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analysis of the patients

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Clinico-chemical Investigation of Chlorobiphenyl Poisoning
 Especially on the Serum Lipid Analysis of
 the Patients.

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(475)

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It was elucidated by the Research Team of Kyushu University for "Yusho" that its etiology is the chlorobiphenyls poisoning and our charge on this problem was to analyze the chemical constituents of serum and urine of the patients. Many analytical data were obtained by the methods, used routinely in clinical laboratories and some findings, which indicated the slight injuries of the liver functions, were obtained; slight elevation of the serum transaminase and alkaline phosphatase activities, decrease of serum iron, urinary excretion of large amounts of amino acids, and so on.

Among these findings, it was assumed to be a significant sign that serum of the patients showed often lactescence, which seemed to be due to lipemia. Thus, our attention was turned to analyze the serum lipids of the patients. When cholesterol, phospholipids and triglycerides of the serum was determined, the former two were in the normal range, but the latter was increased significantly. The ratio of β lipoproteins was also found to be elevated. From these findings, it was elucidated that lactescence of the patients' serum was due to hypertriglyceridemia.

It has been known that free fatty acids of serum are decreased, when carbohydrate are administered to the normal subjects. When we carried out this test for the patients, some of them showed the same interrelationship between blood glucose and serum free fatty acids as that of the normal subjects, but the abnormal figures were found in the other cases. The latter showed the elevated blood glucose level even three hours after glucose administration. In all patients the level of serum free fatty acids was found to be increased at fasting and the decreased level of them after glucose administration was higher than that of the normal.

Next, the gas-chromatographic analysis of each free fatty acid in serum was carried out and it was pointed that palmitic acid was decreased by glucose administration more significantly than the normal did. This experiment was assumed to indicate the abnormal accumulation of palmitic acid in the serum of the patients.

When urinary neutral 17 ketosteroids of the patients were analyzed by gas-chromatography, the elevation of the ratio of etiocholanolone androsterone, which indicated the occurrence of an adrenocortical hyperfunction, was found, though the urinary excretion of total 17 ketosteroids was in the normal range. This finding seemed to be interesting in the relation of fat metabolism and adrenal functions.

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It had been confirmed previously, by the research team of Kyushu University, that the cause of the so-called "Yusho"★ was a chlorobiphenyl poisoning. The authors, who (476) are members of the clinical section of the research team, have been examining blood serum and urine samples of the Yusho patients sent to this central clinical laboratory for some years. In this paper the results of the general biochemical analyses and serum lipid analysis of the samples are reported, the latter analysis being described in particular detail.

Although little was found to be characteristic of Yusho after general biochemical analyses, the patients' serum samples frequently showed clearly visible lactescence, which seemed to be due to lipemia, thus indicating that the chlorobiphenyl poisoning caused abnormal lipid metabolism.

I. GENERAL BIOCHEMICAL ANALYSES.

1. Serum Protein.

As shown in table 1, there was no abnormality in the amounts of total serum proteins. However, when serum proteins were fractionated by an electrophoretic method, increases of α_1 and α_2 -globulin were observed.

2. Serum Non-protein Nitrogen Compounds.

When urea nitrogen, uric acid, α -aminonitrogen (α -aminoacid) and creatinine were determined, no abnormality was found in Yusho patients' serum samples. The results are

★ - Translator's Note: Literally "Oil-disease".

shown in table 2.

3. Urinary Aminoacids.

Since the disease-causing substances of Yusho were chlorobiphenyls, it was thought that some of their degradation products were excreted in the patients' urine. When the patients' urinary aminoacids were analyzed by a Hitachi amino-acid autoanalyzer, Model KLA-3A, taurine, β -amino-isobutyric acid, glycine and histidine were detected in very large quantities and other aminoacids generally were found in rather high concentration, but no abnormal substance anticipated as degradation products of chlorobiphenyls could be found, as shown in table 3.

4. Serum Electrolytes.

As shown in table 4, sodium, calcium and chloride were found in normal concentration in the patients' serum, but potassium appeared to be in somewhat low concentration. Since Yusho was caused by organic chloro compounds, urinary chloride excretion was determined, but, as shown in table 5, no abnormality was observed.

5. Serum Iron.

As shown in table 6, decrease of serum iron was observed in Yusho patients. Some patients indeed had only 50% or less of the normal quantities.

Table 1. Serum Proteins of Yusho Patients.

Item of Analysis	n	Adult x ± S.D.	n	Infant x ± S.D.	Normal Range	Method
Total Protein	35	7.3 ± 0.6 g/dl	8	7.3 ± 0.5 g/dl	6.5~8.2 g/dl	Refractometer
albumin	33	59.4 ± 4.2 %	8	58.1 ± 6.6 %	55~65 %	cellulose acetate electrophoresis
globulin	33	6.2 ± 1.1	8	6.0 ± 1.0	3~5	
"	33	10.3 ± 2.1	8	13.1 ± 3.0	8~10	
"	33	9.0 ± 1.2	8	8.2 ± 0.7	8~12	
"	33	14.8 ± 2.1	8	14.7 ± 4.0	15~21	
Thymol Test	4	1.1 ± 0.4 Unit	0		4 Unit	Maclagan ¹²⁾
Zinc Sulfate Test	17	5.2 ± 1.5	3	4.7 ± 0.8 Unit	4~12	Kunkel ¹¹⁾

Table 2. Serum Non-protein Nitrogen Compounds of Yusho Patients.

Item of Analysis	n	Adult x ± S.D.	n	Infant x ± S.D.	Normal Range	Methods
Urea Nitrogen	22	12.3 ± 3.6	12	12.3 ± 2.4	8 ~ 17	Auto Analyzer
Uric Acid	11	5.0 ± 1.6	8	5.0 ± 1.0	2 ~ 6	Henry et al
α Amino Nitrogen	2	4.9 ± 0.4	1	4.7	3.5 ~ 5.0	Rubinstein-Pryce ¹⁴⁾
Creatinine	5	1.0 ± 0.2	0		0.8 ~ 1.5	Momose, ¹³⁾ Mukai

The figures show mg/dl

Table 3: Analysis of Urinary Aminoacids of Yusho Patients.

Aminoacids	Yusho Patient		Min. and Max. of Normal 5 Specimens
	24 yr. old Female	16 yr. old Male	
	$\mu\text{M}/24 \text{ hr.}$	$\mu\text{M}/24 \text{ hr.}$	$\mu\text{M}/24 \text{ hr.}$
Taurine	5641	8103	35-884
Oxyproline	0	0	0~27
Aspartic Acid	36	15	1~5
Threonine *	548	863	8~83
Serine	366	836	10~53
Glutamic Acid	52	84	18~186
Citrulline	Trace	Trace	9~109
Proline	47	76	88~137
Glycine	1865	2997	82~308
Alanine	538	341	173~467
1/2 cystine	70	135	6~21
Valine	63	61	105~239
Methionine	21	49	15~24
Isoleucine	84	106	22~69
Leucine	47	71	59~139
Tyrosine	104	247	32~61
Phenylalanine	77	125	31~77
β -AIBA	247	1042	0
Tryptophan	160	42	3~27
Ornithine	0	0	0
Lysine	243	118	33~189
Histidine	743	1170	11~57
Arginine	18	Trace	5~67
α -ANBA	13	30	8~28
Methylhistidine**	344	213	2~30
β -Alanine	Trace	Trace	0

β -AIBA = β -Amino-isobutyric acid

α -ANBA = α -Amino-n-butyryric acid

* Threonine contains both glutamine and aspartic acid

(477)

** Methyl histidine is a mixture of 1-Methyl- and 3-Methyl-histidine.

Table 4: Serum Electrolytes of Yusho Patients.

Item Analyzed	*Adult n	$\bar{x} \pm S.D.$	Infant n	$\bar{x} \pm S.D.$	Normal Range	Method
Sodium	54	142±2	17	140±2	135~152	Auto Analyzer
Potassium	53	4.0±0.4	17	4.2±0.5	4.5~5.5	Auto Analyzer
Chloride	53	104±3	16	103±2	96~110	Auto Analyzer
Calcium	29	4.6±0.2	13	4.7±0.1	4.5~5.5	Flame Analysis

The figures are shown in a meq/l unit

Table 5: Urinary Chloride of Yusho Patients.

Patients	1st Determination	2nd Determination
Female 35 yr.	8.3	
" 40 yr.	5.4	9.3
" 24 yr.	6.4	7.6
" 28 yr.	10.0	
" 39 yr.	6.8	
" 15 yr.		7.5
" 37 yr.	5.1	8.0

Normal Range

5~10g/24 hr.

6. Serum Enzyme.

Serum transaminase activity was found slightly elevated in the patients' serum, and a tendency of GOT < GPT was observed. In infant patients, the increase of GOT activity was more pronounced than that in adult patients. While serum lactic acid dehydrogenase activity did not change, alkaline phosphatase activity was generally slightly larger in the patients' serum than in normal serum. These results are presented in table 7.

7. Urinary 17-Ketosteroids.

As described earlier, many Yusho patients showed lipemia. Since lipid metabolism usually was affected by steroid

hormones to a large extent, determination of urinary 17-ketosteroids was conducted to see if there was any significant change in their content. The determination was carried out following Zimmermann's method¹⁵⁾, except hiamine^{*} 10X, that is, one molar methanolic solution³⁾ of p-(diisobutylcresoxyethyl)-dimethylbenzylammonium chloride, was used instead of the usual 5N aqueous potassium hydroxide solution for coloration at the final step.

The results are shown in table 8, which indicate that both male and female patients excreted normal daily amounts of the total steroids. However, since it appeared to be important to know the relative amounts of steroids, the steroids were individually determined. Although absolute amounts of each steroid were commonly considered to be important, it was found that the ratio of etiocholanolone (Et) versa androsterone (As) was even more important.

Table 6: Serum Iron of Yusho Patients.

	Adult		Infant		Normal Range	Method
	n	$\bar{x} \pm S.D.$	n	$\bar{x} \pm S.D.$		
Serum Iron	50	90 \pm 34	17	83 \pm 26	100-170	Kingsley-Getchell ¹⁶⁾

(478)

The unit is $\mu\text{g}/\text{dl}$

* Translator's Note; Transliterated. Probably a trade name of the commercial reagent solution described above.

Table 7: Serum Enzyme Activities of Yusho Patients.

Enzyme ^A	Adult		Infant		Normal Range	Method
	n	$\bar{x} \pm S.D.$	n	$\bar{x} \pm S.D.$		
GOT	70	24 ± 19	12	36 ± 23	5-40	Auto Analyzer
GPT	48	23 ± 16	10	21 ± 11	5-30	Auto Analyzer
Lactic Acid Dehydrogenase	24	278 ± 65	5	284 ± 24	100-450	Cabaud-Wroblewski ⁵⁾
Basic Phosphorynase	65	10.3 ± 3.5	11	17.4 ± 4.9	A = 4-12 I = 9-23	Auto Analyzer (Modified Kind-King Method ⁹⁾)

A-Adult
I-Infant

* - Enzyme unit is as follows.
 GOT, GPT: Karmen Unit
 Lactic Acid Dehydrogenase: Karmen Unit
 Basic Phosphorynase: King-Armstrong Unit.

Table 8: 17-Ketosteroids of Yusho Patients.

A; total 17-Ketosteroids.

Patients		n	$\bar{x} \pm S.D.$	Normal Range
Patients	Female Adult	23	5.7 ± 1.8 mg/24hr	4-12 mg/24hr
	Male Adult	13	7.3 ± 2.8	7-18

Table 8: Continued.

B: Individual Determination of 17-ketosteroids.

			Total Zimmermann Dye *	Andro- sterone (As)	Etiocho- lanolone (Et)	Dehydro- epiandro- sterone (DHA)	Et/As
Nor- mal	M	28	7.6	0.12mg/dl	0.07mg/dl	0.02mg/dl	0.6
	M	28		0.12	0.12	0.05	1.0
	M	28		0.21	0.17	0.09	0.8
	M	20		0.15	0.06	0.06	0.4
Yusho- mal	M	35	6.0	0.09	0.15	0.13	1.7
	M	33	6.0	0.06	0.12	0.09	2.0
	M	39	9.9	0.05	0.13	0.09	2.6
	M	37	7.3	0.11	0.10	0.09	0.9

F- Female

M- Male

* - It should correspond to the total amount of 17-ketosteroids, but due to contaminated dyes, it becomes larger than the total of As + Et + DHA.

The fractional determination of 17-ketosteroid was carried out as follows. Urine was hydrolyzed by shaking with ethyl acetate containing perchloric acid, and the freed steroids were extracted with dichloromethane. Its neutral fraction was purified using a (preparative) * thin layer plate of SiGel H (Merck) developed by a (1:1) mixture of benzene and ethyl acetate. The purified steroid mixture was quantitatively analyzed by a gas chromatographic method using cholesterol as an internal standard, to determine the amounts of androsterone, etiocholanolone (479) and dehydroandrosterone (Unpublished: by Junji NAGAI and Michiyo FURUKAWA).

* Translator's Note: Added by the translator.

The results are given in table 8. High values of the Et/As ratio of both male and female Yusho patients are quite significant. This is perhaps to show that an adrenocortical hyper function took place in the patients.

II. SERUM LIPID ANALYSIS.

As described earlier, the lipemia of Yusho patients was the most significant characteristic of this disease. Therefore, the authors examined the patients' serum lipid in great detail.

1. Serum Cholesterol, Phospholipids and Triglycerides.

From the patients whose Yusho symptoms were confirmed by clinical diagnoses, serum samples were withdrawn and the three title components in the samples were determined. The results were given in table 9. As seen in the table, both serum cholesterol and phospholipids were found in a normal range, but triglycerides were found in larger quantities than normal. Therefore, it was concluded that the lipemia of the patients was due to hypertriglyceridemia.

2. Serum Lipoproteins.

In order to confirm the above conclusion, filter paper electrophoresis of the patients' serum was conducted by Durrum and others' method⁶⁾, and its lipoproteins were dyed with "Oil red O", and the ratio of B/A lipoproteins was determined by a colorimetric method. As shown in table 10, the B/A lipoprotein ratio was remarkably higher than the normal ratio of 1.2-2.5. Moreover, the high values were found to be proportional to the high value of total serum lipids. This may

also be taken as evidence for the serum triglyceride increase in Yusho patients.

3. Carbohydrate Administration and Serum Free Fatty Acids.

Since Yusho was estimated to be not only a kind of skin disease but also a disease caused by a total metabolism disorder, the authors examined the patients' metabolism of carbohydrate by administering a carbohydrate substance.

Following the routine method of carbohydrate administration, 50g of glucose was given orally, immediately after a control serum sample was withdrawn from the patients at their fasting periods. Blood samples were taken at 30, 60, 90, 120, 150 and 180 minutes after the administration of the carbohydrate, their blood sugar was determined by the glucose oxidation method²⁾, and blood sugar curves of Yusho patients were obtained.

Since free serum fatty acids exist in a very close relationship with serum carbohydrates, free fatty acids in serum samples withdrawn at fasting and after 60, 120, and 180 minutes following the administration of glucose were determined. The free fatty acids were determined as follows. The serum sample was extracted with chloroform, and the acids extracted were methylated with diazomethane and the esterified fatty acids were analyzed by a gas chromatographic method using arachidic acid as an internal standard (Junji NAGAI, Yoshiaki YAE and Michiyo FURUKAWA: Unpublished).

Fluctuations of blood sugar and the accompanied free serum fatty acids are shown in table 11. When the blood

sugar increased by administration of glucose, serum fatty acids decreased, and as the blood sugar value approached the standard value at the fasting, the free serum fatty acids approached the standard value at the fasting.

Table 9: Serum Lipids of Yusho Patients

Lipids	"	$\bar{x} \pm S.D.$	Normal Range	Method
Cholesterol	27	181 \pm 43	140-210	Zurkowski ¹⁾
Phospholipids	27	8.1 \pm 1.8	5-10	Baumann ¹⁾
Triglycerides	32	189 \pm 64	50-130	Blankenhorn et al ⁴⁾

* - Analyzed only of adult patients.
The figures are shown in the unit mg/dl.

Table 10: Serum Lipoproteins of Yusho Patients.

Subjects		Ratio of β/α Lipo-proteins	Total Serum Lipids *
Normal	Male 26 yr.	1.9	620 mg/dl
Yusho	Female 24 yr.	2.0	700 mg/dl
	Male 35 yr.	3.5	1000
	Female 60 yr.	4.4	875

* - Determined by the method of de la Huerga et al⁸⁾, and this method gives approximate values only.

Table 11: Fractuation of Blood Sugar and Free Serum Fatty Acids after Administration of Glucose to Normal Adult.

Subject	Items Analyzed	Fasting	After Administration (min.)					
			30	60	90	120	150	180
Male 48 yrs.	Blood sugar	82	134	146	128	125	104	88
	Free Serum Fatty Acids	9.8	-	8.0	-	7.2	-	8.4

Unit is mg/dl.

Table 12: Fractuation of Blood Sugar and Free Serum Fatty Acids after Administration of Glucose to Yusho Patients.

Type	Patients	Items of Analysis	Fast- ing	After Administration (min)					
				30	60	90	120	150	180
A	F-24 yr	Blood Sugar Free Serum Fatty Acids	80	131	156	152	139	93	76
			22.2	-	23.2	-	24.2	-	25.0
B	M-34 yr	(same)	88	108	118	102	102	105	72
			13.9	-	13.3	-	7.2	-	10.0
	F-38 yr	(same)	88	83	107	84	93	117	115
			32.8	-	23.5	-	23.9	-	21.9
	M-39 yr	(same)	89	107	102	108	125	113	111
			30.0	-	13.5	-	14.4	-	18.9

(480)

In a certain number of cases of Yusho patients, nearly the same tendency of changes as in the normal cases was observed, but many cases of abnormal fluctuations were also found. However, no case of excess blood sugar was found in Yusho patients when glucose was administered.

Although the mode of blood sugar fluctuation after administration of glucose to Yusho patients varied a great deal, they could be roughly classified into two groups, A and B. Namely, in the group A of table 12, blood sugar change was quite similar to normal cases, but the serum free fatty acids did not decrease even after the administration of glucose.

In the type B blood sugar change, the sugar value never returned to the fasting period standard value even after 180 minutes of glucose administration, and it increased further than the standard value. However, in group B, the free serum fatty acid value changed just as the normal cases.

It was quite significant to find that the patients' free serum fatty acid level was as high as 1.5-4 times the normal specimen, regardless of the type of fluctuation of blood sugar after administration of glucose, and that the amount of its decrease after glucose administration was also much higher than the normal specimen.

4. Fluctuation of Each Free Serum Fatty Acid after Administration of Glucose.

The individual fatty acids examined are myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), palmito-oleic acid ($C_{16:1}$)

stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$) and linolic acid ($C_{18:2}$), and these are the major fatty acids found as normal serum free fatty acids.

Since the total free fatty acids in the patients' serum were unusually large, and these large total amounts also were found to vary considerably, it was felt important to check how the amounts of individual serum fatty acids fractuate. Individual fatty acid was determined separately as described earlier, using a gas chromatographic method.

Table 13 shows the fractuation of a normal specimen. It reveals that the major contribution to the decrease of free serum fatty acid is done mainly by oleic acid and partly by palmitic acid.

One of the group A cases shown in table 14 had nearly the same serum free fatty acid fractuation as that of the normal specimen, that is, drastic decrease of oleic acid by glucose administration and some decreases of both myristic acid and linolic acid. On the other hand, the other case of group A showed an increase of free serum fatty acid after administration of glucose, and particularly the increases of palmitic acid and palmito-oleic acid were significant.

Table 13: Fractuation of Each Free Fatty Acid in Serum of Normal Adult after Administration of Glucose (Male 48 yrs.)

Hour of Sampling	C _{14:0}	C _{16:0}	C _{18:1}	C _?	C _{18:0}	C _{18:1}	C _{18:2}
Fasting	0.4	3.3	0.8	0.7	1.9	2.6	0.1
After Admin. 60min	0.3	2.6	0.6	1.0	1.8	0.6	1.0
120 "	0.4	2.5	0.4	0.7	1.3	1.4	0.7
180 "	0.6	3.2	0.7	1.1	1.6	0.7	0.7

Unit in mg/dl.

Table 14: Fractuation of Each Free Fatty Acid in Serum of Yusho Patients after Administration of Glucose.

(481)

Type	Patients	Hour of Sampling	C _{14:0}	C _{16:0}	C _{18:1}	C _{18:0}	C _{18:1}	C _{18:2}
A	Female 24 yr.	Fasting	2.7	6.6	1.4	3.8	5.7	2.1
		After Admin. 60M	2.5	8.1	2.4	3.0	5.5	1.7
		120 "	2.2	8.7	2.7	2.7	6.2	1.9
	Male 34 yr.	180 "	2.8	7.9	2.1	3.5	6.4	2.4
		60M	2.5	3.3	1.2	2.0	3.7	1.3
		120 "	1.3	5.1	1.0	2.3	2.9	0.7
B	Female 38 yr.	180 "	0.8	3.2	0.2	1.5	1.2	0.3
		180 "	1.5	4.3	0.4	2.1	2.0	0.5
		60M	3.5	14.0	1.8	5.0	6.1	2.5
	Male 39 yr.	120 "	3.4	13.7	1.5	5.4	4.4	0.1
		180 "	3.3	9.5	2.0	3.9	4.0	1.3
		180 "	2.2	9.5	0.7	3.3	3.6	2.6
Male 39 yr.	60M	3.2	11.5	2.2	4.0	6.9	2.4	
	120 "	2.4	6.8	1.8	2.7	3.4	1.4	
	180 "	3.6	6.7	1.2	2.8	3.9	1.2	
		180 "	1.6	5.8	1.2	2.7	2.8	4.9

M = Min.

Unit in mg/dl of each fatty acid.

In group B, decreases of both palmitic acid and oleic acid were clearly shown in both cases, and stearic acid also appeared to be in the decreasing tendency.

5. Total Fatty Acids in Pimples of Yusho Patients.

The major symptom of Yusho is a pimple^{*}-like appearance of the patients' faces, and as described previously, abnormal fatty acid metabolism has been confirmed in this disease. Therefore, two sets of analytical data of fatty acids were compared; one of the Yusho patients' pimples and the other of ordinary pimples. In all cases, the "core"^{**} of the pimple was pressed out, but since it was extremely difficult to collect a sufficient quantity for analysis from one patient, samples of ordinary pimples were collected from several score people and the samples of Yusho patients were collected from members of one and the same family. Pressing out the "cores" of the patients' pimples was done twice after a certain time interval, and the collected "cores" of pimples were placed in a (2:1) mixture of chloroform and methanol and stored in a cool place. The amounts collected were approximately 200 mg. in both groups.

Extraction of fat was conducted by homogenizing the "cores" of pimples with a (2:1) mixture of chloroform:methanol

* - Translator's Note: a papilla, a comedo, a blackhead, a carbuncle or a pustule. No particular distinction is made by the authors.

** - Translator's Note: The authors use a word "center" or "heart".

using a glass homogenizer, and the homogenate was filtered and the filtrate was combined with the storage solution previously described. The combined fat extract solution was evaporated below 40°C under a nitrogen atmosphere, and the residue was saponified using a 5% methanolic potassium hydroxide. Fatty acid liberated was extracted and methylated by the routine method, and the ester mixture was analyzed by a gas chromatographic method. Even though the largest amount of samples possible was collected as previously described, the amount obtained for the final analysis was insufficient to do anything beyond comparative analyses of the total fatty acids.

The analytical results are shown in table 15. Even when only samples of the same family were combined for the analysis, the fatty acid compositions varied depending on when the samples were collected. Nevertheless, there was undoubtedly a very clear difference between the fatty acids of Yusho patients' pimples and those of ordinary pimples.

Namely, stearic acid and oleic acid were rich in Yusho pimples and particularly linolic acid, which could not be detected in common pimples by gas chromatography, was detected at fairly high concentration in Yusho pimples. On the other hand, palmitic acid and palmito-oleic acid were detected in Yusho pimples at relatively low concentration.

Table 15: Total Fatty Acids of "Pimples" of Yusho Patients.

Sample	C ₁₆	C ₁₈	C _{18:1}	C _{18:2}	C _{18:3}	C _{18:3}
Common Pimples	11.6	41.2	17.0	13.7	16.5	—
Yusho Pimples						
1st sampling	3.0	22.1	10.7	16.6	31.5	16.1
2nd sampling	8.3	23.4	6.1	31.2	23.5	7.5

The figures are % of each fatty acid.

In summary, the C₁₆ fatty acids were found in low concentrations in Yusho pimples, but the C₁₈ acids, particularly the unsaturated C₁₈ acids, were detected at high concentrations. (482)

Gas chromatographic determination of cholesterol in Yusho and ordinary pimples revealed that the former contained nearly ten times as much cholesterol as the latter pimples, but the absolute quantity of the cholesterol could not be determined. UV spectroscopy indicated the presence of a sterol type substance with a conjugated double bond system in Yusho pimple fatty substance fraction, but its identification could not be done due to the lack of a sufficient amount of samples.

III. SUMMARY

The following facts were found to be abnormal, when Yusho patients' various clinical testing samples were analyzed. These are listed below with brief discussions.

- (1) A slight increase of α_2 -globulin was found in serum proteins, but its significance remains unknown.
- (2) Except for a very slight decrease of potassium, serum

electrolytes appeared to be normal. The cause of this slight decrease of potassium is also unknown.

(3) The serum iron content was found lower than usual, and this may be attributed simply to one of the general phenomena of chronic hepatitis.

(4) Even though the serum α -amino nitrogen remained in the normal range of concentration, urinary aminoacid excretion was very large in Yusho patients, and particularly the excretion of taurine and glycine were abnormally large. This may be attributed to a result of a defense action by the liver.

(5) As to the serum enzyme, transaminase activities, and particularly the GOT activity of infant patients, were found to be at an elevated level. Alkaline phosphatase activities were also found at the upper limit of the normal range. These findings indicate that some liver disorders took place owing to chlorobiphenyls, and the infants had higher susceptibility to these chlorocompounds. However, in all cases serum bilirubin remained within a normal range.

(6) The quantity of daily excretion of urinary 17-ketosteroids remained normal in both male and female patients. However, the ratio of etiocholanolone against androsterone was quite high in the case of Yusho patients, indicating that chlorobiphenyl induced excretion of adrenal hormones, directly or indirectly.

(7) While serum cholesterol and phospholipids were found within the normal range, triglycerides were detected in an increased quantity. Increase of a ratio of β/α serum lipoproteins was also detected. The cause of the increase of serum triglycerides

in Yusho is still not clear.

(8) The blood sugar curves could be classified into normal and abnormal types after the administration of glucose. After three hours, in the latter type, the blood sugar quantity was larger than at the fasting period. But in both groups none showed the symptom of excess blood sugar after administering glucose.

(9) Free fatty acids after administration of glucose were found to be normal. However, absolute quantities of free serum fatty acids both at fasting and after glucose administration were of course much larger than the normal specimen. In some cases, the free serum fatty acids maintained their high level of ^{the} fasting period, even after glucose administration.

(10) When individual fatty acid of free serum fatty acid mixture of Yusho patients was analyzed, palmitic acid was found to decrease quite remarkably after administration of glucose. From this finding, it was reasonably concluded that one of the causes of increase of free serum fatty acids in Yusho disease was an accumulation of palmitic acid.

(11) When total fatty acids of "pimples", which are the characteristic skin symptom of Yusho disease, were analyzed, the percentage of C₁₈ fatty acids, particularly unsaturated C₁₈ acids, was found much higher than that in normal pimples. Also the cholesterol content was high in pimples of Yusho.

It is interesting to know how these abnormalities are related to abnormal lipid metabolism in the human body caused by chlorobiphenyls. The authors expect more will be

revealed along this line in the future.

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Addition (to the section on Serum Enzyme) Choline Esterase
Activity.

Choline esterase (ChE) activities of serum and red blood corpuscles (erythrocytes) of Yusho patients were determined by a modified method of S. Hestrin (J. Biol. Chem. 180:249, 1949). The average value and their standard deviation of four cases were as follows.

Serum ChE: 11.6 ± 0.8 (Normal range is 10-15, and the unit is shown by a number of μ M of acetic acid produced by degradation of acetyl choline with 0.05 ml of serum in one hour).

Red Blood Corpuscle ChE: 33.4 ± 1.3 (Normal range is 30-35, and the unit is shown by a number in mg of acetyl choline decomposed by red blood corpuscles contained in 1 ml of whole blood within 30 minutes).

As shown by these data, both choline esterase activities were found within the normal ranges and no abnormality could be detected.