How was the reaction in the liver?

Dr. Deguchi: In the liver, the protoplasm of the hepatic cells showed the diffuse marked reaction.

Dr. Ogawa: (1) The over 0.01^M final concentration of KCN caused the tetrazolium reduction. (2) The reducing efficiency of tetrazoliums by SH groups is dependent on the pH of the solution (Pearse and Zimmerman).

核蛋白質に関する組織化学的研究

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Histochemical Studies on Nucleoproteins

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Ceroid pigment in paraffin sections loses its audanophilia due to methylation following a treatment with peracetic acid. Then the cell nucleus is found PAS reaction positive. In this case, it is observed that the nucleus is almost thionin unstainable. This observation has led to make further experiment in which the section is pre-treated with hydrochloric acid for four hours.

As a result, the removal of basophilia from the nucleus by the pre-treatment makes the nuclear Schiff reaction positive. On further study this positive reaction is assumed to be due to nucleoproteins other than desoxyribonucleic acid (DNA).

Material and Method

Alcohol or formalin fixed normal rat liver paraffin sections are mostly employed. Liver samples obtained from three starved rats also are used.

They are exposed to N HCl at 37°C. for four hours and treated with peracetic acid to be methylated^{*)}; exposed to water at 60°C. for 24 hours^{*)}; methylated^{*)}; exposed to N HCl at 37°C. for 8-20 hours; treated with 4% trichloroacetic acid (TCA) at 90°C. for 15 minutes^{*)} and deaminated^{*)}. As staining, Feulgen reaction^{*)}, periodic acid-Schiff (PAS) reaction (Lillie^{*)}, Schiff reaction (half an hour exposed), Yasuma-Ichikawa's method^{*)}, thionin staining (Highman^{*)}) and fastgreen staining are performed.

In preparing peracetic acid, 20cc of acetic anhydride is well mixed with 5 cc. of 30% H₂O₂ and allowed to stand for two hours. Before use, the mixture is diluted to 50cc. with distilled water. In this mixture section are immersed at room temperature for ten minutes. *For methylation, 99.2cc. of methanol mixed with 0.8cc. of concentrated HCl is employed for 24 hours at 60°C. § Van

Slyke's reagent ; a solution made of 6 g. of sodium nitrate dissolved in 10cc of distilled water is mixed to with 5 g. of glacial acetic acid and 25 cc. of distilled water. The section prepared is immersed in Van Slyke's reagent for 1-12 hours at 20-25°C.

Table 1.

Animal No.	Sex	Weight (g.)		Weight loss	Experimental period	Period of life	Strains
		Initial	Terminal				
Rat 60	♀	230	160	30%	7 days	about one year	Mixed Gifu
Rat 69	♂	300	190	37%	14 days	about half a year	Wistar Strain
Rat 70	♀	230	100	52%	19 days	about half a year	Wistar Strain

Results

Following deamination, Feulgen reaction and thionin staining are found unaffected, while fast green staining is found negative. Following a pre-treatment with N HCl at 37°C. for four hours, the results of staining are obtained as shown in Table 3.

It is revealed that the treatment with N HCl for four hours almost results in absence of a positive thionin staining in the nucleus (Fig. 2) and that the nucleus becomes PAS and Schiff positive (Fig. 3), Feulgen reaction and fast green staining being still maintained markedly positive (Fig. 4). Following deamination these staining reactions become negative except for thionin staining and fast green staining. The results obtained of an 8-20 hour treatment with N HCl and a treatment with TCA are shown in Table 4.

It is revealed that the fast green staining enhances its positive reaction following the 8-20 hour treatment with N HCl and TCA (Fig. 5) and that Schiff reaction and thionin staining prove negative. The results obtained

Table 2.

Pre-treatment	Staining method	
none	Feulgen reaction	+
	PAS reaction	—
	Schiff reaction	—
	Yasuma-Ichikawa's method	—
	thionin staining	+
	fast green staining	+
deamination	Feulgen reaction	+
	PAS reaction	—
	Schiff reaction	—
	Yasuma-Ichikawa's method	—
	thionin staining	+
	fast green staining	—

Table 3.

Pre-treatment		Staining method	
N HCl at 37°C for 4 hours	none	Feulgen reaction	+
		PAS reaction	+
		Schiff reaction	+
		Yasuma-Ichikawa's method	+
		thionin staining	÷
		fast green staining	÷
	deamination	Feulgen reaction	—
		PAS reaction	—
		Schiff reaction	—
		Yasuma-Ichikawa's method	—
		thionin staining	÷
		fast green staining	—

Table 4.

Pre-treatment	Staining method	
N HCl at 37°C for 8-20 hours	Feulgen reaction	—
	PAS reaction	—
	Schiff reaction	—
	Yasuma-Ichikawa's method	—
	thionin staining	—
	fast green staining	+
TAC at 90°C for 15 minutes	Feulgen reaction	—
	PAS reaction	—
	Schiff reaction	—
	Yasuma-Ichikawa's method	—
	thionin staining	—
	fast green staining	+

Table 5.

Staining methods	Pre-treatment		
	Peracetic acid and methylation	Peracetic acid and water at 37°C for 24 hours	Methylation
Feulgen reaction	+	+	+
PAS reaction	+	—	—
Schiff reaction	—	—	—
Yasuma-Ichikawa's method	—	—	—
thionin staining	—	÷	—
fast green staining	+	+	+

following methylation subsequent to a treatment with peracetic acid are shown in Table 5.

Methylation following a treatment with peracetic acid results in positive PAS reaction of the nucleus (Fig. 6) which is found unaffected following a treatment with TCA.

It is demonstrated that the use of methylation instead of the warm water treatment or omission of the peracetic acid treatment results in negative PAS reaction of the nucleus.

Discussion

A three hour treatment with N HCl is meant, according to Dempsey and others²⁾, to remove basophilia from the nucleus. The thionin staining which is performed following this treatment shows a marked decrease in stainability. In this case, DNA is considered to be almost absent. Schiff reaction of

Fig. 1 Thionin Stain.

Fig. 2 Treated with N HCl at 37°C. for 4 hours followed by Feulgen reaction.

Fig. 3 Treated with N HCl at 37°C. for 4 hours followed by Schiff reaction.

Fig. 4 Treated with N HCl at 37°C. 4 hours followed by Feulgen reaction.

Fig. 5 Treated with N HCl at 37°C for 20 hours followed by fast green stain.

Fig. 6 Treated with peracetic acid and methylation followed by PAS reaction.

the nucleus is found markedly positive following a four treatment with N HCl (Table 2). Thus it is assumed that a positive substance in this case is nucleoproteins other than DNA.

However, it is said that nucleohistone is extracted by a short time treatment with diluted HCl (according to Mori and others⁹⁾ 0.02 N HCl at 37°C. for two hours).

Therefore, a positive substance in this case is considered to be nonhistone nucleoprotein. Feulgen reaction remains positive following this pretreatment, however this reaction seems to lose its specificity to DNA and seems only to show presence of the nucleoprotein as is case with Schiff reaction following this pre-treatment.

It is understood that deamination blocks amino acids of proteins in collagen fibers and others to eliminate their stainability and blocks the acidophilic substance (e.g., histone which belongs to basic proteins) which resists extraction of nucleic acids¹⁰⁾. But it is demonstrated that deamination following a four hour treatment with N HCl results in the blocking of various reactions including Schiff reaction except for thionin staining (Table 3). This finding seems to support an interpretation that the stained substance is the nucleoprotein other than DNA.

If a treatment with HCl is prolonged to 8-20 hours or TCA is employed at 90°C. for 15 minutes, thionin staining and other reactions including Schiff reaction of the nucleus become negative except for fast green staining (Table 4).

It has been already stated^{5,7)} that fast green method markedly stains the nucleus following the removal of DNA. So, in this case, the feature of a nonhistone basic nucleoprotein which is an acidophilic component of the nucleus is presented. Regarding the results of Table 4, an 8-20 hour treatment with N HCl results in extraction of DNA and nucleohistone, but it seems that the nucleoprotein itself which should be the substance to be stained is not extracted. It is considered that an aldehyde radical which is produced during the four hour treatment with N HCl becomes Schiff reaction negative through hydration or oxidation during a treatment prolonged to 8-20 hours.

It is assumed^{7,8)} that a treatment with TCA results in extraction of DNA but well preserves nucleoproteins. Regarding the results of Table 5, it appears to be due to non-production of aldehyde radical through the treatment that no positive reaction of the nucleoproteins in question occurs to Schiff reaction or other techniques following a treatment with TCA.

Concerning Table 6, only the experimental results are presented, i.e., PAS reaction of the nucleus being positive following a treatment with peracetic acid and methylation. Schiff reaction and others following a treatment with HCl show no changes for the hepatic cell nuclei during the fasting experiment. This finding can be interpreted on the some basis as a report¹⁾ that DNA basis as a report¹⁾ that DNA shows no changes in the fasting experiment.

Conclusion

Following a four hour treatment with N HCl, Schiff reaction and other

reactions of the nucleus become positive with a marked fast green staining of the nucleus but a very little thionin staining of DNA. Deamination following a four hour treatment with N HCl results in negative nuclear Schiff reaction and other reactions. The above-mentioned results lead to an assumption that such positive nuclear reactions are due to the nucleoprotein other than DNA. Since nucleohistone is very likely to be extracted following a treatment with HCl, the nucleoprotein in question seems to be a non-histone basic nucleoprotein. In starved rats, no influences of starvation are observed on Schiff reaction and other reactions of the hepatic cell nuclei following a treatment with HCl.

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血球ハイドロペルオキシダーゼの組織化学的研究

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Histochemical Demonstration of Hydroperoxidase in Blood Cells

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Theorell (1), from the point of view in his own and Chence's research (2), introduced hydroperoxidase as a general name for catalase and peroxidase, because crystallized catalase was not a catalytic enzyme in release of oxygen from hydrogen peroxide, but an oxidative enzyme (3). The histochemical demonstration of this enzyme in blood and bone marrow cells have been attempted from this point in this paper.

Material and Methods

Human beings and various animals were examined. Formalin gas fixed smears of the normal blood and bone marrow were used. The modified te-