bonate and saline produced no rise in the average level of glutamine in the group of animals.

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17278. Effect of Administration of Sodium Benzoate on the Level of Glutamine in Blood. VI.

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In previous publications we reported on the effect of the administration of insulin, glucose,[1] amino acids and sodium bicarbonate[2] on the level of glutamine in the blood. It was suggested that the effect of insulin in lowering the level of glutamine in the blood might be due, in part, to the depression of oxidative deamination of amino acids by insulin. Since it has been reported that benzoic acid depresses the oxidation of d-amino acids[3] and other intermediary metabolites[4,5] the effect of its administration on the level of glutamine was investigated.

Procedure and methods. Male rabbits in the post absorptive state were used for these studies. The effect of the administration of sodium benzoate was studied also in a normal human female subject. The benzoate was administered either intravenously or orally as indicated in the table. Blood was collected in tubes containing sodium oxalate and the plasma separated by centrifugation. Glutamine was determined by the method described in a previous publication.[6] The total amino acids were determined by the method of Simonelli.[7] The blood sugar (total reducing substance) was determined by the Folin-Wu method[8] which includes other non-glucose reducing substances. In some cases the blood was allowed to glycolize at 37°C overnight in order to determine the change in non-glucose reducing substances.

Observations. Doses of 0.25 g, 0.5 g, and 0.75 g of benzoic acid neutralized with sodium hydroxide and injected intravenously produced a fall in the level of glutamine in the plasma both in the 30 min. and 60 min. period after injection. The extent of the fall varied in different animals and was not related to the size of the dose of benzoic acid. Statistical analysis of the fall showed that the drop was statistically significant for both periods (see table).

The level of the total amino acid nitrogen in the blood plasma tended to fall in all animals; however, this was negligible in some animals. The extent of the change in the level of glutamine was not parallel with the changes in total amino acid nitrogen. The blood sugar (total reducing substances) also rose in all the animals which received benzoic acid intravenously. The extent of the rise also was variable and two of the animals which showed the most marked rise (Rabbits nos. 1 and 3) in the "blood sugar" received the smallest dose of benzoic acid. By incubating the blood at 37°C overnight it was found

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3 Klein, J. R., and Kamin, H., *J. Biol. Chem.*, 1941, **138**, 507.

4 Griffith, W. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 279.

5 Jowett, M., and Quastel, J. H., *Biochem. J.*, 1935, **29**, 2143 and 2159; Quastel, J. H., and Wheatley, A. H. M., *ibid.*, 1935, **29**, 2773.

6 Harris, M. M., Roth, R. T., and Harris, R. S., *J. Clin. Invest.*, 1943, **22**, 569.

7 Simonelli, U., Clinical Colorimetry with the Pulfrich Photometer, by W. Krebs, p. 24, Carl Zeiss, Jena.

8 Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

TABLE I.
Effect of Administration of Sodium Benzoate on the Level of Glutamine in the Blood.

Rabbit No.	Wt., kg	Substance administered*	Time, min.	Glutamine in plasma, mg per 100 ml	Amino acid nitrogen in plasma, mg per 100 ml	Blood sugar, mg per 100 ml
1	2.5	0.25 g benzoic acid in 8 ml water	0	8.0	9.4	132
			30	6.3	8.7	155
			60	6.8	9.1	220
2	2.6	0.25 g benzoic acid in 8 ml water	0	7.9	10.0	118
			30	5.9	9.7	124
			60	6.0	10.1	152
3	2.9	0.25 g benzoic acid in 5 ml water	0	8.6	9.9	126
			30	6.5	9.8	218
			60	6.6	9.2	260
4	2.9	0.5 g benzoic acid in 10 ml water	0	7.5	10.5	118
			30	6.5	9.8	148
			60	7.5	10.0	172
5	2.9	0.75 g benzoic acid in 10 ml water	0	8.0	8.0	113
			30	7.3	7.3	122
			60	6.9	6.9	119
6	2.9	0.75 g benzoic acid in 10 ml water	0	8.3	7.8	122
			30	7.3	7.3	134
			60	6.9	7.3	163
7	4.2	2.0 g in gelatin capsules + 15 ml water (orally)	0	8.1	7.2	—
			75	5.7	7.1	95
			175	6.1	7.4	96
Human subject		5.9 g sod. benzoate in	0	6.6	9.1	89
59.1		capsules + 200 ml	45	7.0	8.5	77
		water	85	6.0	8.4	84
			135	4.8	8.7	92
8		No treatment	0	8.3	9.0	
			30	8.4	9.0	
			60	8.1	9.4	
		Saline controls, avg.	0	6.4	6.3	101
		for 6 animals	30	6.5	6.2	105
		see previous paper	60	6.5	6.2	109

* Sodium benzoate was administered intravenously except where otherwise indicated.

Statistical analysis of the effect of the intravenous administration of sodium benzoate gave a Fisher "t" value of 6.64 and 6.30, with $P < 0.01$ for the changes in the level of glutamine for the 30 and 60 min. periods respectively, which is highly significant. The corresponding changes in the level of amino acid nitrogen gave a Fisher "t" value of 2.03 with $P > 0.05$ and 1.81 with $P > 0.1$ which were not statistically significant.

in some of the experiments that the rise was due in part to an increase in a reducing substance which did not undergo glycolysis and was undoubtedly due to glycuronic acid.

Sodium benzoate was administered orally in capsules to one rabbit and one human female subject in doses of 2.0 g and 5.9 g respectively. The level of glutamine dropped markedly in both cases. However, the level of amino acid nitrogen remained practically unchanged in the rabbit. In the human subject the glutamine level dropped 27% and the total amino acid nitrogen only about 4% at the end of 135 min. after the administration of sodium benzoate. The "blood glucose" showed very little change in both cases and in fact was slightly lower in the human subject after 45 minutes.

Discussion. It is claimed that benzoic acid is detoxified by forming a glycuronide in the

rabbit and hippuric acid in man. If this is so, it would mean that the level of glutamine is depressed irrespective of the mode of detoxification used by the animal organism. It is interesting to note the variable effect of the administration of benzoic acid on the level of the total amino acids and the total reducing substances in the blood. Although glycine was withdrawn from the metabolic mixture as hippuric acid in the experiment on the human subject (several grams of crystalline hippuric acid were isolated from her urine), still the level of the total amino acids in the blood was lowered to no greater extent than in some of the rabbits. These variable effects in the different animals suggest that variable metabolic changes, besides that involved in the formation of benzoyl glycuronide, probably occur in different rabbits. The fall in the level of glutamine may be due, in part, to the inhibition of the oxidative deamination of amino acids beside the inhibition of other oxidative processes.[4,5] This would reduce the supply of nitrogen for amidation. However, where there was a fall in the level of the total amino acids in the blood it is possible that certain amino acids were withdrawn from the metabolic mixture thus reducing the supply of nitrogen for amidation. The inhibition of deamination alone should tend to raise the level of the amino acids in the blood. Since in some cases the level of glutamine was depressed without any significant change in the level of the total amino acids (Rabbits nos. 2, 3 and 7) it is possible that the process of amidation was directly depressed.

Christensen and his co-workers[9] claim that special mechanisms are present in cells for concentrating amino acids and that in the presence of high concentrations of amino acids there is competition between the various amino acids for these mechanisms. If the depression in the level of glutamine in the plasma were due to a shift of glutamine into tissue cells due to the removal of glycine by conjugation with benzoic acid then one should obtain a significant drop in the level of the total amino acid nitrogen in the plasma. This did not occur in a number of experiments. Furthermore, the statistical analysis indicated that the changes in the level of amino acid nitrogen were not significant while those for glutamine were highly significant (see additional discussion in previous paper and table). This problem requires further study.

It is of interest to note that the smaller doses of sodium benzoate (Rabbits nos. 1 and 3) produced the most pronounced rise in blood sugar (total reducing substances). The reason for this effect is not apparent at the present time.

Summary and conclusions. 1) Sodium benzoate administered intravenously and orally to rabbits and one human subject was found to depress the level of glutamine in the blood plasma.

2) The intravenous administration of sodium benzoate produced a rise in the total reducing substances in the blood which was highest with the smallest doses of benzoate.

3) The changes in the glutamine level were not related to either the change of the total amino acid level or the level of total reducing substances in the blood.

4) It is suggested that amidation may be either directly or indirectly depressed by the administration of sodium benzoate.

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[9] Christensen, H. N., Streicher, J. A., and Elbinger, R. L., *J. Biol. Chem.*, 1948, **172**, 515.