ON THE INFLUENCES OF THE SPLENIC COMPONENTS OF VARIOUS ANIMALS ON THE EXPERIMENTAL HEPATIC DISTURBANCES

I. INFLUENCES OF SPLENIC COMPONENTS OF CATTLE*

YOSHIO TOGAMI

Pharmacological Department, Showa Medical School, Tokyo

Received for publication April 17, 1952

INTRODUCTION

Since the studies by Tsunoo and Koizuka (1, 2) on the specific components of spleen (so-called splenic hormone), numerous studies on various functions of spleen of various animals have been performed in our laboratory. Fujita (3, 4) and Abe (5, 6) and others have reported on substances inducing leucopenia and leucocytosis contained in the spleen of cattle and hog, and further discussed on the relationships of these isolated substances to aromatic aldehydes confirming an important role played by the splenic components to the leucocytosis or leucopenia in the circulating blood among the actions of aldehydes to rabbit. Meanwhile, Yoshimatsu (7, 8) has proved that the Crystal (A) $C_6H_{13}O_2N$, which was isolated by Fujita and Abe from cattle and hog spleen, is a substance to cause leucopenia and also is a blood coagulation promoting substance. Later, Sekino (9, 10, 11) and Sakai (12, 13) have histologically investigated the influence of spleen on the toxicity of nitro-compounds to rabbit. Roughly in parallel to the studies by Sekino and Sakai and others, Irisawa (14) has investigated in detail the relationship between the extracts of various splenic fractions as well as crystalline substances of spleen and the toxicity of cocain. In 1932, Tanakaya (15) has reported that spleen can exert a certain protective action on the hepatic disturbances induced by carbon tetrachloride.

Basing on the above facts, experiments were planned to investigate the influences of splenic components of various animals on the hepatic disturbances induced by carbon tetrachloride, whereby to study further on the correlation of the above influences to various splenic functions which had been clarified in the past.

EXPERIMENTAL MATERIALS AND METHODS

Mice weighing approximately 10 g were selected as test animals. Those mice

*Director: Prof. Dr. Sh. Tsunoo

overgrown were not taken in order to avoid the risk that they may have certain changes in their livers due to age. For one experimental group of animal, mice which are in a same stage of growth were selected primarily. They were fed on barley and a small amount of vegetable before they were subjected to the experiments. In general, one group consisted of 10 mice. They were kept for 15 days after the experiment was initiated, then their carotid arteries were cut through for bleeding to death. Livers were taken out and fixed in 1:10 formalin solutions, and sections were made as usual and stained with hematoxylin and eosin.

In the case of carbon tetrachloride alone, 0.05 cc per 10 g mice was injected subcutaneously in the back every other day. In other words, the 1st injection was given on the 1st day of experiment, and the 7th injection was given on the 14th day of experiment, and on the 15th day, the mice were bled to death. In the case of combined injection, 0.1 cc per 10 g mice of various extracts of spleen

FIG. 1

or various other substances subcutaneously in the back every other day and carbon tetrachloride was injected 15 minutes later in the same manner as in the case of carbon tetrachloride alone.

The isolation method of the alcohol soluble component of spleen followed Fujita's method in general (Fig. 1). Cattle spleen containing blood was minced and ground, infused in 94% alcohol to separate into alcohol extract and solid splenic component. Alcohol contained in the former was distilled off and the residue was combined with the latter. The combination was then thoroughly extracted with 5 volumes of warm water. The filtrate thus obtained was coagulated by heat and concentrated to syrupy state and added with a large amount of alcohol until the solution demonstrated no turbidity. The solution was then kept standing before it was filtrated by suction and distilled under reduced pressure for concentration. When the concentrate was kept standing at room temperature, crystalline substance settled out. Crystal (A) was obtained when this crystal was recrystallized from hot solution under added animal-charcoal.

The melting point of Crystal (A) was 241°C with decomposition; it was Millon negative, ninhydrin positive litmus neutral. This crystal had no leucine-like lustre, and it was tasteless or had very mild bitterness. The results of elementary analysis conferred with that of leucine, but it was optically inactive, and its space formula is still unknown.

The isolation of alcohol insoluble component of bovine spleen was performed under the cooperation by Irisawa and Katada (15). The splenic substance of cattle, which had been stored as alcohol insoluble substance (seen in Fig. 1), was added with water and dissolved on a water bath and then was added with lead subacetate solution. After filtration, H₂S gas was bubbled and H₂S gas was removed as according to the usual procedures to settle cut crystal. The resulting filtrate was then concentrated and the solution first obtained was named as Solution (A) of alcohol insoluble componet of cattle spleen. The remaining Solution (A) was added with phosphotungstic acid, and the resulting precipitate was filtered off. The extract obtained from the precipitate was named as Solution (B), and the extract obtained from the filtrate was named as Solution (C). During this process, numerous crystalline substances were isolated, but the inhibitory action on the hepatic disturbances of the following 5 substances were investigated.

Crystal (I)—melting point 271°~272°C with decomposition, white needle crystal like tyrosine

Crystal (II)—melting point 263°~264°C with decomposition and sublimation, Millon positive (++)

Crystal (III)—melting point 259°~260°C with decomposition, Millon (±)

Crystal (IV)—melting point 255°~256°C with decomposition and sublimation

Crystal (V)—melting point 255°~256°C with decomposition and sublimation
Each 1 cc of the extracts prepared corresponded to 1 g of fresh organ.

RESULTS OF EXPERIMENTS

A. Subcutaneous Injection of Carbon Tetrachloride Alone

For the purpose of control test, histological sections prepared from 10 normal mice were examined microscopically. Excepting 3 cases, in which very mild round cells infiltration was observed in Glisson's capsule, the arrangement of hepatic cells was normal, and no hyperplastic tendency of connective tissue was observed (Plate 1: A, B).

The mice subcutaneously injected with carbon tetrachloride as described in the above demonstrated poor growth during the experiment, and their gain in weight was not distinct as compared with the control. In 2 cases of mice, they lost weight and became fatal on the 9th day of experiment.

When histological sections of liver intoxicated by carbon tetrachloride were microscopically examined, a high degree of hepatic disturbances was observed. The arrangement of hepatic cells was irregular, albuminoid, vacuolar or lipoid degenerations were also observed. Specifically, vacuolar degeneration was found intensive, and the lipoid degeneration was also fairly in a high degree, and necrosis was also found in certain cases. The nuclei of the hepatic cells were found extremely swollen. In Glisson's capsules, round cells infiltrations were observed, and as the result, formation of pseudo biliary ducts was also recognized. All of the Kupffer's stellate endothelial cells were found enlarged and hyperplastic. Around the central veins as well as all the blood vessels, cell infiltrations were observed, in particular, round cells infiltrations were recognized in the interstitial tissues accompanied by hyperplastic tendency of the connective tissue (Table 1, Plate 1: C, D).

B. Subcutaneous Injection of Carbon Tetrachloride Combined with Splenic Components of Cattle

I. In the case of alcohol soluble component of bovine spleen

In this experiment, influence of the following 9 extracts of bovine spleen combined with carbon tetrachloride on the hepatic disturbances induced by carbon tetrachloride were investigated.

- Alcohol soluble extract
- Acetone soluble extract
- Acetone insoluble extract
- Benzol soluble extract
- Benzol insoluble extract
- Ether soluble extract
- Ether insoluble extract

TABLE 1. Subcutaneous injection of carbon tetrachloride alone

No. of animals		313	314	315	316	317	318	319	320	321	322		
Body-weight before injection (g)		12.8	11.1	8.9	10.2	8.3	10	14.7	11.7	7.8	8.3		
Terminal body-weight (g)		13.7	12.0	9.5	9.6	6.9	8.1	14.6	9.3	6.4	6.2		
Survival period (day)		15	15	15	15	15	15	15	15	9	9		
Weight of liver (mg)		800	850	700	520	550	450	900	500	450	390		
Microscopic appearance	Findings in the interstitial tissues	Connective tissue		+	+	+	-	-	+	++	+	±	-
		Glisson's capsules	Hyperplasia of the connective tissue	±	±	-	-	-	-	-	-	-	-
			Round cells infiltration	+	+	+	-	±	+	±	+	+	±
			Formation of pseudo biliary ducts	+	+	+	-	+	+	+	+	+	+
			Arterial and venous hyperemia	-	-	-	-	-	-	-	-	-	-
	Findings in acini	Kupffer's stellate endothelial cells	Enlargement	+	+	+	+	+	+	+	+	+	+
			Hyperplasia	++	+	++	+	+	+	++	+	+	+
		Hepatic cells	Arrangement	I R	I R	I R	I R	I R	I R	I R	I R	I R	I R
			Albuminoid degeneration	+	+	+	+	+	+	+	+	+	+
			Vacuolar degeneration	++	++	++	+	+	+	++	+	+	+
			Lipoid degeneration	+	+	+	+	+	+	+	+	+	-
			Necrosis	-	-	+	+	-	-	±	+	-	-
			Atrophia of nuclei	-	-	-	+	-	-	-	-	-	-
			Swelling of nuclei	+	+	+	±	+	+	+	+	±	-
		Round cells infiltration	Around the blood vessels	++	++	+	±	-	+	-	++	-	±
			Around the hyperplastic connective tissue	+	+	+	-	-	+	+	+	-	-
			In intraacinous area	-	-	-	-	-	-	-	-	-	-
		Cells infiltration around the central veins		+	+	+	±	-	±	++	+	+	±

R: Regular, IR: Irregular, SIR: Slightly irregular

Ethyl acetate soluble extract

Ethyl acetate insoluble extract

In comparison with the cases of the injection of carbon tetrachloride alone, in all the cases of various extracts in combination with carbon tetrachloride, the hepatic disturbances were milder. In other words, each of the extracts acted

inhibitory against hepatic disturbances in mice induced by carbon tetrachloride intoxication. Above all, acetone insoluble extract demonstrated a strong inhibitory action against hepatic disturbances, showing no irregular arrangement of hepatic cells. Albuminoid or vacuolar degenerations were extremely mild, and lipid degeneration or necrosis were recognized only in one or two cases. The round cells infiltration in Glisson's capsules and pseudo biliary duct formation were also very rare (Table 2, Plate 2: G, H).

TABLE 2. Subcutaneous injection of carbon tetrachloride combined with acetone insoluble extract isolated from bovine spleen

No. of animals		333	334	335	336	337	338	339	340	341	342		
Body-weight before injection (g)		11.7	13.5	9.3	8.1	12.9	12.4	9.3	11.1	10.7	11		
Terminal body-weight (g)		12.2	11.7	10.6	6.9	12.3	8.1	10.6	11.7	10.8	9.2		
Survival period (day)		15	15	15	15	15	15	15	15	15	15		
Weight of liver (mg)		800	600	700	700	700	400	600	600	600	500		
Microscopic appearance	Findings in the interstitial tissues	Connective tissue		±	-	±	-	-	-	±	-	-	
		Glisson's capsules	Hyperplasia of the connective tissue	-	-	-	-	-	-	-	±	-	
			Round cells infiltration	-	±	±	±	±	±	±	±	±	
			Formation of pseudo biliary ducts	±	-	+	-	-	-	-	±	±	-
			Arterial and venous hyperemia	-	-	-	-	-	-	-	-	-	-
	Kupffer's stellate endothelial cells	Enlargement	+	+	+	+	+	+	+	+	+	+	
		Hyperplasia	+	+	+	+	±	+	+	+	+	±	
	Findings in acini	Hepatic cells	Arrangement	SIR	I R	I R	SIR	SIR	SIR	SIR	I R	SIR	R
			Albuminoid degeneration	+	+	+	+	+	+	+	+	+	-
			Vacuolar degeneration	+	+	+	±	+	+	+	+	+	-
			Lipoid degeneration	-	-	+	-	-	-	-	±	-	-
			Necrosis	-	+	+	-	-	-	-	-	-	-
			Atrophia of nuclei	-	+	+	-	-	-	-	-	-	-
			Swelling of nuclei	+	±	+	+	+	+	+	+	+	+
	Round cells infiltration	Around the blood vessels	±	+	+	±	-	-	±	+	+	±	
		Around the hyperplastic connective tissue	-	-	-	-	-	-	-	+	-	-	
		In intraacinous area	-	+	+	-	-	+	-	-	-	-	
	Cells infiltration around the central veins		-	+	+	±	±	-	±	±	-	±	

R: Regular, IR: Irregular, SIR: Slightly irregular

Next to the above was alcohol soluble extract. In this case, the irregularity of the arrangement of hepatic cells was much milder in comparison with the case with carbon tetrachloride alone, and albuminoid, vacuolar and lipoid degenerations were also much milder, and in no cases, necrosis was met.

The inhibitory action against hepatic disturbances of the benzol insoluble extract was found to be not much different from that of the former. In the cases with each of the other extracts, they demonstrated slight inhibitory action, and none of them promoted the hepatic disturbances induced by carbon tetrachloride.

II. In the case of Crystal (A) $C_6H_{13}O_2N$ isolated from the alcohol soluble fraction of bovine spleen

One per cent and one tenth per cent solutions of Crystal (A) $C_6H_{13}O_2N$ were made, and they were injected in the same quantity and in the same manner.

At both of the above concentrations, Crystal (A) did not inhibit the hepatic disturbances induced by carbon tetrachloride.

III. In the case of alcohol soluble extract combined with Crystal (A)

An equal amount mixture of alcohol soluble extract of bovine spleen and 1% solution of Crystal (A), which demonstrated almost no inhibitory action against the hepatic disturbances, was injected to investigate the grade of the inhibition against the hepatic disturbances induced by carbon tetrachloride.

Although the quantity of alcohol soluble extract was only one half of the quantity in the case with the same extract alone, the hepatic disturbances were clearly inhibited when the former was added with the Crystal (A) solution. Especially, vacuolar degeneration of hepatic cells was found much milder compared with that in the case with alcohol soluble extract alone. In other words, the Crystal (A) is not capable of demonstrating inhibitory action against hepatic disturbances by itself, but it enhances the inhibitory action of alcohol soluble extract (Table 3, Plate 3: I, J).

IV. In the case with alcohol insoluble component of bovine spleen

1) The inhibitory action against the hepatic disturbances of Solution (A), (B) and (C)

In the same manner as was in the cases with various extracts isolated and prepared from the alcohol soluble fraction of bovine spleen, the influence of the solutions of the above three on the hepatic disturbances induced by carbon tetrachloride was investigated.

Solution (A) and (B) demonstrated inhibitory action against hepatic disturbances approximately in the same degree, next to benzol insoluble extract of bovine spleen. Both of the above solutions inhibited lipoid degeneration of he-

patic cells, and they inhibited also albuminoid and vacuolar degenerations except only in a small number of cases. Necrotic state of hepatic cells was only rarely recognized.

TABLE 3. Subcutaneous injection of carbon tetrachloride with alcohol soluble extract combined with Crystal (A) from alcohol soluble fraction of bovine spleen

No. of animals		587	588	589	590	591	592	593	594	595	596		
Body-weight before injection (g)		10.6	10.5	12.5	10.8	12.8	10.6	9.2	10	10.1	10.1		
Terminal body-weight (g)		13.3	14.0	11.3	14.6	14.8	14.9	10.7	11.4	10	14.5		
Survival period (day)		15	15	15	15	12	15	15	15	15	15		
Weight of liver (mg)		1150	1050	650	1150	1600	1300	900	800	750	1050		
Microscopic appearance	Findings in the interstitial tissues	Connective tissue		-	±	-	-	-	+	-	±	±	±
		Glisson's capsules	Hyperplasia of the connective tissue	-	-	-	-	-	-	-	-	-	-
			Round cells infiltration	+	+	±	-	+	+	+	-	-	-
			Formation of pseudo biliary ducts	+	+	-	-	+	+	+	-	-	-
			Arterial and venous hyperemia	-	-	-	-	-	-	-	-	-	-
		Kupffer's stellate endothelial cells	Enlargement	+	+	+	+	+	+	+	+	+	+
	Hyperplasia		+	+	+	+	+	+	+	+	+	+	
	Findings in acini	Hepatic cells	Arrangement	I R	I R	SIR	I R	I R	I R	SIR	I R	I R	I R
			Albuminoid degeneration	+	+	+	+	+	+	+	+	+	+
			Vacuolar degeneration	+	±	±	±	-	+	±	+	+	+
			Lipoid degeneration	+	±	-	±	±	±	-	-	±	+
			Necrosis	±	-	-	±	-	+	-	-	-	+
			Atrophia of nuclei	-	-	-	+	-	±	-	-	-	+
			Swelling of nuclei	+	+	+	+	+	+	+	+	+	+
	Round cells infiltration	Around the blood vessels	+	+	+	+	+	+	+	+	+	+	
		Around the hyperplastic connective tissue	-	-	-	-	-	+	-	+	+	+	
		In intraacinous area	+	+	-	+	+	+	+	+	-	-	
	Cells infiltration around the central veins		+	-	+	+	+	±	+	+	+	+	

R: Regular, IR: Irregular, SIR: Slightly irregular

Contrary to Solutions (A) and (B), the inhibitory action of Solution (C) against the hepatic disturbances was found to be fairly strong. Its inhibitory action

seemed to be superior to that of alcohol soluble extract, although not as much as that of acetone insoluble extract. The albuminoid or vacuolar degeneration of hepatic cells was restricted to a slight degree by Solution (C), and lipid degeneration was totally prevented (Table 4, Plate 3: K, L).

TABLE 4. Subcutaneous injection of carbon tetrachloride with Solution (C) from alcohol insoluble fraction of bovine spleen

No. of animals		1081	1082	1083	1084	1085	1087	1088	1089	1090
Body-weight before injection (g)		10.5	10	10.1	8.9	9.9	6.1	10.6	9.3	10.2
Terminal body-weight (g)		17.1	12.0	17.2	12.2	14.7	10.1	13.3	14.5	13.8
Survival period (day)		15	15	15	15	15	15	15	15	15
Weight of liver (g)		1400	1000	1500	1000	1400	800	1000	1000	1100
Microscopic appearance	Findings in the interstitial tissues	Connective tissue		±	-	±	-	-	-	-
		Glisson's capsules	Hyperplasia of the connective tissue	-	-	-	-	-	-	-
			Round cells infiltration	+	+	+	±	+	±	±
			Formation of pseudo biliary ducts	+	+	+	-	+	±	-
			Arterial and venous hyperemia	-	-	-	-	-	-	-
	Kupffer's stellate endothelial cells	Enlargement		+	+	+	+	+	+	+
		Hyperplasia		+	+	+	+	+	+	-
	Hepatic cells	Arrangement		I	R	I	R	I	R	I
		Albuminoid degeneration		+	+	+	+	±	+	+
		Vacuolar degeneration		+	+	+	+	±	+	+
		Lipoid degeneration		-	-	-	-	-	-	-
		Necrosis		-	-	-	-	-	±	-
		Atrophia of nuclei		-	-	-	-	-	-	-
		Swelling of nuclei		+	+	+	+	+	+	+
	Round cells infiltration	Around the blood vessels		+	+	+	±	+	±	-
		Around the hyperplastic connective tissue		+	-	-	-	-	-	-
		In intraacinous area		-	-	-	-	-	-	-
	Cells infiltration around the central veins		+	+	+	-	+	±	±	-

R: Regular, IR: Irregular, SIR: Slightly irregular

2) *The inhibitory action of alcohol soluble extract combined with Solution (A) against the hepatic disturbances*

As for an equal amount mixture of alcohol soluble extract and Solution (A), the inhibitory action against the hepatic disturbances was investigated.

In this case, the inhibitory action was found to be much stronger compared with the case with Solution (A) alone, but the action was considered to be slightly inferior to the case with twice amount of alcohol soluble extract alone. However, lipoid degeneration of the hepatic cells was clearly inhibited, showing the specificity of the Solution (A) in its action.

3) *The inhibitory action of the combination of alcohol soluble extract, Solution (A) and Crystal (A) against the hepatic disturbances*

An equal amount mixture of alcohol soluble extract and Solution (A), 0.1 cc in volume, was mixed with 0.1 cc of 1% solution of the Crystal (A). Each of the mice was injected with 0.2 cc of the above mixture per 10 g, and carbon tetrachloride was injected 15 minutes later.

In this case, the inhibitory action was recognizable, but it was not so strong as compared with the case with the alcohol soluble extract alone. However, the lipoid degeneration of hepatic cells was found fairly distinctly inhibited.

4) *In the cases with various crystals isolated from alcohol insoluble fraction of bovine spleen*

For this experiment, the following 5 concentrations of the crystals isolated was adopted as according to their respective solubility.

Crystal (I)	0.1%
Crystal (II)	0.5%
Crystal (III)	1.0%
Crystal (IV)	1.0%
Crystal (V)	1.0%

In this experiment, these crystals inhibited the hepatic disturbances induced by carbon tetrachloride distinctly. Above all, the action of Crystal (I) which is insoluble and tyrosine-like substance, demonstrated most intensive action. In the case of Crystal (I), the arrangement of hepatic cells was generally normal, and only very slight albuminoid and vacuolar degenerations were recognizable. Further, not a single case of lipoid degeneration was encountered. No histological pictures of necrotic state were observed. The degree of the enlargement of the nuclei of hepatic cells was also slight. Round cells infiltration in Glisson's capsules and formation of pseudo biliary ducts were also recognized, but no hyperplasia of connective tissue was observed. In short, the inhibitory action of the Crystal (I) was found to be stronger than that of the acetone insoluble extract (Table 5, Plate 2: E, F).

TABLE 5. Subcutaneous injection of carbon tetrachloride with Crystal (I) isolated from alcohol insoluble fraction of bovine spleen

No. of animals		1141	1142	1143	1144	1145	1147	1148	1149	1150
Body-weight before injection (g)		6.4	9.3	10.8	8.7	9.0	6.8	8.0	8.3	5.8
Terminal body-weight (g)		8.3	10.9	13.7	11.4	11.5	9.9	9.7	10.3	7.2
Survival period (day)		15	15	15	15	15	15	15	13	15
Weight of liver (mg)		500	650	850	650	650	500	500	700	450
Microscopic appearance	Findings in the interstitial tissues	Connective tissue		-	-	-	-	-	-	-
		Glisson's capsules	Hyperplasia of the connective tissue	-	-	-	-	-	-	-
			Round cells infiltration	+	+	+	±	+	±	-
			Formation of pseudo biliary ducts	+	+	+	+	+	-	-
			Arterial and venous hyperemia	-	-	-	-	-	-	-
	Kupffer's stellate endothelial cells	Enlargement		+	+	+	+	+	+	+
		Hyperplasia		+	+	+	+	+	±	+
	Findings in acini	Hepatic cells	Arrangement	SIR	SIR	SIR	SIR	I R	I R	SIR
			Albuminoid degeneration	±	±	±	±	+	+	±
			Vacuolar degeneration	±	±	±	±	+	+	±
			Lipoid degeneration	-	-	-	-	-	-	-
			Necrosis	-	-	-	-	-	-	-
			Atrophia of nuclei	-	-	-	-	-	-	-
			Swelling of nuclei	+	+	+	±	±	±	±
	Round cells infiltration	Around the blood vessels		+	+	+	±	+	±	+
		Around the hyperplastic connective tissue		-	-	-	-	-	-	-
		In intraacinous area		-	-	-	-	-	-	-
			Cells infiltration around the central veins	+	+	+	±	±	±	±

R: Regular, IR: Irregular, SIR: Slightly irregular

Following the Crystal (I), was the Crystal (III). The inhibitory action of Crystal (III) was not as strong as acetone insoluble extract, but seemed to be stronger than alcohol soluble extract or Solution (C). Albuminoid and vacuolar degenerations of hepatic cells were mild, and almost no lipoid degeneration was recognized.

The enlargement of the nuclei of hepatic cells was also slight, and no hyperplasia of connective tissue was noticed.

Though slight in strength, the Crystal (IV) demonstrated recognizable inhibitory action against hepatic disturbances, but the action of the Crystals (II) and (V) was found to be extremely weak.

Among the inhibitory action against the hepatic disturbances demonstrated by the above 5 crystals, the inhibitory action against lipid degeneration of hepatic cells, which is considered to be in common among the above 5 crystals, was observed also in the cases of Solutions (A), (B) and (C), which were isolated from alcohol insoluble fraction of bovine spleen. This is a matter of great interest.

SUMMARY

For the purpose of investigating the influences, particularly the inhibitory action of components of bovine spleen on the experimental hepatic disturbances induced by carbon tetrachloride, 9 extracts and 1 crystal from alcohol soluble fraction, 3 extracts and 5 crystals from alcohol insoluble fraction of bovine spleen were prepared, and the action demonstrated by each of the above substances alone or combined with one or more substances of the above was investigated in detail to obtain the above described results.

The Crystal (I), isolated from alcohol insoluble fraction, demonstrated the most intensive inhibitory action against the hepatic disturbances induced by carbon tetrachloride, followed by acetone insoluble extract from alcohol soluble fraction, Crystal (III), Solution (C), the combination of alcohol soluble extract and Crystal (A) from alcohol soluble fraction, alcohol soluble extract and benzol insoluble extract.

The Crystal (A) $C_6H_{13}O_2N$, which had been isolated by Tsunoo and Fujita from bovine spleen and reported as leucopenia inducing substance, and later reported by Yoshimatsu as a strong blood coagulation promoting substance, did not demonstrate any inhibitory action against the hepatic disturbances. In spite of the above facts, Crystal (A) was proved to have a fostering action to the inhibitory action of alcohol soluble extract in the experiment with the combination of these two substances. On the other hand, Irisawa has investigated the influence of the splenic components of cattle on the average survival period of the mice administered with cocain, and recognized that Crystal (A) prolongs the average survival period fairly intensively, and further that its action is stable. In his report, he also reported that various extracts and crystals isolated from the alcohol insoluble fraction almost do not prolong the average survival period. He also recognized the fact that the alcohol soluble extract prolongs the average survival period most intensively among various extracts. As antidotal

mechanism of the above various extracts against cocain, Irisawa has the activating action of these substances, especially of Crystal (A) and alcohol soluble extract, to the reticuloendothelial system in his mind.

Tanakaya has investigated the significance of the reticuloendothelial system to the cause of the experimental induction of hepatic cirrhosis, particularly its relationship to splenic hormone, and concluded as follows. "The reticuloendothelial system in general, particularly spleen, has a certain definite relationship to the cause of the experimental induction of hepatic cirrhosis, therefore, in case the function of the reticuloendothelial system is imperfect or without the spleen, the affects of the invading toxic substance to the liver is rapid and intensive." Further, he recognized that the above change was remarkably alleviated by transplantation of spleen tissue or by injection of splenic extract, and he claimed that the specific secretion - hormone - of spleen lessens the change in the liver caused by the toxic substance through the medium of reticuloendothelial system, particularly through the medium of Kupffer's stellate cells.

In short, it was made clear that the tyrosine-like Crystal (I) demonstrates an extremely strong inhibitory action against the hepatic disturbances. As to the mechanism of action, it might be attributable to the activation of the reticuloendothelial system, but the over-all picture of the inhibitory action will not be visualized unless other unknown factors are taken into consideration.

CONCLUSIONS

1. The hepatic disturbances induced by carbon tetrachloride is not influenced at all by Crystal (A), which was isolated from the alcohol soluble fraction of bovine spleen, and which is leucopenia inducing and blood coagulation promoting substance.

2. Crystal (I), which was isolated from the alcohol insoluble fraction of bovine spleen, and which is tyrosine-like substance, demonstrates the most intensive inhibitory action against the hepatic disturbances.

3. Among various extracts, acetone insoluble extract demonstrates the strongest inhibitory action. The action of the other substances is weaker in the order of Solution (C), alcohol soluble extract, benzol insoluble extract.

4. Crystal (III), which was isolated from the alcohol insoluble fraction and which is Millon ($\pm$) without tyrosine-like lustre, demonstrates inhibitory action next to acetone insoluble extract in strength.

Acknowledgement. Sincere appreciations are due to the Ministry of Education through which the fund to support the performance of the experiments contained in this paper was granted.

REFERENCES

- 1) TSUNOO, SH. AND KOIZUKA, H.: *Folia pharmacol. japon.* 40, Proceedings 86§ (1944)
- 2) TSUNOO, SH. AND KOIZUKA, H.: *J. Showa Med. Assoc.* 9, No. 2, 10 (1949)
- 3) FUJITA, W.: *Folia pharmacol. japon.* 44, Proceedings 56§ (1948)
- 4) FUJITA, W.: *Medical Reports of Showa Medical School* 1, 50 (1949)
- 5) ABE, S.: *Folia pharmacol. japon.* 44, Proceedings 54§ (1948)
- 6) ABE, S.: *Medical Reports of Showa Medical School* 1, 8 (1949)
- 7) YOSHIMATSU, S.: *Folia pharmacol. japon.* 44, Proceedings 57§ (1948)
- 8) YOSHIMATSU, S.: *J. Showa Med. Assoc.* 10, No. 2, 31 (1950)
- 9) SEKINO, H.: *Folia pharmacol. japon.* 44, Proceedings 14§ (1949)
- 10) SEKINO, H.: *J. Showa Med. Assoc.* 10, No. 1, 42 (1950)
- 11) SEKINO, H.: *Medical Reports of Showa Medical School* 2, 87 (1950)
- 12) SAKAI, T.: *Folia pharmacol. japon.* 44, Proceedings 14§ (1949)
- 13) SAKAI, T.: *J. Showa Med. Assoc.* 11, No. 3, 21 (1951)
- 14) IRISAWA, K.: *Ibid.* 10, No. 3, 26 (1950)
- 15) TANAKAYA, K.: *Okayama Igakkai Zasshi* 44, 2567 (1932)
- 16) KATADA, T.: *Folia pharmacol. japon.* 46, Proceedings 109§ (1950)

EXPLANATION OF PLATES

- PLATE 1: A. Section of normal liver. Hematoxylin and eosin stain. $\times 100$
 B. Section of normal liver. Hematoxylin and eosin stain. $\times 400$
 C. Section of liver intoxicated by carbon tetrachloride alone. Hematoxylin and eosin stain. $\times 100$
 D. Section of liver intoxicated by carbon tetrachloride alone. Hematoxylin and eosin stain. $\times 400$
- PLATE 2: E. Section of liver intoxicated by carbon tetrachloride with Crystal (I) isolated from alcohol insoluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 100$
 F. Section of liver intoxicated by carbon tetrachloride with Crystal (I) isolated from alcohol insoluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 400$
 G. Section of liver intoxicated by carbon tetrachloride with acetone insoluble extract from alcohol soluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 100$
 H. Section of liver intoxicated by carbon tetrachloride with acetone insoluble extract from alcohol soluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 400$
- PLATE 3: I. Section of liver intoxicated by carbon tetrachloride with alcohol soluble extract combined with Crystal (A) from alcohol soluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 100$
 J. Section of liver intoxicated by carbon tetrachloride with alcohol soluble

extract combined with Crystal (A) from alcohol soluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 400$

- K. Section of liver intoxicated by carbon tetrachloride with Solution (C) from alcohol insoluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 100$
- L. Section of liver intoxicated by carbon tetrachloride with Solution (C) from alcohol insoluble fraction of bovine spleen. Hematoxylin and eosin stain. $\times 400$

E

F

G

H

I

J

K

L

A

B

C

D