Mutagenic and Clastogenic Activities of the Browning Reaction Model Systems

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ABSTRACT-Two short-term bioassays were employed to asses the mutagenic and clastogenic activities in browning reaction of pentose-creatine, pentose-glycine and pentose-creatine -glycine browning reaction model system. Methylene chloride extract of rhamnose-creatine -glycine browning reaction solution exhibited the strongest mutagenicity toward *Salmonella typhimurium* TA98 with S-9. Methylene chloride extract of pentose-creatine and pentose-glycine browning reaction solutions was also tested for mutagenicity, with positive responses. Methylene chloride extract of pentose-creatine-glycine browning reaction solutions induced significant increase in chromosome aberrations in the treated Chinese hamster ovary(CHO) cells. Each of pentose-creatine and pentose-glycine browning reaction solutions induced a relatively low frequency of chromosome aberrations in the treated cells.

Keywords□ Mutagenic and Clastogenic Activities, Browning reaction, *Salmonella typhimurium* TA98, Chromosome aberration

Maillard [1] advanced the hypothesis that brown pigments of nonenzymatic browning reaction occur when the amino acid and the carbonyl group of a sugar react in the presence of heat. Hodge [2] organized the nonenzymatic browning reactions that simple model systems could be used to study on complex food system. The browning model systems between sugars and amino acids have been widely used to study the properties of processing foods [3-6]. Maillard reaction produced tremendous number of chemicals. These chemicals range from volatile alcohols, aldehydes, ketones, ethers, esters and sulfur and nitrogen-containing heterocyclic compounds to nonvolatile Amadori compounds and complex browning pigments of medium to high molecular weight. Maillard reaction products showed in these food systems are undoubtly the mutagens [7]. Some mutagenic studies have been carried out on amino-reducing sugar model systems such as L-rhamnose $-NH_3-H_2S$ system [8], cysteamine-glucose system [9], maltol-NH_3 system [10]. Certain browning products obtained from the reaction in an aqueous solution exhibited some mutagenicity. Spingarn and Garvie [11] reported that browning model system of monosaccharide-NH_3 formed some mutagenic activities toward the Ames, *Salmonella/microsome* assay [12]. Recently, browning pigments obtained from browning reaction has been isolated from pyrolysates of amino acids and protein [13-15] and proved to be carcinogenes [13,14,16] Actually, 2-amino-3-methylimidazo [4,5] quinoline(IQ) and 2-amino-3,4-dimethylimidazo[4,5] quinoline were originally isolated from broiled fish[17,18] and 2-amino 3,8-dimethylimidazo[4,5] quinoxaline (MeIQx) was isolated from fried beef and beef extracts[19-21] It has been suggested that creatine or creatinine, other amino acids and sugars usu-

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ally present in meat of fish[22, 23] are involved in the formation of IQ, MeIQ and MeIQx, which are imidazoquinoline and imidaz-oquinoxaline derivatives[24-26]. Negishi, et al.[27] found a mixture of creatinine, glucose and glycine as a model system were formed MeIQx. The objective of this study was to investigate the formation of mutagenic and clastogenic compounds during the heating of mixture solutions of pentose, glycine and creatine. Two short-term test assays of Maillard reactions solutions were used; assessment of the induction of reverse mutation to histidine independence by browning compounds in *Salmonella* strain TA98, determination of mutagenic and testing of clastogenic activity of browning compounds by chromosome aberration in Chinese hamster ovary(CHO) cells. The clastogenic activity of those compounds implies that the chromosomes are damaged by chromatid breakage and exchange. Chromosome aberration is synonymous with chromosome damage.

MATERIALS AND METHODS

Preparation of samples - Test materials prepared for this study were arabinose, ribose, xylose and rhamnose, glycine or creatine. Each reaction solution was prepared by dissolving equal mole (2 mole) of each sugar and equal mole of glycine or creatine in 5ml of distilled water. The reaction solution was heated at 150℃ for 1hr., 2hrs. and 3hrs. in round flask which was kept in connected reflux condenser with or without equal mole of glycine or creatine.

Browning index— Determination of browning degree was carried out according to Palombo, R. et al.[28] with minor modification. The reaction mixture was diluted with distilled water. The optical density of the clear filterates was determined on a spectrophotometer. Water was used as blank. Browning index, OD, was calculated as; $OD = A_{420nm} - A_{550nm}$.

Mutagenicity test—Mutation test: Mutagens assay was carried out using Ame's strain of *Salmonella typhimurium* TA98 and histidine reversion were measured by his standard method [12, 29] with some modification. Using standard mutagen of 0.00125 ppm MeIQ as indicator, each mutagen extracted by methylene chloride was dissolved with dimethyl sulfoxide to a concentration of 2,000ppm. The reaction mixture of 0.1ml of bacterial culture of TA98 and 0.5ml of S-9 were incubated at 37℃ for 20min and added 2ml of 0.6% molten top agar containing 0.5% sodium chloride, 12.4μg of histidine and 9.6 μg of biotin. Vortex for 30 sec at low speed and poured on selective minimal glucose agar plate. All the plate were incubated at 37℃ His^+ revertants were counted. All experiments were run in triplicate and the results were interpreted from the average of the triplicate runs.

Extract of mutagens: Heated sample was dissolved with diluted hydrochloric acid and basic fraction was extracted with methylene chloride after adjusting pH 11 with aqueous sodium hydroxide. The methylene chloride extract was evaporated to dryness in vacuo and the residue was taken up in dimethyl sulfoxide to make 21000ppm concentration. Aliquots of these extracts were tested for mutagenicity and clastogenicity.

Chromosome aberration test — Cell culture: A clonal sub-line of the Chinese hamster ovary(CHO) was used. This cell line was obtained from J.B. Lee, Pusan San-Ub University. The cells had been maintained by 5 days passages and grown in a monolayer in petri dishes with Eagle's minimum essential medium(MEM, Grand Island Biological CO., Grand Island, N.Y.) supplemented 10% calf serum and antibiotics (Penicillin G, 100units/ml ; Streptomycin, 10μg/ml) and were added 10mM Hepes buffer and 7.5% sodium bicarbonate. The stock cultures were maintained in 240ml culture bottle at 37℃ in

Table 1. Browning index in browning model systems of pentose, pentose-creatine, pentose-glycine and pentose-creatine-glycine.

Sugars / Reaction times	Arabinose			Xylose			Ribose			Rhamnose		
	1	2	3	1	2	3	1	2	3	1	2	3
Control	0.052	0.076	0.094	0.068	0.092	0.117	0.076	0.095	0.112	0.098	0.139	0.187
Creatine	0.390	0.455	0.609	0.375	0.467	0.518	0.415	0.488	0.531	0.557	0.632	0.696
Glycine	0.503	0.663	0.801	0.556	0.688	0.822	0.597	0.691	0.754	0.682	0.796	0.899
Crcatine Glycine	0.921	0.952	0.976	0.931	0.955	0.983	0.920	0.935	0.956	0.972	0.022	1.152

Determination of browning index was performed described in Materials and Methods. Pentose measured at 420nm or 550nm without dilution. Pentose-creatine, pentose-glycine and pentose-creatine-glycine systems diluted with distilled water 200 fold and then measured at 420nm or 550nm.

CO_2 incubator and incubated again for every 2 or 3 days using the 0.05% trypsin -EDTA · 2Na.

Chromosome aberration test: CHO cells were treated with 2,000ppm of each mutagens for 1hr and this cells added new culture solution were incubated at 37℃ for 24 hrs Cells were treated with colcemid(0.06μg/ml) for 3hr. They were incubated in 0.075M -KCl hypotonic solution for 8 min at 37℃. The cells were fixed with ice cold fixative ethanol:glacial acetic acid(3 : 1) which was 3 times. Air-dried slides were stained with 4.0% Giemsa (Gurr's R66) solution. The number of cells with chromosome aberrations was recorded on 100 wellspread metaphase[30]. No chromosome exchanges were observed in any control treatments.

RESULTS AND DISCUSSION

Browning index— Nonenzymatic browning index of model systems were shown the varous pentose-creatine-glycine borwning model system(Table 1). To assess the reproducibility of the suggested browning determination method, five seperate determinations were carried out on the samples of browning model systems. The browning index was shown that rhamose-creatine-glycine model system was highest the color intensity among the model systems, whereas no significant differences were observed in any of others browning model systems. It was found that these model systems brought about the most intense brown color at any reaction time followed, in order of effectiveness, by pentose-creatine-glycine, pentose-glycine system and pentose-creatine system. Shinogara, K. et al.[31] reported that the relationship between the time-course of color intensity and that of formation of the mutagenicity on the reaction mixture of glucose and lysine at 100℃. D-glucose-glycine (0.5M) solution attained a more intense brown color during heating at 100℃ for than a D -glucose-glycine(0.05M) solution[32]. Song and Chichester[33] also found D-fructose to be more reactive than D-glucose in the presence of glycine. Pentose were selected as sugar reactants in this study with idea that each sugar may produce different amounts and perphaps types of mutagens and clastogens.

Mutagenicity of browning model systems— The influence of the pentose-glycine-creatine of Maillard reaction model systems in the *Salmonella* strain TA98 susceptible to base -pair substitution was investigated[12]. *Salmonella* strain TA98 was used exclusively since *Salmonella* other strains were not as sensitive to the browning model system[34]. All data are taken from the response curves of reaction times of 1hr., 2hrs. and 3hr. Mutagens

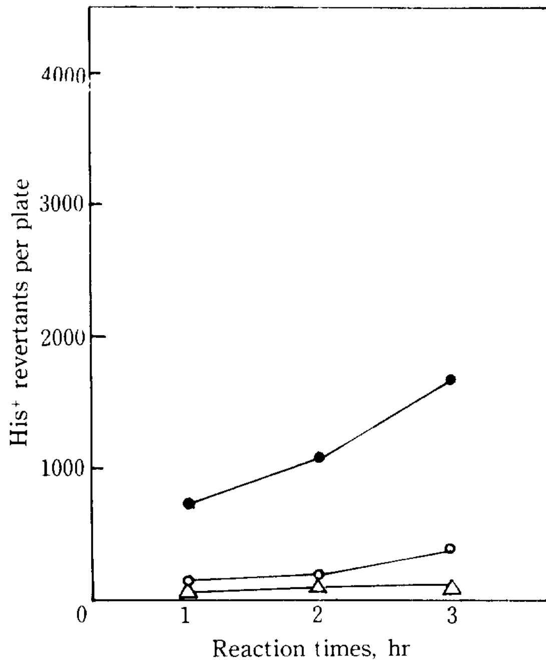

Fig.1. Mutagenicity browning model systems of arabinose-glycine, arabinose-creatine and arabinose-creatine-glycine as shown by revertant colonies per plate using *Salmonella typhimurium* TA98.

The browning systems were compared with a spontaneous mutation frequency of 620 colonies per plate using 0.00125 ppm MeIQ as indicator. △—△, arabinose-glycine; ○—○, arabinose-creatine; ●—●, arabinose-creatine-glycine system.

formed in this experiments required the metabolic activation with S-9 mix. A wide variety of promatagens can be activated by microsomal enzymes in a homogenized liver centrifugate called S-9 fraction[35]. Methylene chloride extracts obtained from the reaction products of arabinose-glycine, arabinose-creatine and arabinose-creatine-glycine model systems yielded 32, 400 and 1631 for 3 hr revertant colonies on *Salmonella thypimurium* TA98(Fig.1). These results were compared with 0.00125ppm of MeIQ per plate as mutagenic indicator. Arabinose-creatine-glycine model system was approximately 620 fold of mutagenic activity of MeIQ, respectively. Arabinose-creatine-glycine model system was intensively mutagenicity of the arabinose-creatine-glycine model system was 1631 fold than 420 of that arabinose-creatine model system. Fig.2. were shown the mutagenicities of methylene cholride extracts xylose-glycine, xylose-creatine an and xylose-creatine-glycine model systems. The mutagenic activities of xylose-glycine and xylose-creatine model systems were low, whereas the xylose-creatine-glycine model system possessed fairly highest mutagenic activities as an 3373 revertants his⁺ colonies. In case of ribose-glycine, ribose-creatine and ribose-creatine-glycine model systems, the

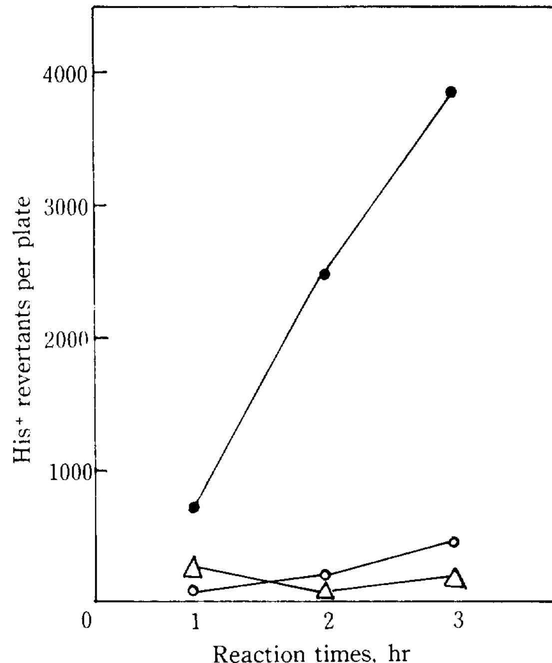

Fig.2. Mutagenicity browning model systems of xylose-glycine, xylose-creatine and xylose-creatine-glycine as shown by revertant colonies per plate using *Salmonella typhimurium* TA98.

The browning systems were compared with a spontaneous mutation frequency of 620 colonies per plate using 0.00125 ppm MeIQ as indicator. △—△; xylose-glycine; ○—○, xylose-creatine; ●—●, xylose-creatine-glycine system.

mutagenic activities of ribose-glycine and ribose-creatine model systems were very low, however the ribose-creatine-glycine model system was highest mutagenic activities(Fig.3). Mutagenicity of rhamnose-glycine, rhamnose-creatine and rhamnose-creatine glycine model system were shown in Fig.4. The mutagenic activities was increased with increasing the reaction time. Peak on the formation of his$^+$ revertant by rhamnose-creatine-glycine model systems occurred at such reaction time-course as 3hr. Rhamnose-glycine model system was lower than that of rhamnose-creatine model system. In this experiments the mutagenic activities of browning model systems by rham-

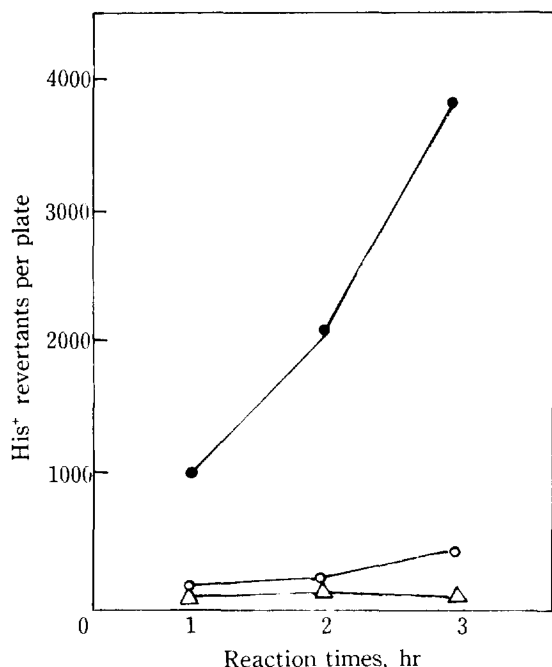

Fig.3. Mutagenicity browning model systems of ribose-glycine, ribose-creatine and ribose-creatine-glycine as shown by revertant colonies per plate using *Salmonella typhimurium* TA98.

The browning systems were compared with a spontaneous mutation frequency of 620 colonies per plate using 0.00125 ppm MeIQ as indicator. △—△ ; ribose-glycine; ○—○, ribose-creatine; ●—●, ribose-creatine-glycine system.

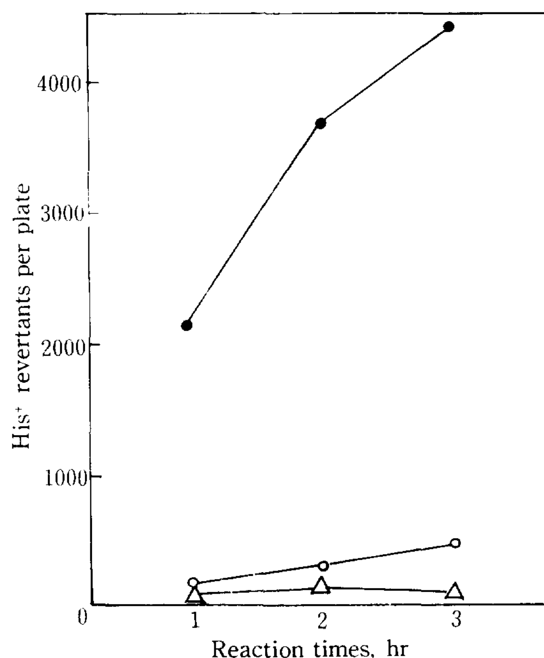

Fig.4. Mutagenicity browning model systems of rhamnose-glycine, rhamnose-creatine and rhamnose-creatine-glycine as shown by revertant colonies per plate using *Salmonella typhimurium* TA98.

The browning systems were compared with a spontaneous mutation frequency of 620 colonies per plate using 0.00125 ppm MeIQ as indicator. △—△ ; rhamnose-glycine; ○—○, rhamnose-creatine; ●—●, rhamnose-creatine-glycine system.

nose-creatine-glycine by system was highest than those of pentose such as arabinose, xylose and ribose. Spingarn and Gravie[11] reported that a methylene chloride extract of refluxed (6 hr) glucose-ammonia solution had a mutagenic activity with *Salmonella typhimurium* TA98 after S-9 activation. Nagao, et al.,[36] Commoner, et al[37]. Spingorn and Weisburger[38] have described the formation of mutagens during the cooking of meat. It has found that the mutagens are formed by the pyrolysis of sugar-amino acids model system. It has been considered that the mutagenicity are increased to the aquous solution of amino acids or some nitrogenous compounds in the presence of

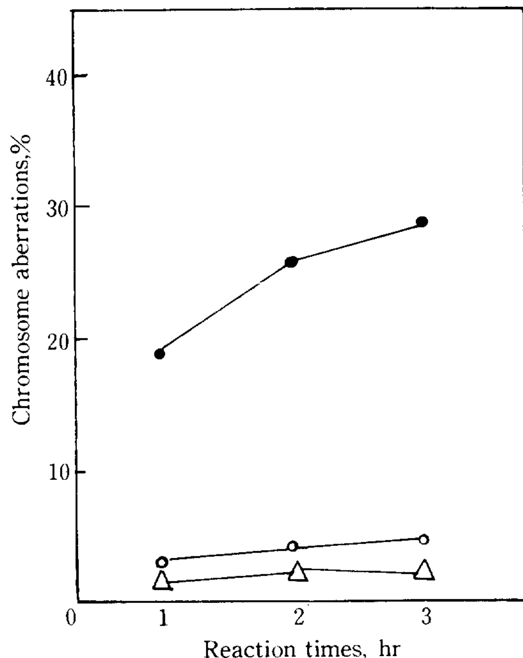

Fig.5. Effect of arabinose-glycine, arabinose -creatine and arabinose-creatine-glycine browning model systems on the percentage of metaphase plates showing aberrant chromosome in treated Chinse hamster ovary(CHO) cells.

The browning systems compared with 10.2% aberrant chromosomes formed from 0.00125 ppm MeIQ as indicator. △—△, arabinose-glycine; ○—○, arabinose -reatine; ●—●, arabinose-creatine -glycine model sgetem.

sugars. Mutagenicity was observed clearly by heating products of various amino acids with creatine[34]. Yoshida, D., and Okamoto, H[39]. reported that creatine was heated with glucose or oleic acid, the numbers of revertant colonies were 1.9×10^4 or 1.0×10^4 per mmole of creatine, respectively. The mutagenic material in the reacted solution of creatine and glucose showed that the mutagens are basic substances and are chromatographically distinguishable from benzo(a)pyrene, amino-α and amino-γ -carbolines which are mutagenic principles in the pyrolysis products of protein[40]. Therefore the formation of mutagens by the reaction between creatine and other amino acids would be expected during the cooking of meat.

Clastogenic activity in CHO cells The freguency of chromosome aberration at metaphase in CHO cells was estimated following a 24hr exposure to dilutions of various pentose -creatine, pentose-glycine and pentose -creatine-glycine browning model systems. The abilities of compounds in these systems to induce chromosome aberrations are shown Figures 5-8 for heated at 150℃ for 1hrs., 2hrs. and 3hrs. The cell treated with methylene chloride extracts of rhamnose-creatine model systems at 2,000ppm per ml for 1hr. Each of

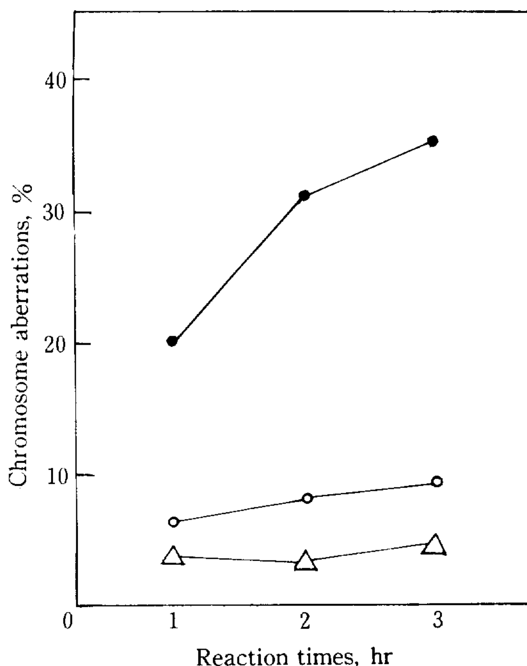

Fig.6. Effect of xylose-glycine, xylose-creatine and xylose-creatine-glycine browning model systems on the percentage of metaphase plates showing aberrant chromosome in treated Chinse hamster overy(CHO) cells.

The browning systems compared with 10.2% aberrant chromosomes formed from 0.00125 ppm MeIQ as indicator. △—△, xylose-glycine; ○—○, xylose-creatine; ●—●, xylose-creatine-glycine model system.

these browning systems induced chromosome aberrations in the treated cells. Examples of the scored chromosome aberrations are shown in Figures 6-9. Using the mutagenic MeIQ for clastogenic estimation, MeIQ at the concentration of 0.00125ppm induced chromosome aberration in 10.2% of the metaphase plates of CHO cells. At even pentose-glycine and pentose-creatine browning model systems, the degree of chromosome aberration dropped to near zero, presumely because of the toxic levels of browning reaction compounds. The clastogenic activities of the arabinose-creatine -glycine browning model system was similiar

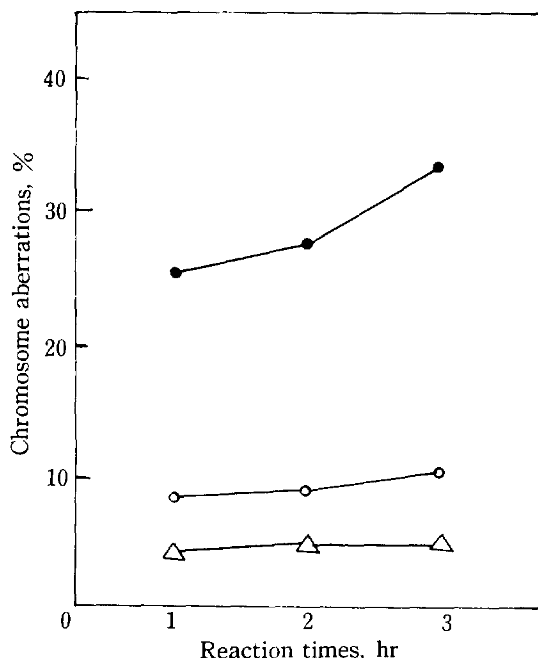

Fig.7. Effect of ribose-glycine, ribose-creatine and ribose-creatine-glycine browning model systems on the percentage of metaphase plates showing aberrant chromosome in treated Chinse hamster ovary(CHO) cells.

The browning systems compared with 10.2% aberrant chromosomes formed from 0.00125 ppm MeIQ as indicator. △—△, ribose-glycine; ○—○, ribose-creatine; ●—●, ribose-creatine-glycine model system.

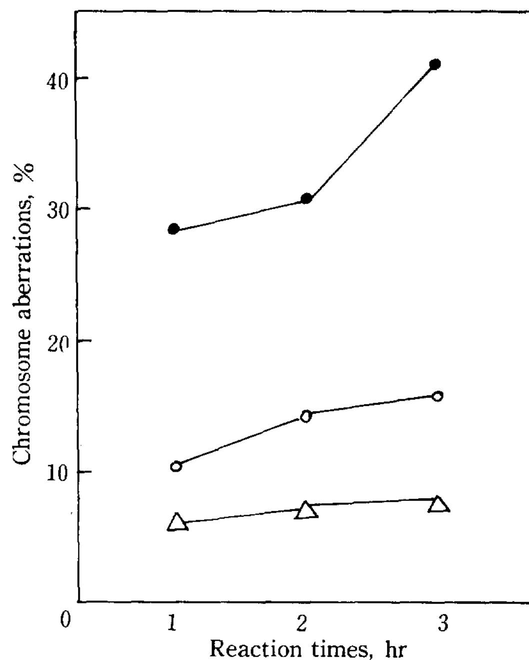

Fig.8. Effect of rhamnose-glycine, rhamnose -creatine and rhamnose-creatine-glycine browning model systems on the percentage of metaphase plates showing aberrant chromosome in treated Chinse hamster overy(CHO) cells.

The browning systems compared with 10.2% aberrant chromosomes formed from 0.00125 ppm MeIQ as indicator. △—△, rhamnose-glycine; ○—○, rhamnose -creatine; ●—●, rhamnose-creatine -glycine model system.

to those of xylose-creatine-glycine systems or ribose-creatine-glycine browning model systems. Presumably similar clastogenic compounds are formed in the pentose-creatine -glycine systems. Especially, for rhamnose -creatine-glycine system, the browning compounds has highest clastogenic activities for 1 hr, 2hr or 3hr among the others browning occur only with potent clastogens. Comparable levels of chromosome aberrations has been observed in mammalian cells exposed to 250-400 rad of x-ray[41], 2-8$\times 10^{-5}$M of aflatoxin B_1[42], 2-4$\times 10^{-6}$M N-methyl -N^1-nitro-N-nitros-quanidine[43]. According

to Powire et al.,[44] Maillard reaction solutions have clastogenic compound which caused high levels of chromosome aberration. The results of the two bioassays demonstrated the presence of Maillard reacton and chromosome aberration. All of the pentose-creatine, pentose-glycine and pentose-creatine glycine browning reaction solutions possessed clastogenic compounds which caused high level of chromosome aberration.

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