Their experiments indicated that the anti-thyroid activity of this compound in humans is comparable to that of 6-n-propylthiouracil.

Summary. A new method for the detection of compounds which have anti-thyroid activity is described. This method has been used to isolate a crystalline anti-thyroid compound from rape seed having the empirical formula C_5H_7ONS.

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17277. Effect of Amino Acids and Sodium Bicarbonate on the Level of Glutamine in Blood. V.

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In a previous publication[1] we reported on the depressing effect of glucose and insulin administration on the level of glutamine and amino acids in the blood plasma and some of the possible mechanisms involved in producing this effect were discussed. In a subsequent publication[2] it was shown that this was not due to a mere diffusion of amino acids and glutamine into the tissues as had been suggested by Hamilton.[3]

In this paper we wish to report the effect of the intravenous administration of some amino acids, sodium bicarbonate and physiological saline solution on the level of glutamine in the blood plasma of rabbits.

Procedure and methods. Male rabbits, 18 hours post absorptive, were used for these experiments. Six animals were used for each substance which was tested. The amino acids and sodium bicarbonate were made up as a 10 percent solution in distilled water and 10 ml or the equivalent of 1 g of substance per 3 kg of body weight was administered intravenously. The saline solution was administered in quantities of 10 ml. Since the rabbits were very similar in weight (Table I) the volumes varied by only one or two ml. The rabbits were bled from the marginal vein of the ear just before injection and 30 and 60 minutes after injection. About 12 ml of blood were drawn at each bleeding into tubes containing sodium oxalate to prevent coagulation. The blood was immediately centrifuged for about 10 minutes and the plasma was used for the determination of total amino acids and glutamine. The amino acids were determined colorimetrically by Russell's modification of Frame's method[4] and the glutamine by our method as described in a previous publication.[5] The determinations were carried out in duplicate or triplicate and statistical analysis of the data showed that the variations between duplicate determinations were negligible.

Observations. It will be seen from Table I that the initial level of glutamine in the post-absorptive state bore no relation to the level of the total amino acids during this period.

dl-Alanine. Following the intravenous administration of dl-alanine there was a marked rise in the level of glutamine in the first 30 minutes. This amounted to an average increment of 3.3 mg per 100 ml of blood plasma. Although the level tended to decrease in the following 30 minutes it still was above the initial level by an average of 2.0 mg. Statistical analysis by Fisher's "t"[6] method for a

1 Harris, M. M., Roth, R. T., and Harris, R. S., *J. Clin. Invest.*, 1943, **22**, 577.

2 Harris, M. M., and Harris, R. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 471.

3 Hamilton, P. B., *J. Biol. Chem.*, 1945, **158**, 397.

4 Russell, J. A., *J. Biol. Chem.*, 1944, **156**, 467.

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

6 Mainland, D., Treatment of Clinical and Laboratory Data, p. 147, Oliver and Boyd, London, 1938.

TABLE I.
Effect of Administration of Various Amino Acids, Saline and Sodium Bicarbonate on Level of Glutamine in Blood Plasma.

Rabbit No.	Wt., kg	Substance administered	Amino acid nitrogen in plasma, mg per 100 ml			Glutamine in plasma, mg per 100 ml		
			Before	30 min.	60 min.	Before	30 min.	60 min.
1	3.1		7.0	14.4	10.2	6.0	8.9	6.8
2	3.4	dl-alanine	5.9	14.8	12.7	7.2	10.4	10.6
3	3.5		6.5	16.8	13.4	7.1	9.4	8.4
4	4.0		8.4	19.0	13.2	8.8	12.7	10.5
5	4.4		6.9	14.5	12.8	7.3	11.1	9.0
6	4.0		6.8	14.7	11.5	7.5	11.2	10.3
Average	3.73		6.9	15.7	12.3	7.3	10.6	9.3
						Fisher's "t"	13.6	6.43
				(8.8)*	(5.4)	P	<0.01	<0.01
7	3.9		5.2	17.4	14.4	6.1	8.3	8.3
8	3.9		7.4	18.2	15.3	7.5	9.3	9.5
9	4.1	glycine	7.7	18.7	15.5	8.1	9.6	9.3
10	3.8		6.9	16.8	15.2	11.4	12.5	10.9
11	3.6		6.6	14.1	10.5	7.4	7.7	8.2
12	4.1		6.6	14.6	11.5	8.0	10.1	8.7
Average	3.9		6.7	16.6	13.7	8.1	9.6	9.2
						Fisher's "t"	4.67	4.34
				(9.9)	(7.0)	P	<0.01	<0.01
13	3.4		7.8	19.5	17.1	7.6	8.1	8.3
14	4.0		6.3	21.2	16.7	7.4	8.1	9.3
15	4.2	β-alanine	6.1	19.5	14.9	5.8	8.5	8.0
16	4.3		7.1	21.1	17.6	9.5	10.6	10.1
17	4.2		6.9	21.7	16.3	8.7	8.5	8.7
18	3.6		5.9	21.2	18.9	8.4	9.2	8.8
Average	3.95		6.7	20.7	16.9	7.9	8.5	8.9
						Fisher's "t"	2.35	3.01
				(14.0)	(10.2)	P	>0.05	<0.05
19	4.4		6.6	6.6	6.4	8.0	7.8	6.5
20	4.2		7.4	6.8	6.6	8.4	7.6	8.1
21	3.5	Sodium bicarbonate	7.2	6.8	6.6	7.0	7.7	8.5
22	4.0		6.9	6.2	—	9.8	8.6	8.7
23	4.4		7.2	7.2	7.1	7.6	7.8	8.0
24	3.8		6.4	7.0	7.3	8.3	7.2	8.8
Average	4.05		6.7	6.8	6.8	8.2	7.8	8.1
						Fisher's "t"	1.71	0.160
				(0.1)	(0.1)	P	>0.1	>0.8
25	3.9		6.4	6.3	6.0	6.6	7.0	6.1
26	4.1		6.7	6.1	6.3	7.7	7.1	7.7
27	4.5	Saline	6.4	6.3	6.3	6.5	6.3	5.6
28	3.9		6.9	6.8	6.9	6.9	7.2	7.4
29	2.4		5.9	5.5	6.1	5.0	5.1	5.5
30	3.5		5.9	6.4	5.7	5.9	6.0	6.0
Average	3.72		6.4	6.2	6.2	6.4	6.4	6.4
				(0)	(0)	Fisher's "t"	.206	.096
						P	>0.8	>0.9

* The figures in parentheses represent the increments, above the initial level, of the average level of amino acid nitrogen per 100 ml of plasma.

small number of experimental animals showed that the increments obtained were highly significant for both periods with $P < 0.01$.

Glycine. The administration of glycine produced an average rise in the level of glutamine of 1.5 mg for the 30 minute period and

1.1 mg for the 60 minute period. The "t" values for both periods were significant with $P < .01$. Only one of the 6 animals, (No. 10) showed a slight drop below the initial level in the 60 minute period.

β-Alanine. Following the administration of β-alanine there was an average rise in the level of glutamine of 0.6 mg in the 30 minute period and 1.0 mg after 60 minutes. The "t" value was not significant for the 30 minute period but was significant for the 60 minute period. The value of P was greater than 0.05 for the former and less than 0.05 for the latter period.

Sodium Bicarbonate and Saline. There was a slight drop in the average level of glutamine following the administration of sodium bicarbonate and no change following saline. The "t" values, however, were not significant for any of the periods.

Total Amino Acids Nitrogen. The average values of the total amino acid nitrogen in the different groups were very similar in the initial period before injection. Following the injection of saline and sodium bicarbonate the average values remained practically unchanged in the 30 and 60 minute periods. The administration of the various amino acids resulted in a rise in the level of the total amino acid nitrogen in the plasma which remained elevated with a tendency to fall in the second 30 minute period. The smallest rise occurred with dl-alanine and the highest rise occurred with β-alanine.

Statistical Analysis. The data were analyzed by Fisher's "t" method and the P values for significance were determined from Fisher's table. Any values of P equal to or less than 0.05 were considered significant.

Owing to the fact that the initial average level of glutamine in the plasma was not the same for the various groups, the data were analyzed by the method of variance and covariance by which statistically "adjusted means"[7,8] are obtained.

TABLE II.
Changes in the Level of Glutamine in Blood Plasma Resulting from the Administration of Various Substances Compared with Statistically Adjusted Results by the Method of Variance and Covariance.

Substance administered	Obtained average values of glutamine in plasma, mg per 100 ml					Statistically adjusted mean values of glutamine in plasma, mg per 100 ml				
	Before	30 min.	60 min.	Increment 30 min.	Increment 60 min.	Before*	30 min.	60 min.	Increment 30 min.	Increment 60 min.
dl-alanine	7.3	10.6	9.3	3.3	2.0	7.58	10.81	9.46	3.23	1.88
Glycine	8.1	9.6	9.2	1.5	1.1	7.58	9.20	8.90	1.62	1.32
β-alanine	7.9	8.5	8.9	0.6	1.0	7.58	8.27	8.71	0.69	1.13
Sod. bicarb.	8.2	7.8	8.1	—0.4	—0.1	7.58	7.33	7.74	—0.25	0.16
Saline	6.4	6.4	6.4	0.0	0.0	7.58	7.30	7.09	—0.28	—0.49
Average	7.58	8.58	8.38	1.0	0.8		8.58	8.38	1.0	0.82
F	1.8	12.1	7.5				29.0	8.6		
P	>0.05	<0.01	<0.01				<0.01	<0.01		

* In order to equate for initial level, it is assumed that all of the individual animals showed a "before" average of 7.58 equal to the grand average of all the animals in the "before" determination.

[7] Rider, P. R., Introduction to Modern Statistical Methods, Analysis of Covariance, p. 150, John Wiley and Sons, Inc., New York, 1939.

[8] Snedecor, G. W., Statistical Methods, p. 116, 215, 318, Iowa State College Press, Ames, Iowa, 1946.

The results of both methods of analysis (unadjusted averages and "adjusted means") were essentially similar. (Table II)

It will be noted that the most marked effect was obtained from the administration of dl-alanine and lesser effects were produced by glycine and β-alanine in this order. Since the average values for the initial level of glutamine of the groups of animals used for the administration of sodium bicarbonate and saline, which may be considered as the controls, consisted of both a high and low average value, this also acted as a control for the effect of the variation in the initial level of glutamine.

Discussion. It will be noted that the increment in the level of the amino acid nitrogen after 30 and 60 minutes remained much higher following the administration of β-alanine than that of dl-alanine. This probably indicates that the latter entered the metabolic processes of the tissues more rapidly and is in keeping with the more marked effect on the level of glutamine produced by it.

Christensen and his coworkers[9] have reported that the oral administration to guinea pigs of various α-amino acids, except glutamic acid, will produce a rise in glycine in the plasma with a decrease in the ratio of the concentration of glycine in the liver and muscles to that in the plasma. This was interpreted by them as due to competitive inhibition between some of the amino acids for the means by which the cells concentrate amino acids. However, from a recalculation of their data it is apparent that although the distribution ratio for glycine fell the actual concentration of glycine in the liver cells rose above the fasting level in some cases. It would seem that the rise in the level of glycine in the plasma could be interpreted as resulting from a marked production or accumulation of glycine accompanied by an increased burden placed upon the concentrating power of the liver cells. The authors give no data regarding glutamine except that following the administration of glutamate which resulted in an increase in glutamine production.

If the claims of Christensen and his coworkers are correct, it is possible that the rise in the level of glutamine in the plasma may be due to a displacement of glutamine from the tissues by dl-alanine or glycine. We are inclined to believe, however, that this is probably due to an increased production of glutamine. l-Alanine is known to play an important role in the transamination of glutamic acid to l-alanine (glutamic acid + pyruvic acid ↔ alanine + ketoglutaric acid). An increase in the supply of alanine would tend to reverse this reaction and thus hinder transamination and favor amidation. Braunstein[10] has indicated that there is an interrelation between transamination and amidation and where transamination is decreased amidation or glutamine formation is increased and vice versa. Since glycine, as far as is known, does not enter into transamination mechanisms the process whereby it produces a rise in the level of glutamine requires further elucidation. It is possible that it may result from the amidation of free ammonia liberated by the amino oxidase of glycine. Various investigators[10,11] have indicated that amidation is probably an important means whereby ammonia is rapidly fixed by the formation of glutamine in the animal organism.

In connection with our observations it may be of interest to call attention to the recent findings of Awapara and his coworkers[12] that the dicarboxylic amino acids are decreased and alanine is increased in the liver of adrenalectomized rats given 17 hydroxydehydrocorticosterone (Compound E of Kendall).

Summary. Studies were carried out regarding the effect of the intravenous administration of dl-alanine, glycine, β-alanine, sodium bicarbonate and saline on the level of glutamine in the blood of rabbits. dl-Alanine was found to produce a marked rise in the level of glutamine. Glycine produced a smaller and β-alanine the smallest rise. Sodium bicar-

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

10 Braunstein, A. E., Advances in Protein Chemistry, Vol. 3, p. 1, Academic Press, Inc., New York, 1947.

11 Krebs, H. A., *Biochem. J.*, 1935, **29**, 1951.

12 Awapara, J., Marvin, H. N., and Wells, B. B., *Endoc.*, 1949, **44**, 378.

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

1 Harris, M. M., Roth, R. T., and Harris, R. S., *J. Clin. Invest.*, 1943, **22**, 577.

2 Harris, M. M., and Harris, R. S., PROC. SOC. EXP. BIOL. AND MED., preceding paper.

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.