mixed with P.P.A. (6 g.) and the mixture was heated at 180° for 5 min. The product was worked up as for (XIV), and was purified from EtOH, giving colorless plates of m.p. 142~143°. Yield, 0.56 g. *Anal.* Calcd. for $C_{17}H_{12}N_2$: C, 83.58; H, 4.95; N, 11.47. Found: C, 83.12; H, 5.29; N, 11.65.

Picrate: Orange minute pillars, m.p. 246~247° (decomp.). *Anal.* Calcd. for $C_{17}H_{12}N_2 \cdot C_6H_3O_7N_3$: C, 58.35; H, 3.19; N, 14.80. Found: C, 57.88; H, 3.43; N, 14.66.

2-(4-Quinolyl)indole Methiodide—The above-mentioned 2-(4-quinolyl)indole (XXXII) (0.48 g.) was dissolved in an excess of MeI and the whole was allowed to stand overnight at room temperature, separating a bulky orange-red precipitate. The excess of MeI was evaporated, the residue was washed with benzene, and recrystallized from MeOH, affording wooly orange needles of m.p. 242~243° (decomp.). Yield, 0.45 g. *Anal.* Calcd. for $C_{18}H_{15}N_2I$: C, 55.97; H, 3.91; N, 7.26. Found: C, 56.24; H, 4.20; N, 7.46. U.V. (a) In acid and neutral soln.: U.V. $\lambda_{max}^{95\%EtOH}$ mμ (log ε): 327(3.70), 435 (4.37); $\lambda_{shoulder}$ 260(4.06), 314(3.59); λ_{min} 303(3.43), 342(3.48). (b) In alkaline soln. (0.01*N* KOH): $\lambda_{max}^{95\%EtOH}$ mμ (log ε): 292(3.97), 470(3.74); λ_{min} 287(3.95), 376(3.24).

Summary

The synthesis of β-carboline derivative reported in the preceding paper[9] was now successfully extended to include the syntheses of two new salts of pentacyclic β-carbolinium derivatives (III and IV), which formed the anhydronium bases (XXVIII and XXIX) by the agency of alkali. The reduction of (III) to the corresponding tetrahydro base (XXXVII) was only possible with sodium borohydride, directly followed by catalytic reduction over Raney nickel catalyst, while the tetrahydro base (XXXVIII) of (IV) could be obtained with an excess of sodium borohydride in boiling hydrous ethanol in one step. The stability of (XXXVII) and (XXXVIII) in the air was compared with that of tetrahydrodibenzoquinolizines.

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79. Koiti Kimura,[*1] Kazuko Yamauchi,[*2] and Shigeaki Kuwano[*2]: Studies on Tannins. VIII.[1] Effect of Tannins and Related Compounds on Cysteine Desulfhydrase Activity of *Escherichia coli*.

(*Pharmaceutical Faculty, Osaka University*[*3])

In the preceding report[2] of this series an investigation was made on the inhibitory action of tannins and related compounds towards the production of diamines by *Escherichia coli*. The present paper deals with the effect of these compounds on the enzymatic formation of hydrogen sulfide from cysteine. This investigation was primarily undertaken with the object of comparing the effects of the same series of compounds on the two different enzymes requiring the same coenzyme. It is well known that pyridoxal phosphate is the common cofactor for the enzyme systems responsible for the diamine formation (amino acid decarboxylases) and for the hydrogen sulfide liberation (cysteine desulfhydrase).

The formation of hydrogen sulfide by the action of microörganisms occurs in the large intestine and this causes some injurious effects on the host organism. Therefore, it was also hoped that such an investigation might shed some light on the mechanism of pharmacological and even medicinal action of tannins.

*1 Present address: Pharmaceutical Institute, Medical Faculty, University of Kyoto, Kyoto.
*2 Present address: Kotaro Institute for Physiological Chemistry of Crude Drugs, Oyodo-ku, Osaka.
*3 Hotarugaike, Toyonaka, Osaka-fu (木村康一, 山内和子, 桑野重昭).

1) Part VII: Yakugaku Zasshi, **78**, 327(1958).
2) K. Kimura, S. Kuwano, H. Hikino: *Ibid.*, **78**, 236(1958).

Microbial production of hydrogen sulfide from cysteine has so far been studied by various investigators, among which are Fromageot,[3] Smythe,[4] Delwiche,[5] Ichihara, *et al.*,[6] and Tamiya.[7] Its enzymatic mechanism has, however, not yet been fully elucidated. Tamiya[7] suggested that the liberation of hydrogen sulfide is preceded by the detachment of the amino group of cysteine. Metaxas and Delwiche[8] found that pyridoxal phosphate was lost from cysteine desulfhydrase if the bacterial cells were ground with alumina. Artman, *et al.*[9] reported that cysteine desulfhydrase was almost completely inhibited by dihydrostreptomycin and pointed out that this inhibition was of a irreversible and noncompetitive nature.

In the present paper, it is shown that most of the tannins and related compounds are effective inhibitors towards the cysteine desulfhydrase of *E. coli*. The mechanism of inhibition is also discussed based on the results obtained in the present investigation.

Materials and Methods

Cell Suspension—*E. coli* UKT-B strain, obtained from the Research Institute for Microbial Diseases, University of Osaka, was grown at 37° for 18 hr. in a medium containing 10 g. each of meat extract, yeast extract, and peptone, 5 g. of K_2HPO_4, 1 g. of NaCl, 500 mg. of $MgSO_4 \cdot 7H_2O$, and 100 mg. of L-cystine in 1000 cc. of tap water. The initial pH of the medium was adjusted to approximately 7.4. After cultivation the cells were collected, washed twice with physiological saline, and suspended in the saline. The suspension containing approximately 3.2 mg. of cells (dry weight) was used in almost all the experiments.

Reaction Conditions—Reaction vessels employed were similar to the one described by Suda, *et al.*[10] One cc. of the cell suspension, 1 cc. of the inhibitor solution,*4 and 2.5 cc. of 0.1*M* phosphate buffer (pH 7.0) were placed in the main chamber of the vessel, and 0.5 cc. of 0.025*M* cysteine hydrochloride in the side chamber. After preincubating the vessel at 37° for 10 min., the reaction was started by adding the cysteine solution into the main chamber. The reaction was allowed to proceed under aeration at 37° for 60 min. The reaction mixture was continuously aerated according to the procedure described by Tamiya.[7]

Determination of Hydrogen Sulfide—H_2S was determined by Tamiya's modification[11] of the method of St. Lorant.[12]

Compounds Tested—The following compounds were tested for their inhibitory action on the enzymatic liberation of H_2S by *E. coli*: Gallotannin, gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, salicylic acid, benzoic acid, methyl gallate, ethyl protocatechuate, pyrogallol, phloroglucinol, pyrocatechol, resorcinol, hydroquinone, phenol, and *d*-catechin were either commercial products or prepared as reported in a previous paper.[2] *m*-Digallic acid was kindly supplied by Professor O. Th. Smidt of University of Heidelberg. Trimethylgallic acid,[13] gallic acid ethanolamide,[14] ellagic acid,[15] ethyl gallate,[16] propyl gallate,*5 and caffeic acid[17] were newly synthesized in this laboratory for use in the present investigation.

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*4 Dissolved in 0.1*M* phosphate buffer (pH 7.0) and, if necessary, neutralized with 0.1*M* Na_2HPO_4.
*5 Synthesized by the procedure similar to that for ethyl gallate.

3) C. Fromageot, P. Desnuelle : Enzymologia, **6**, 80(1939); C. Fromageot : "The Enzyme" **I**, 1237 (1951).
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16) A. Bogojawlensky, J. Narbutt : Ber., **38**, 3344(1905).
17) F. Hayduck : Ber., **36**, 2935(1903).

Results

Survey of Inhibitory Effect—The effect of tannins and related compounds on the cysteine desulfhydrase activity of *E. coli.* was examined at inhibitor concentrations ranging from 0.005 to 0.02*M*. Fig. 1 illustrates the results obtained when the concentration of inhibitors was fixed at 0.01*M*.

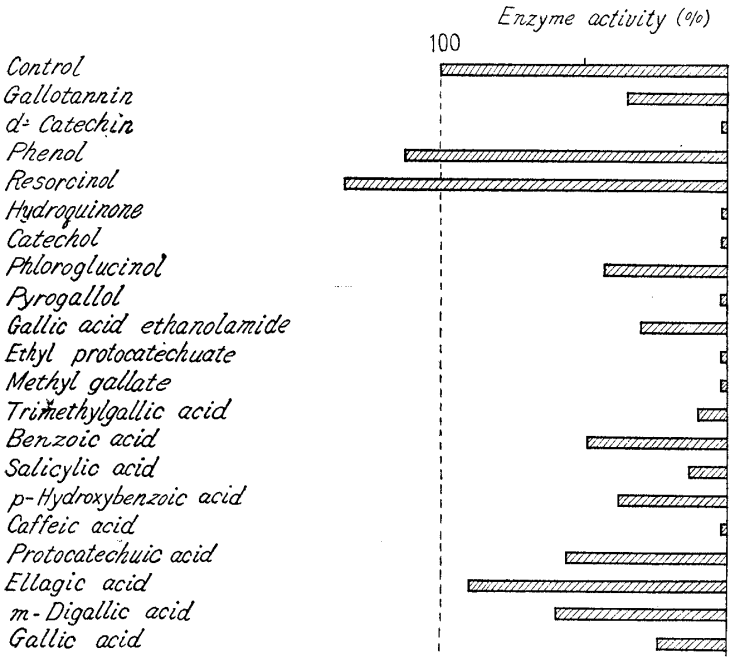

Fig. 1.
Effect of Various Inhibitors at 0.01*M* concn.

As can be seen, all the compounds tested, except phenol and resorcinol, were found to be inhibitory towards the hydrogen sulfide production at all the concentrations employed. Phenol was slightly accelerative to the reaction at concentrations lower than 0.01*M*, but became inhibitory when the concentration was increased to 0.02*M*. Similarly, resorcinol exhibited considerable stimulation at concentrations lower than 0.01*M*. Ellagic acid inhibited the enzyme activity only to a slight extent probably due to its limited solubility.

A survey of the results shown in Fig. 1 reveals that the inhibitory action was considerably reduced by the presence of free carboxylic group in the inhibitor molecule. Thus, for example, methyl gallate and ethyl protocatechuate were more powerful inhibitors than gallic acid and protocatechuic acid, respectively. Such inhibition-reducing effect of free carboxylic group is more clearly understandable from Fig. 2 in which the inhibition by gallic acid, methyl gallate, pyrogallol, and *d*-catechin is compared at varying concentrations.

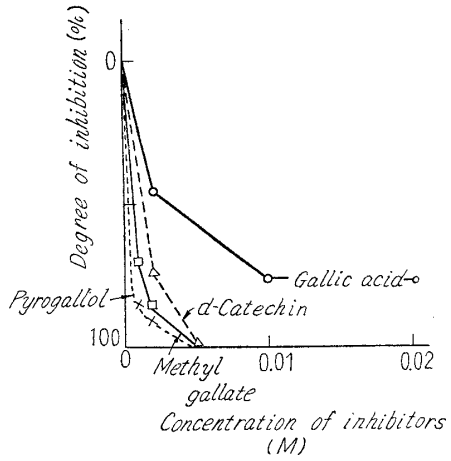

Fig. 2. Inhibitory Effect of Gallic Acid, Methyl Gallate, Pyrogallol, and *d*-Catechin

Dry weight of cells, 3.2 g.

When the effects of three alkyl esters of gallic acid, e.g. methyl, ethyl, and propyl gallates, were compared, the inhibitory action was found to increase with the increasing number of carbon in the alkyl group (Fig. 3).

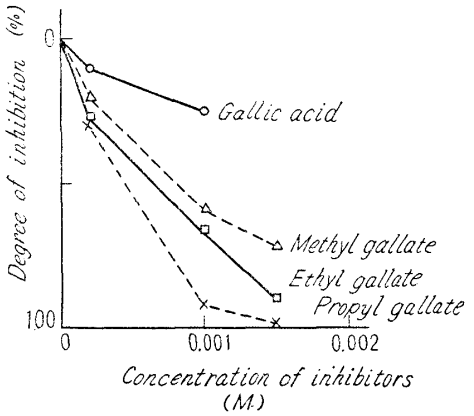

Fig. 3. Inhibitory Effect by Alkyl Esters of Gallic Acid

Dry weight of cells, 3.2 mg.

The fact that the presence of free carboxyl group in the molecule decreased the inhibitory action of the compound towards cysteine desulfhydrase activity is quite in contrast to the results obtained with the amino acid decarboxylation system of the same organism, which was more strongly inhibited by compounds with free carboxyl group.[2]

Although free carboxylic acids generally exerted weaker actions, trimethylgallic and caffeic acids were found to be stronger inhibitors than gallic and protocatechuic acids, respectively. Such anomalous behavior may be explained by the presence of three methoxyl groups in trimethylgallic acid and of a double bond in the side chain of caffeic acid.

Of the polyphenols, *o*- and *p*-polyhydroxy compounds such as pyrogallol, catechol, and hydroquinone acted as more powerful inhibitors when compared with *m*-polyhydroxy compounds such as phloroglucinol and resorcinol. These findings are completely in accord with those obtained with the amino acid decarboxylating system.[2]

Gallotannin was less inhibitory than gallic acid. This may be due to the smaller permeability of the former compounds into the cells.

Effect of Buffers on Inhibition by Gallic Acid—In order to determine if buffers have any effect on the inhibition, inhibition by gallic acid was compared in phosphate and citrate buffers (pH 7.0 and 0.07*M* final concentration). As recorded in Table I, no essential difference was detected in the

Table I. Effect of Buffers on Inhibition by Gallic Acid
(Dry weight of cells, 3.6 mg.)

	Phosphate buffer (0.07*M*)		Citrate buffer (0.07*M*)	
Gallic acid added (mmole)	0	0.01	0	0.01
Hydrogen sulfide formed (μmole)	0.841	0.396	0.417	0.199
Inhibition (%)	—	52.9	—	52.3

degree of inhibition in these two buffers. The concentration of phosphate buffer similarly showed no effect on inhibition in the range of 0.035~0.07*M*.

Effect of Cell Concentrations on Inhibition by Gallic Acid and *d*-Catechin—Inhibition by a fixed concentiation of gallic acid was found to be dependent on the concentration of bacterial cells employed. As can be seen from Fig. 4, less inhibition was observed with increasing cell concentrations. A

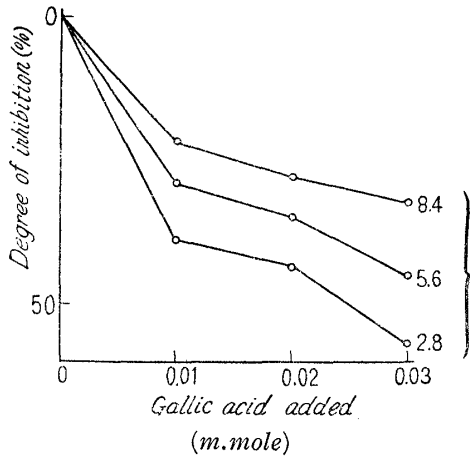

Fig. 4. Changes of the Degree of Inhibition of Gallic Acid according to varying Cell Concentration

Dry weight of cells (mg.)

Table II. Inhibition by Gallic Acid and *d*-Catechin with Constant Ratio of Cell Concentration to Inhibitor Concentration

			H_2S formed (μmole)				Inhibition (%)
Expt. 1	Gallic acid added (m.moles)		—	0.01	—	0.02	—	0.03	
	Dry weight of cells (mg.)	3.2	1.211	0.753					37.8
		6.4			2.800	1.606			42.7
		9.6					3.675	2.283	38.0
Expt. 2	*d*-Catechin added (m.moles)		—	0.005	—	0.01	—	0.015	
	Dry weight of cells (mg.)	2.4	0.871	0.600					31.1
		4.8			2.320	1.262			45.6
		7.2					3.366	1.013	70.0

similar relationship was also found in *d*-catechin as inhibitor. As may be deduced from Table II, the degree of inhibition by gallic acid seems to remain constant if the ratio of cell concentration to inhibitor concentration is kept at a constant value regardless of the absolute cell concentrations in the experimental vessels. This relationship, however, does not hold for inhibition by *d*-catechin as shown in Table II. It is, therefore, suggested that the mechanism of inhibition by *d*-catechin might be more complicated than that by gallic acid.

Effect of Substrate, Coenzyme, and Other Compounds on Inhibitory Actions—The inhibition caused by gallic acid seems to be of a non-competitive nature with respect to the substrate concentration as judged from the analysis of data by the method of Wilson[18] (Table III). It was also shown,

Table III. Mode of Action of Gallic Acid
(Dry weight of cells, 2.8 mg.)

	Final concn. (*M*)	H_2S formed (μmole)				
Gallic acid		0	0.001	0.002	0.005	0.01
Substrate[a]	0.0020	1.055	0.830	0.810	0.645	0.562
	0.0025	1.090	0.850	0.642	0.489	0.398
	0.0050	0.841	0.650	0.520	0.507	0.335
	0.0100	0.730	0.456	0.436	0.386	0.325
				Value of V/Vi[b]		
		1.00	1.26	1.30	1.63	1.88
		1.00	1.28	1.69	2.23	2.74
		1.00	1.29	1.62	1.66	2.50
		1.00	1.60	1.67	1.88	2.25

a) Although pH was maintained, the enzyme activity was reduced at concentrations of substrate higher than 0.005*M*.

b) V is the reaction velocity and Vi the velocity in the presence of the inhibitor. In this case, if V/Vi is plotted against different values of inhibitor concentration, a straight line may be obtained with a slope independent of substrate concentration. Therefore, the inhibition is non-competitive. Similar results were obtained by methyl gallate, pyrogallol, and *d*-catechin.

Table IV. Effect of Pyridoxine plus ATP

Pyridoxine hydrochloride and ATP-Na_4 added (μg.)*	Final concn. of inhibitor (*M*)	H_2S formed (μmole)			Dry wt. of cells(mg.)
		—	250	2500	
Gallic acid	—	1.269	1.299	1.359	3.6
	0.0005	0.964	0.984	0.949	
Methyl gallate	—	1.229	1.179	1.299	3.6
	0.0005	1.059	1.089	1.089	
Pyrogallol	—	0.939	1.022	1.035	3.2
	0.0005	0.672	0.642	0.691	
d-Catechin	—	1.026	1.161	1.286	2.8
	0.001	0.741	0.866	0.856	

* Equal amounts of pyridoxine hydrochloride and ATP-Na_4 were added.

as recorded in Table IV, that the inhibition could not be reversed by the addition of excess amounts of pyridoxine and adenosine triphosphate (ATP). These two compounds were added in expectation that they would be converted by the cells to pyridoxal phosphate, the coenzyme of cysteine desulfhydrase.

18) P.W. Wilson: "Respiratory Enzymes," Chapt. II (1949), Burgess Publishing Co.

Attempts to reverse the inhibition by SH compounds were abandoned since it was found that thioglycollic acid was rather inhibitory and that glutathione accelerated the enzyme reaction considerably in the absence of inhibitors. Glucose and glucose 6-phosphate also appeared to promote the enzymatic activity and reverse the inhibition to some extent. Kinetic behavior of these reversal phenomena was difficult to explain. None of the metal ions tested showed any reversal effect.

Effect of Preincubation and Aeration—The degree of ihibition by gallic acid, methyl gallate, pyrogallol, and *d*-catechin was found to be considerably affected by the time of preincubation of the cells in the presence of these inhibitors. As shown in Fig. 5, inhibition by gallic acid gradually

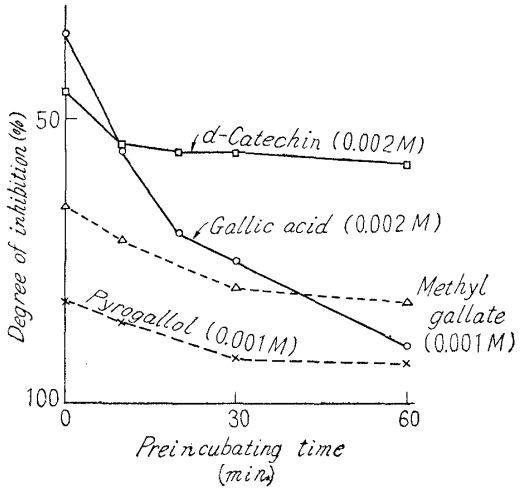

Fig. 5. Effect of Preincubating Time of Contact between Polyphenolic Compounds and Cells on Inhibitory Action

Dry weight of cells, 3.2 mg.

increased with increasing preincubation time, at least up to 60 min. The inhibition by *d*-catechin, on the other hand, increased to a certain extent by preincubating the cells for 10 min., but remained at this level even if preincubation was prolonged to 120 min.

In order to obtain a clue to the cause of such a preincubation effect, the inhibitor was removed from the cells by centrifugation as completely as possible after preincubation for 10~60 min. and the residual enzyme activity of the cells was determined. As can be seen from Fig. 6, it was

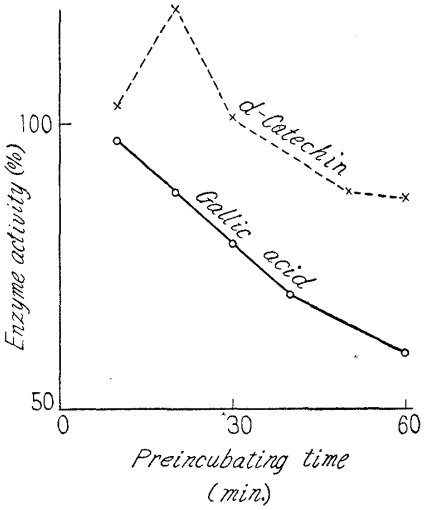

Fig. 6. Residual Enzyme Activtty after Inhibitors were removed

Dry weight of cells, 3.2 mg.
Concn. of inhibitors, 0.002*M*

found that enzyme activity of the cells had decreased to 60% of the control after 60-min. preincubation with gallic acid. With *d*-catechin as inhibitor, almost 90% of the activity was still detected after 60 min. and, furthermore, even a slight increase of the activity over that of the control was observed after contact of the cells with the inhibitor for a short period. It appears possible that gallic acid combines more firmly than *d*-catechin with the enzyme protein or cell walls.

Preincubation of gallic acid with the cells at 37° under aeration resulted in progressive inhibition and it was even more pronounced than the non-aerated. A similar, but less pronounced, inhibitory effect was also observed even when the phosphate buffer containing gallic acid alone was aerated prior to addition of the cells and the substrate (Fig. 7). It seemed unlikely, however, that the powerful inhibition caused by oxidized gallic acid was due to its conversion to ellagic acid since this acid and

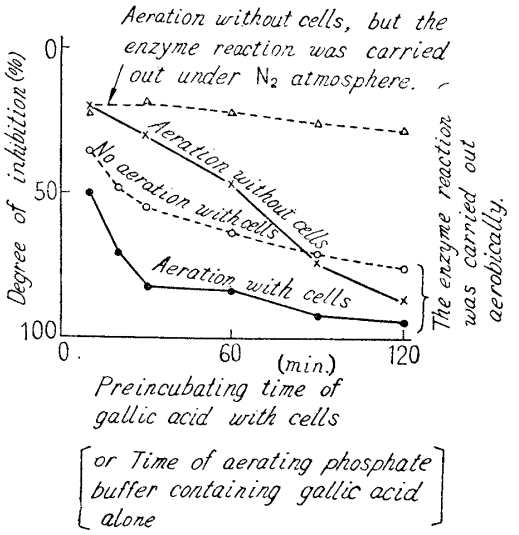

Fig. 7. Effect of Aeration of Gallic Acid Solution before Reaction starts

Dry weight of cells, 3.0 mg.
Final concn. of gallic acid, 0.001*M*

m-digallic acid, which could give rise to ellagic acid by oxidation, showed far less inhibitory action than gallic acid itself.

With *d*-catechin as the inhibitor no difference was detected in the degree of inhibition between the cells preincubated under aeration and under non-aerated conditions. Furthermore, when the phosphate buffer containing only *d*-catechin was aerated in the absence of the cells, no increased inhibition was observed as compared with non-aerated conditions. It was, therefore, inferred that in the case of *d*-catechin the preoxidation of the inhibitor has nothing to do with the emergence of inhibitory action.

When gallic or protocatechuic acid was preincubated with cells both under aerobic conditions and in nitrogen atmosphere, and then subjected to the enzymatic reaction under continuous bubbling of nitrogen,*6 only a slight difference was observed in the degree of inhibition between the two sets of preincubation conditions. When, however, the reaction was carried out under aeration, preincubation conditions strongly influenced the results. Thus, preincubation of gallic acid with cells

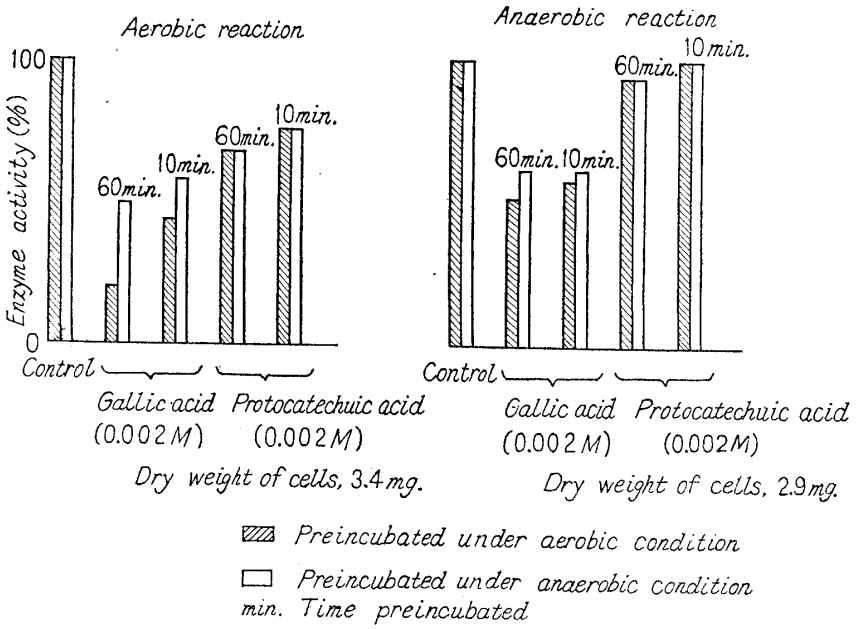

Fig. 8. Fffect of Preincubating Condition and Reaction Condition on Inhibition by Gallic or Protocatechuic Acid

*6 Through alkaline pyrogallol solution. Under these conditions no difference was observed in the enzyme activity as compared with aerobic conditions, although Tamiya[7] suggested that no enzyme activity was observed under anaerobic conditions.

under aeration caused much more remarkable inhibition in comparison with that in a nitrogen atmosphere. Such a behavior was not observed with protocatechuic acid. It will be clear from Fig. 8 that the presence of oxygen during the reaction is necessary for gallic acid to cause pronounced inhibition, but is not necessarily required during the preincubation period. Even the increased inhibition detectable with the preoxidized gallic acid could be attained with the non-oxidized acid if the reaction was conducted under aerobic conditions. Since the oxidized product of protocatechuic acid, on the other hand, did not show such an effect, it seems that the inhibition mechanism by gallic acid is apparently different from that by protocatechuic acid and *d*-catechin.

Protection by Substrate—It was revealed that less inhibition was produced to the cysteine desulfhydrase activity when gallic acid or *d*-catechin was simultaneously added with cysteine to the reaction mixture than when the inhibitor was added prior to the substrate. As shown in Table V,

Table V. Protection by Substrate

Preincubation for 10 min.	Dry wt. of cells (mg.)	H_2S formed (μmole)		
		Inhibitor with cells	Inhibitor, cells, and cysteine separately	Inhibitor with cysteine
Gallic acid (0.002*M*)	3.2	0.398	0.464	0.710
d-Catechin (0.002*M*)	3.0	0.367	0.526	0.769

this was especially so in the case of preincubation experiments. Moreover, it was found that little inhibition was observable if the inhibitor was added to the system after the reaction started. From the results obtained in experiments in which each mixture indicated in Table VI was aerated and then added to the cell suspension, it was evident that cysteine protected gallic acid from oxidation, while cysteine itself was probably oxidized.

Table VI. Interaction between Gallic Acid and Cysteine under Aeration
(Dry weight of cells, 3.8 mg.)

H_2S formed (μmole)			
Control[a]	Gallic acid[b] added	Aerated gallic acid solution added[c]	Aerated gallic acid solution with cysteine added[d]
0.876	0.620	0.273	0.631

a) The reaction velocity is maximum when final concn. of substrate is 0.0025*M* (cf. Table III).
b) Final concn., 0.001*M*.
c) Aerated in phosphate buffer (pH 7.0) for 45 min. Final concn., 0.001*M* gallic acid.
d) Aerated under the above condition. Final concn., 0.001*M* gallic acid and 0.00125*M* cysteine.

Discussion

The data presented in this paper indicate that polyhydroxyl compounds in general are more or less inhibitory to the liberation of hydrogen sulfide from cysteine in *E. coli*. Despite a number of attempts made in the present investigation, no conclusions can be drawn as yet concerning the mechanism of inhibition. It is certain, however, that the inhibition is not competitive with respect to the substrate concentration, although cysteine can partially protect the enzyme from inhibitors. The inhibition also seems to be very difficult to be reversed. In fact, it was not possible to substantially reverse the inhibition by any means tried.

Evidence obtained in this investigation shows that the mechanisms of inhibition by gallic acid and *d*-catechin differ considerably in several points. It was revealed that while gallic acid has to be oxidized to become sufficiently inhibitory, *d*-catechin inhibits the reaction by itself. Since gallic acid and *d*-catechin are the fundamental components of pyrogallol-tannins and catechol-tannins, which are widely distributed in nature, it is possible that these two different types of tannins exhibit different pharmacological actions on the living organism.

The fact that inhibition by both gallic acid and *d*-catechin decreases with increasing cell concentration suggests that a stoichiometric combination of the inhibitor with the enzyme is needed for the attainment of full inhibition. More experiments are required,

however, before this conclusion can be definitely established.

As mentioned above, gallic acid is unique as an inhibitor in that it requires pre-oxidation for maximum inhibition although the presence of oxygen is necessary during the reaction. The nature of the active oxidized product has not yet been elucidated. It should be pointed out, however, that gallic acid itself is also responsible for a part of the inhibition observed.

The fact that preincubation of the cells with inhibitor is necessary to attain a maximal inhibition may be explained by the time required for the inhibitor to penetrate the cells and, in the case of gallic acid inhibition, also by the time needed to produce the active oxidized form.

The authors are indebted to Dr. R. Sato of the Institute for Protein Research, University of Osaka, for valuable suggestions. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged.

Summary

The enzymatic production of hydrogen sulfide by *E. coli* was inhibited by most of the tannins and related compounds tested. Inhibition by polyhydroxyl compounds lacking free carboxyl group is greater than that by polyhydroxycarboxylic acids. The inhibition is not competitive with substrate, nor is it reversed by the addition of a coenzyme.

In order to attain a maximal inhibition it is necessary to incubate the cells with an inhibitor for a certain length of time prior to addition of the substrate. Cysteine is able to partially protect the enzyme from the action of inhibitors. This protecting effect is more pronounced when the substrate and inhibitor are incubated and then added to the reaction mixture.

Inhibition by gallic acid is considerably enhanced by aerating the inhibitor solution. This suggests that the oxidized form of gallic acid is more inhibitory than gallic acid itself. There are many differences between the mechanisms of inhibition by gallic acid and *d*-catechin.

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80. Hisao Tsukamoto and Akira Yamamoto: Metabolism of Drugs. XIX.[*1] Metabolic Fate of *p*-Aminosalicylic Acid in the Rabbit. (3).[*2]

(*Pharmaceutical Institute, Medical Faculty, University of Kyushu*[*3])

In the previous papers of this series,[1,7] the occurrence of seven metabolites was reported in the urine of rabbit receiving *p*-aminosalicylic acid (PAS). They were PAS, N-acetylated PAS (Ac-PAS), *p*-aminosalicyluric acid (PASU, M_1), ester-type PAS-glucuronide (ester-PASG, M_2), *m*-aminophenyl sulfate (MAPS, M_3), ether-type PAS-glucuronide (ether-PASG, M_4), and *m*-aminophenyl glucuronide (MAPG, M_5). PAS, Ac-PAS, and PASU have been isolated by Venkataraman[2] and Way,[3] respectively.

*1 Part XVIII. H. Tsukamoto, H. Yoshimura, S. Toki: This Bulletin, **6**, 88(1958).
*2 Part (2). H. Tsukamoto, A. Yamamoto, O. Kamata: *Ibid.*, **5**, 565(1957).
*3 Katakasu, Fukuoka (塚元久雄, 山本 陽).
1) H. Tsukamoto, A. Yamamoto: This Bulletin, **3**, 427(1955).
2) A. Venkataraman, *et al.*: J. Biol. Chem., **173**, 6(1948).
3) E.L. Way, *et al.*: J. Am. Pharm. Assoc., Sci. Ed., **44**, 65(1955).