行なわれているが，オイルパテの塗装以下の作業はすべて何ら隔壁のない一つの工場内で行なわれている。われわれが対象としたのはこの塗装工場である。ここでは1日平均約 15 台の自動車（主としてダンプカー，トラック）が取り扱われ，常時溶剤に曝露されている労働者は約100名であった。

II. 作業環境及び環境中のトルエン濃度の測定

1) 作業環境

第1図は作業場の略図である。図に示されているように，作業場は三方が壁に囲まれ，壁には小窓があるが常時閉まっている。残りの一方は自動車の出入りするスライディングドアーになっているが，このスライディングドアーは常時 ¼ 以上は開放されていないので，換気は極めて悪いと推察される。



( ) 番号は気中濃度を測定した場所

第 1 図　塗装工場見取図

2)　環境中のトルエン濃度の測定法

スプレーガンによる塗装作業及び塗料収納位置から蒸発した塗料溶剤（その組成はトルエン 71.5%，酢酸エチル 20.4%，キシレン 4.3%，ベンゼンらしきもの 3.8% でいずれも重量パーセントである）の環境中の濃度を，作業場内の 6 ヵ所（第 1 図）について三日間にわたり測定した。測定法としては，空気中の蒸気を捕集瓶を用いて捕集溶剤中に溶解捕集し，この捕集溶液をガスクロマトグラフにより分析した。

a.　サンプリング

分析の主な目的がトルエン蒸気の濃度決定であったため，捕集溶剤としては，ガスクロマトグラム上でピークがトルエンのそれから出来るだけ離れた位置に現われること，トルエン及び混合している可能性のある他の蒸気成分に対する溶解度が大であることが必要なので，アセトンを用いた。但し，採気中にアセトンが蒸発減少するので，捕集率が低下することを防ぐためアセトンの補充を行なって採気を続けた。捕集瓶としては，長いミゼットインピンジャーの底部に，径 3 mm のガラス玉 20 cc を充填し，溶剤 20 cc を入れたもの 2 本をシリーズに連結して用いた。2 本同時に用いたのは，各瓶中に捕集された蒸気成分の濃度から捕集効率を求め，その補正を加えた蒸気濃度を求めるためである。採気速度は 2 l/min，全採気量は 30 l であった。

b.　ガスクロマトグラフによる分析

熱伝導度セルを検知部とした装置を用いた。記録計のフルスケールは 60 mV であり，上記方法で採取した試料の分析において，環気中濃度約 10 ppm まで分析可能である。分析条件は，分離カラム：径 4.5 mm，長さ 2 m の銅管の D.O.P. カラム (30—65 メッシュのセライトを担体としている)，カラム温度：150°C，キャリャーガス：ヘリウム，流速：約 40 cc/min で，捕集溶液の 0.1c.c. をツベルクリン用注射器で注入した。溶剤であるアセトンのピークは，感度 $\frac{1}{100}$ で完全に描き，その後感度を最高 $\left(\frac{1}{1}\right)$ にしてトルエンその他のピークを得た。各蒸気成分の濃度は，相当するピークの面積と，アセトンピークの面積との比と，別に求めた濃度——ピーク面積比の関係曲線とから求めた。

3)　分析結果

第 1 表に示されている如く，トルエン蒸気の最低濃度は 11 ppm，最高濃度は 101 ppm であり，平均値は 49.6 ppm で，その信頼限界は 35.7 ppm から 63.5 ppm であった。これらの濃

第 1 表　各測定場所における気中トルエン濃度　(単位 ppm)

測定場所 / 日次	1	2	3	4	5	6
1*	97	52	47	53	36	31
2**	11	12	72	23	71	44
3***	45	20	42	101	46	89

*　天候：晴天，　気温：15.2～16.0°C，湿度：50～60%，採取時間：2～4 p.m.
**　天候：曇天，　気温：14.3～14.8°C，湿度：63～70%，採取時間：2～4 p.m.
*** 天候：雨上り，気温：17.2～18.0°C，湿度：83～89%，採取時間：2.30～4.30 p.m.

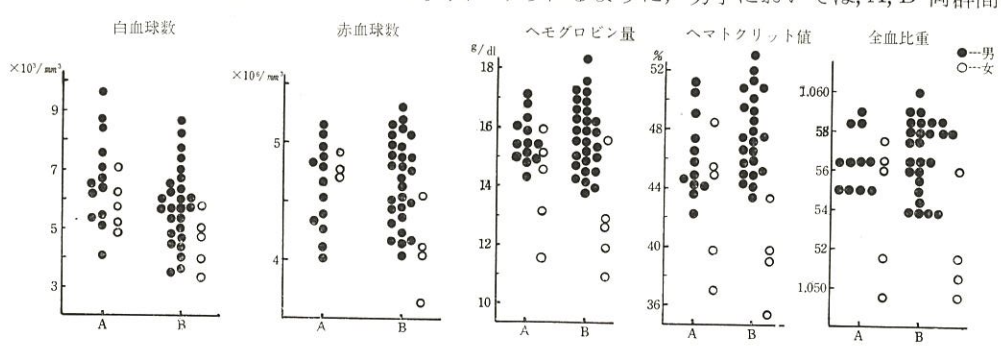
度は ACGIH のトルエン恕限度 200 ppm を下廻るものである。(1) の場所はドアーに近い所にあるが，その数値にみられるように自然換気の影響を概して受けていないようである。従ってこの平均値は，比較的本工場の平均曝露濃度に近いものを示しているのではないかと考える。

III. 血液検査成績

上記のような作業環境下に塗装作業に従事している男子 41 名，女子 10 名，計 51 名について血液検査を行なった。前述のようにこの工場では昭和 34 年 8 月，ベンゼンからトルエンの使用にきりかえているので，対象者を，34 年 8 月以降に始めて塗装作業に従事しトルエンのみに曝露したと考えられるもの（A 群）と，それ以前から従事しベンゼンにも曝露していたことのあるもの（B 群）との 2 群に分けて観察した。

1)　一般的血液所見

一般的検査として，赤血球数，白血球数，ヘモグロビン量，ヘマトクリット値，全血比重を測定したが，その結果は第 2 図の如くである。図にみられるように，男子においては，A, B 両群間に



（A は 34 年 8 月以降塗装作業に従事したもの，B は以前よりのものを示す）

第 2 図　血 液 検 査 成 績

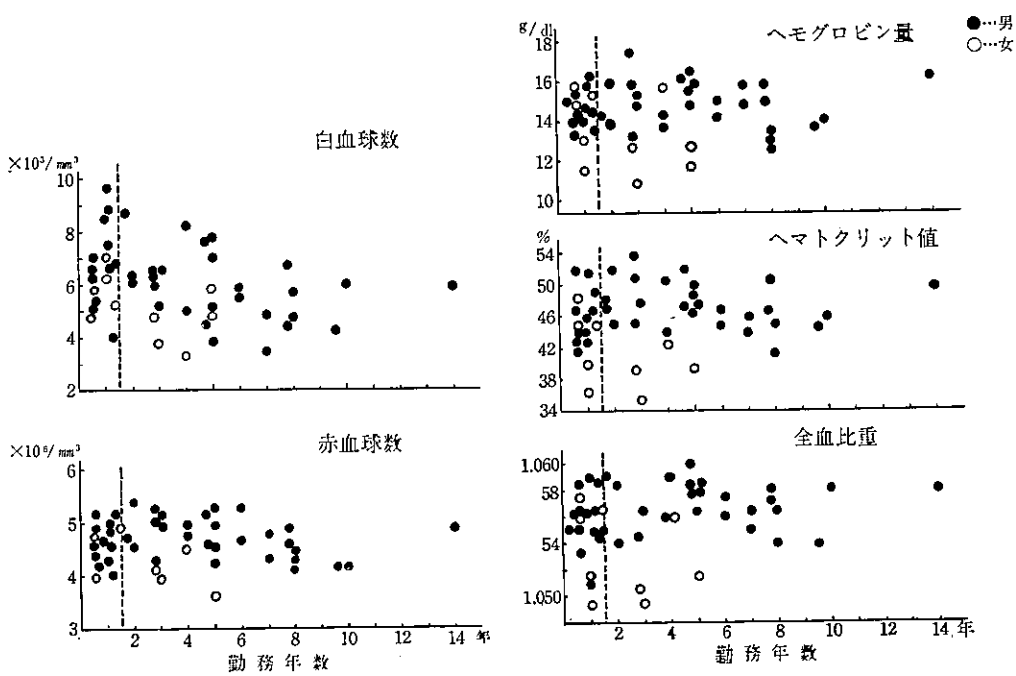
著明な差異は認められず，又両群とも大体正常範囲内の値を示している。しかし，白血球数のみは B 群は A 群に比していくらか低値を示し，減少傾向にあると思われる。女子については，例数が少ないのではっきりしたことは云えないが，ヘモグロビン，ヘマトクリット，全血比重の値はかなり分散が大きく且つ低値を示すものが両群ともに認められ，又血球数ではB群においてかなりの低値を示している。

石津[1] はベンゼンから代用溶剤にきりかわった場合の作業者の健康状態は，やや好転の傾向がみられたのみで全般にまだ顕著な影響を見出し得ないこと，又数年来トルエン等を主体とする溶

剤を使用している場合でも貧血，白血球減少などの所見がみられることを述べている。Greenburg 等[2] は，100—1100 ppm のトルエン濃度に2週間から5年以上曝露されている作業者について検査を行ない，血液検査では，赤血球の減少がわずかながらみられること，ヘモグロビン量が増加していること，平均赤血球容積 (M. C. V.)，平均赤血球血色素量 (M. C. H.)，平均赤血球血色素濃度 (M. C. H. C) 等の増加がみられること，リンパ球が増加していること，白血球の減少はなかったこと等を認め，特に著明な異常をみなかったと述べている。

われわれの調査対象では，ベンゼンを使用していた当時の作業者の検診及びベンゼンの気中濃度の測定は行なっていないので，ベンゼン曝露時の影響を知ることはできない。しかし，女子のB群の血球数がA群に比して減少していることと，後に述べる赤血球の生化学的変化から考察すると，トルエンにきりかえてから約1年半経過しても，ベンゼンの影響はいまだに残っているとも考えられる。但し，この場合，使用している塗装用溶剤中に3.8% のベンゼン様のものが含まれているので，この少量のベンゼン様物質が，いまだに影響を持つ場合も考えられるであろう。トルエンのみに曝露されている作業者では，男子では特に影響を受けていると思われるものは認めなかった。女子ではヘモグロビン，ヘマトクリット，全血比重の数値にかなりのばらつきがみられるが，これはベンゼン同様トルエンでも女子が感受性が高いことを示しているのではないかと考える。

次に第3図はそれぞれの実測値と塗装勤務年数との関係を示したものであるが（点線は溶剤を変更した昭和34年8月を示す），勤務年数と血液所見との間には関係はみられなかった。白血球数においても勤務年数による相関はみられなかった。なお，Greenburg 等[2] はトルエンについて勤務年数及び曝露の強さと血液所見との関係をみているが，完全に満足出来る結果を得ていない。しかし赤血球の減少及び M. C. V. の異常と，この勤務年数及び曝露強度とは関係づけるこ



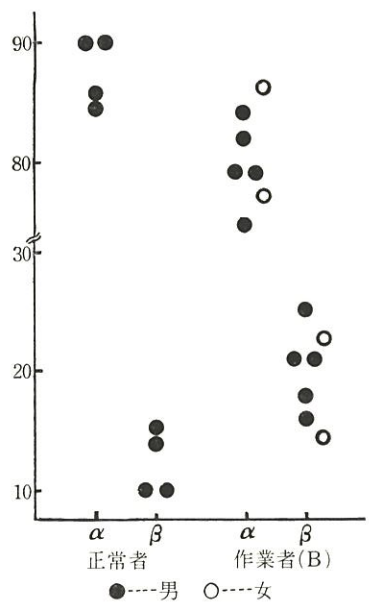
第 3 図　勤務年数別血液所見

とが出来るようであると云っている。

2) 血液の生化学的性状

共同研究者の長谷川，佐藤[3),4)]は，先にベンゼン中毒の実験的研究において，赤血球内漿の電気泳動像及び非ヘム蛋白区分値，血液中カタラーゼ活性値に著明な変化の起ることを報告している。この知見に基づき，われわれはこれらの変化を本工場作業者を対象として検討を加えた。

赤血球内漿の電気泳動像の面積百分率の変化は第4図に示した。正常者のこの泳動像は，かなり早く動く小さい β ピークと，遅く動く大きな α ピークからなっており，α ピークの面積百分率は平均 88%，β ピークは 12% であった。これに対し B 群作業者の α ピークは 80% と低下



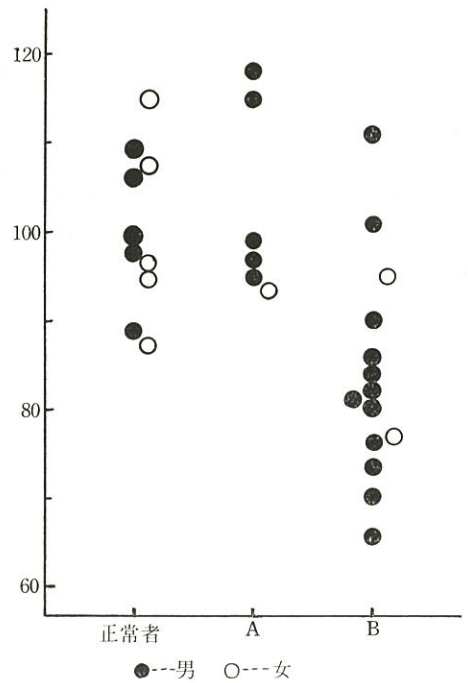
第 4 図 赤血球内漿の電気泳動像の面積百分率の変化

し，β ピークは 20% と増加しており，正常者のそれとかなりな差異を示している。

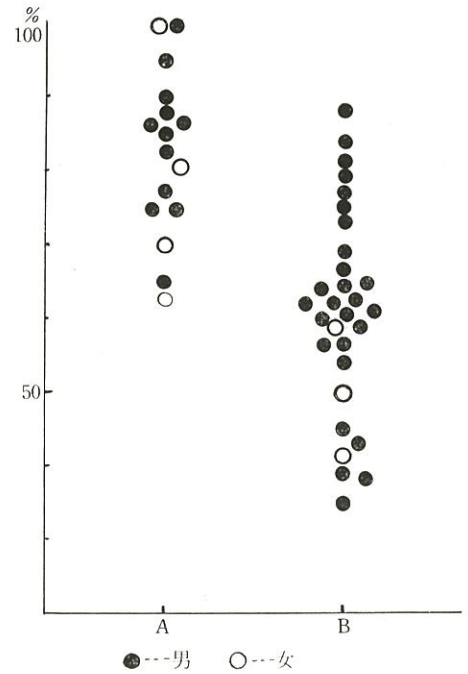
赤血球内漿の非ヘム蛋白区分値は，内漿の全蛋白量に対する非ヘム蛋白量の量比で表現したもので，測定成績は第5図に示した。これは正常者の平均値 2.31% を 100 に換算して各測定値を表現した。A 群の平均値は 103，B 群の平均値は 86 であり，トルエンのみに曝露していた作業者は正常者と差異を認めないが，ベンゼンに曝露した経験のある者では 86 と低下していた。即ちB群では非ヘム蛋白量が相対的に減少していることを示している。

血液のカタラーゼ活性値は第6図に示した。これは正常者の活性値を100% として換算したものであるが，A 群では平均 83%，B 群では平均 65% となり，前者は 17%，後者は 35% の減少がみられた。この場合，血中の全カタラーゼ量は正常者のそれと差異はみられなかったので，A, B 両群の活性値の低下は，当然，活性を阻害する妨害物質が血液中に存在することを意味している。

以上の結果から，以前にベンゼンに曝露していたことのある者では，一般の血液所見で殆んど正常範囲にありながら，赤血球内漿の電気泳動及び非ヘム蛋白区分，血液のカタラーゼ活性にはなおかなり影響を蒙っているのではないかと考えられる。



第 5 図　赤血球内漿の非ヘム蛋白区分の変動



第 6 図　カタラーゼ活性値の変動

要　　約

昭和 34 年 8 月にラッカーの溶剤をベンゼンからトルエンにきりかえてから，1 年半を経過している自動車車体塗装工場の環境調査及び塗装作業者の血液検査を行なった。

i）　トルエンの平均曝露濃度は 63.5 ppm＞m＞35.7 ppm であった。

ii）　調査対象者を，トルエンのみに曝露した者と，以前にベンゼンにも曝露したことのある者の 2 群に分けて検討すると，前者ではカタラーゼ活性の低下が認められたがその他の所見では著しい異常を認めず，後者においては赤血球内漿の電気泳動像及び非ヘム蛋白区分値，血液のカタラーゼ活性値にかなり著明な変化を認め，又この群の女子では血球数の減少が認められた。

iii）　われわれの調査したようなトルエン濃度中で，少なくとも 1 年半位の勤務年限では，この調査における血液の形態学的検査からは著しい影響はみられなかった。

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