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# Effect of Insulin Hypoglycemia and Glucose on Various Amino Acids in Blood of Mental Patients.

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In previous publications[1,2] we reported that the level in the blood of the total amino acids and also of glutamine is markedly depressed during the insulin hypoglycemic shock treatment of mental patients. It was also shown that the administration of glucose[2] produces a similar effect although it is not so marked as that produced during insulin hypoglycemic shock.

As part of an investigation regarding the metabolism of various amino acids in mental patients, determinations were made of the effect of insulin and of the administration of glucose on the level in the blood of the following amino acids: arginine, histidine, leucine, lysine, phenylalanine, tryptophane and valine.

It is known that the metabolism of some specific amino acids may be disturbed in certain clinical conditions as for example, phenylalanine in oligophrenia phenylpyruvica.[3,4] Gjessing[5] and others have reported on alterations in nitrogen metabolism in certain types of periodic psychotic episodes. In some pathological states certain amino acids may serve some special functions as for example, methionine in the protection of the liver against some poisons.[6,7] Furthermore it is known that some amino acids can be synthesized in the animal organism in amounts adequate for its needs while others are essential and must be supplied in the diet for normal nutrition.[8]

No study regarding a group of individual amino acids has thus far been carried out in mental patients owing to the lack of adequate methods. The recent development of microbiological methods has made such study feasible.

*Procedure and Methods.* A group of 15 patients undergoing or about to undergo a course of insulin hypoglycemic shock treatment was used for this study. The patients were about 16 hours postabsorptive when the observations were begun. They were kept in bed during the entire period of study.

After a preliminary specimen of venous blood was taken, the patient was given either a dose of insulin subcutaneously which produced hypoglycemic coma, or 75 g of glucose in 200 ml of tap water which was flavored with lemon juice. Nothing was administered to 4 patients who were used as controls.

Blood was withdrawn from the anterior cubital vein in an oiled syringe and discharged into a flask containing heparin. The blood samples were taken to the laboratory immediately and centrifuged for 10 minutes and the plasma separated. The plasma was again centrifuged for 5 minutes to remove any stray red blood cells. The plasma proteins were then immediately precipitated with tungstic acid according to the method of Hier and Bergeim.[9] The precipitate was removed by centrifugation and filtration. The filtrate was adjusted to pH 7 with 1N NaOH using the Beckman pH meter.

1 Harris, M. M., Blalock, J. R., and Horwitz, W. A., *Arch. Neurol. and Psychiat.*, 1938, **40**, 116.

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

3 Penrose, L. S., and Quastel, J. H., *Bioch. J.*, 1937, **31**, 266.

4 Jervis, G. A., Block, R. J., Bolling, D., and Kanz, E., *J. Biol. Chem.*, 1940, **134**, 105.

5 Gjessing, R., *Arch. f. Psychiat. u. Nervenkr.*, 1935, **104**, 355.

6 Miller, L. L., Ross, J. F., and Whipple, G. H., *Am. J. Med. Sc.*, 1940, **200**, 739.

7 Miller, L. L., and Whipple, G. H., *J. Exp. Med.*, 1942, **76**, 421.

8 Sahyun, M., *Outline of the Amino Acids and Proteins*, 1944, p. 223.

9 Hier, S. W., and Bergeim, O., *J. Biol. Chem.*, 1945, **161**, 717.

Each determination was carried out in triplicate. Leuconostoc mesenteroides P-60 and the improved medium D of Dunn *et al.*[10] were used for the determination of lysine and histidine. Tryptophane was determined according to Dunn *et al.*[11] using *Lactobacillus arabinosis*. The medium of Hac, Snell and Williams[12] and *Lactobacillus casei* were used for the determination of arginine. Leucine and valine were determined with the medium of Shankman[13] and *Lactobacillus arabinosis*. Phenylalanine was determined with the medium of Dunn *et al.*[14] and *Lactobacillus casei*.

One milliliter of basal medium was employed in all the determinations except that of histidine. For the determination of lysine, valine and phenylalanine, 1 ml of blood filtrate was used and for arginine, tryptophane, and leucine only 0.5 ml of filtrate plus 0.5 ml of water was employed. For the determination of histidine it was found necessary to increase the volume of basal medium to 2.5 ml to which was added 1 ml of filtrate and 1.5 ml of water.

A set of standards was run with each determination instead of depending upon standard curves. This acted as a check as to any changes in the behavior of the microorganisms.

Tryptophane and histidine were determined turbidometrically with the Coleman spectrophotometer with the light band set at 540. Electrometric titration, with the Beckman pH meter, of the acid production was used for the determination of the other amino acids.

The total amino acid nitrogen was determined by the colorimetric method of Frame *et al.*[15,16]

The blood sugar was determined by the colorimetric method of Somogyi.[17]

The Coleman spectrophotometer was used for all colorimetric determinations.

*Observations.* Table I shows the effect of insulin hypoglycemia on the level of the total amino acid nitrogen and of various amino acids in the blood plasma. It will be noted that the total amino acids and all of the individual amino acids studied thus far, were significantly depressed during insulin hypoglycemic coma. However, the various amino acids were not depressed to the same degree. This is indicated by the marked variations in the percentage drop from the initial levels of the various amino acids in the same patient (Table I). Thus for example, in patient (J.B., No. 1) lysine is depressed 70% whereas the level of the total amino acids is depressed only 33%. It will also be seen that the levels of leucine and lysine are the 2 amino acids most markedly depressed during insulin hypoglycemic coma. The drop in the levels of leucine ranged from 63 to 81% of the initial level, and that of lysine ranged from 47 to 77%. With a few exceptions, the percentage drop in the level of the individual amino acids was equal or greater than the drop in the level of the total amino acids (Table I).

The initial level of the amino acids appeared to bear no relationship to the dose of insulin required to produce hypoglycemic coma, and the extent of the drop of the level of the amino acids was not directly related to the size of the dose of insulin which was administered.

Table II shows the effect of the administration of 75 g of glucose, orally, on the blood sugar level and the corresponding changes in the level of the total amino acids and of some of the individual amino acids in the blood plasma. The greatest drop in the level of the amino acids occurred in the first hour after the administration of glucose, at which time the level of the blood sugar was the highest. There was, with an occasional exception, a further drop at the end of 2 hours at which time the blood sugar level was either

10 Dunn, M. S., Shankman, S., Camien, M. N., Frankl, W., and Rockland, L. B., *J. Biol. Chem.*, 1944, **156**, 703.

11 Dunn, M. S., Schott, H. F., Frankl, W., and Rockland, L. B., *J. Biol. Chem.*, 1945, **157**, 387.

12 Hac, L. R., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1945, **159**, 273.

13 Shankman, S., *J. Biol. Chem.*, 1943, **150**, 305.

14 Dunn, M. S., Shankman, S., and Camien, M. N., *J. Biol. Chem.*, 1945, **161**, 643.

15 Frame, E. G., Russell, J. A., and Wilhelmi, A. E., *J. Biol. Chem.*, 1943, **149**, 255.

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

17 Somogyi, M., *J. Biol. Chem.*, 1945, **160**, 69.

TABLE I.
Effect of Insulin Hypoglycemia on the Level of Various Amino Acids in the Blood Plasma.

| Patient and No. | Sex | Age, yr | Wt, kg | Dose of insulin units | Total amino acid nitrogen mg/100 ml | | Arginine γ/ml | | Histidine γ/ml | | Leucine γ/ml | | Lysine γ/ml | | Tryptophane γ/ml | | Valine γ/ml | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *B | *A | B | A | B | A | B | A | B | A | B | A | B | A |
| J.B. | M | 29 | 81 | 220 | 6.4 | 4.3 | 23.8 | 13.5 | 15.3 | 6.9 | 57.6 | 20.5 | 25.4 | 7.6 | 14.6 | 9.0 | 34. | 17.4 |
| 1 | | | | | | (33) † | | (43) | | (55) | | (64) | | (70) | | (38) | | (49) |
| J.M. | M | 29 | 64 | 160 | 7.2 | 4.1 | 22.1 | 13.5 | 16.5 | 6.7 | 63.4 | 15.8 | 32.8 | 7.6 | 15.8 | 9.6 | 35.6 | 13.5 |
| 2 | | | | | | (43) | | (39) | | (59) | | (75) | | (77) | | (39) | | (62) |
| W.B. | M | 34 | 66 | 150 | 6.4 | 4.0 | 21.4 | 12.8 | 12.3 | 7.0 | 52.8 | 19.6 | 29.1 | 15.3 | 11.3 | 9.2 | 45.9 | 27.9 |
| 3 | | | | | | (38) | | (40) | | (43) | | (63) | | (47) | | (19) | | (39) |
| J.B. | M | 32 | 91 | 200 | 7.3 | 4.8 | 34. | 16.2 | 16.4 | 8.0 | 60.6 | 17.0 | 42. | 17.4 | 13.7 | 9.1 | 46.5 | 27.8 |
| 4 | | | | | | (34) | | (52) | | (51) | | (72) | | (59) | | (34) | | (40) |
| A.J. | M | 29 | 72 | 340 | 6.0 | 3.9 | 23.8 | 11.8 | 13.0 | 7.5 | 52. | 10.8 | 15.7 | 5.2 | 12.8 | 8.4 | 41.2 | 23.1 |
| 5 | | | | | | (35) | | (50) | | (42) | | (79) | | (67) | | (34) | | (44) |
| A.M. | M | 23 | 65 | 130 | 7.2 | 3.8 | 29.6 | 10.8 | 14.3 | 8.1 | 57. | 11.1 | 28.9 | 7.4 | 12.8 | 6.8 | 44.4 | 23.1 |
| 6 | | | | | | (47) | | (64) | | (43) | | (81) | | (74) | | (47) | | (48) |
| R.A. | F | 21 | 72 | 100 | 8.9 | 4.1 | 25.5 | 8.7 | 13.4 | 6.7 | 62.8 | 20.1 | 36.6 | 11.9 | 21.6 | 14. | 38.2 | 17.8 |
| 7 | | | | | | (54) | | (66) | | (50) | | (68) | | (68) | | (35) | | (53) |
| L.S. | F | 31 | 49 | 120 | 7.8 | 4.8 | 33.9 | 16.8 | 12.1 | 7.6 | 54.9 | 14.7 | 35.0 | 13.5 | 16.5 | 10. | 34.5 | 14.6 |
| 8 | | | | | | (39) | | (51) | | (37) | | (73) | | (61) | | (39) | | (58) |
| F.DeG. | M | 19 | 91 | 0 | 6.5 | 6.6 | 30.9 | 28.2 | 12.2 | 13.1 | 63.6 | 57.0 | 26.5 | 26.5 | 14.1 | 13.0 | 44.3 | 40.4 |
| 12 | | | | | | | | | | | | | | | | | | |
| R.L. | M | 18 | 83 | 0 | 6.5 | 6.4 | 24.3 | 24.9 | 10.0 | 11.3 | 61.8 | 59.4 | 21.2 | 24.0 | 12.4 | 12.2 | 39.6 | 38.1 |
| 13 | | | | | | | | | | | | | | | | | | |
| N.D. | F | 18 | 46 | 0 | 5.9 | 5.8 | 22.0 | 22.2 | 14.4 | 13.7 | 51.9 | 54.9 | 34.5 | 33.5 | 15.1 | 12.7 | 35.7 | 34.5 |
| 14 | | | | | | | | | | | | | | | | | | |
| R.L.‡ | M | 23 | 51 | 0 | 5.9 | 5.9 | 24.9 | 24.6 | 13.1 | 13.2 | 49.8 | 48.9 | 21.7 | 23.6 | 10.8 | 10.0 | 31.8 | 27.3 |
| 15 | | | | | | | | | | | | | | | | | | |

* B = Level before the administration of insulin.

* A = Level 3½ hours after insulin, at which time patients were in hypoglycemic coma. Controls, without insulin, show the effect of 3½ hours rest in bed.

† = Figures in parentheses indicate the depression of the level of the amino acids in per cent of the initial level.

‡ = Phenylalanine was determined in this patient and was 7.2 γ and 7.1 γ per ml before and after 3½ hours of rest, respectively.

TABLE II.

Effect of the Oral Administration of Glucose on the Level of Various Amino Acids in the Blood Plasma.

| Patient and case No. | Sex | Age yr | Wt kg | Time hr | Blood sugar mg/100 ml | Total amino acid nitrogen mg/100 ml | Arginine γ/ml | Histidine γ/ml | Leucine γ/ml | Lysine γ/ml | Phenyl-alanine γ/ml | Trypto-phane γ/ml | Valine γ/ml |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S.W. | M | 15 | 72 | 0* | 92 | 6.1 | 24.4 | 14.2 | 55.8 | 24.8 | — | 14.0 | 33.6 |
| 9 | | | | 1 | 152 | 5.3 (13) † | 20.4 (16) | 12.2 (14) | 42.0 (25) | 21.3 (14) | — | 11.5 (18) | 26.7 (21) |
| | | | | 2 | 80 | 5.1 (16) | 18.0 (26) | 11.3 (20) | 32.1 (42) | 19.8 (20) | — | 11.3 (19) | 21.8 (35) |
| | | | | 3½ | 79 | 5.1 (16) | 19.2 (21) | 12.0 (15) | 37.5 (33) | 19.1 (23) | — | 10.7 (24) | 21.1 (37) |
| J.F. | M | 20 | 67 | 0 | 90 | 6.4 | 29.4 | 12.5 | 63.8 | 30.3 | 6.5 | 12.2 | 45.2 |
| 10 | | | | 1 | 127 | 5.3 (17) | 19.5 (34) | 11.0 (12) | 48.4 (24) | 24.5 (19) | 5.6 (14) | 10.2 (16) | 33.6 (26) |
| | | | | 2 | 109 | 5.4 (16) | 17.7 (40) | 10.4 (17) | 37.8 (41) | 24.5 (19) | 5.1 (22) | 9.2 (24) | 26.7 (41) |
| | | | | 3½ | 75 | 5.3 (17) | 17.2 (41) | 11.0 (12) | 38.1 (40) | 25.2 (17) | 4.5 (31) | 10.1 (16) | 25.1 (44) |
| C.C. | F | 17 | 52 | 0 | 85 | 5.4 | 22.8 | 12.7 | 37.8 | 19.4 | 5.1 | 9.4 | 24.0 |
| 11 | | | | 1 | 138 | 4.8 (11) | 15.6 (32) | 12.2 (4) | 25.2 (33) | 16.1 (17) | 4.5 (12) | 8.5 (10) | 17.5 (27) |
| | | | | 2 | — | — | — | — | — | — | — | — | — |
| | | | | 3½ | 74 | 4.4 (19) | 13.8 (39) | 12.0 (6) | 25.6 (32) | 17.4 (10) | 5.1 (0) | 9.9 (−5) | 15.0 (38) |

* = Time interval after the administration of glucose.

† Figures in parentheses represent the depression of the amino acids in per cent of the initial level.

lower or higher than the initial postabsorptive level. After 3½ hours the blood sugar fell below the initial level while the amino acids all remained significantly depressed below the initial level and tended to show either a slight rise or fall from the level reached at the 2-hour period.

The findings in 4 control patients who did not receive insulin are shown at the bottom of Table I. After 3½ hours the levels of the amino acids in the blood of these patients either remained practically unaltered or tended to rise or fall by a few per cent from the initial postabsorptive level. The findings were in marked contrast to those observed after the administration of insulin or glucose. The controls also might be considered an added check on the methods used in this study.

*Discussion.* Our report in a previous publication[2] that there was a marked depression in the level of glutamine and total amino acids in the blood plasma of patients receiving insulin hypoglycemic shock treatment, was confirmed by Hamilton[18] in experiments on dogs. Since he found that the percentage drop of glutamine and of the total amino acids was the same he interpreted these findings as indicating that the drop in the level of glutamine was due merely to a diffusion of the amino acids from the blood into the tissues. We have suggested[19] that other interpretations of the data were possible and have discussed the possibilities in a previous publication.[2]

The findings in this study indicate that various amino acids in the blood differ in the extent to which they are affected during insulin hypoglycemic coma. Such differences are also found following the oral administration of glucose. It is apparent that some pronounced alterations in nitrogen metabolism, other than the uniform diffusion of amino acids from the blood into tissues, must occur which would account for the results obtained.

[18] Hamilton, P. B., *J. Biol. Chem.*, 1945, **158**, 397.

[19] Harris, M. M., Roth, R. T., and Harris, R. S., *J. Nerv. and Ment. Dis.*, 1945, **102**, 466.

Bouckaert and his coworkers[20] and Mirsky[21] were of the opinion, from their studies on nephrectomized, hepatectomized, and eviscerated animals, that insulin inhibits deamination by the liver and also affects the synthesis and hydrolysis of proteins in the various tissues of the body. Since the injection of insulin into eviscerated animals resulted in the disappearance of endogenous amino acids from the blood stream, Mirsky[21] favored the view that this was due to an increase in synthesis rather than a decrease in hydrolysis of proteins in the peripheral tissues. Frame and Russell[22] have recently carried out similar studies on eviscerated rats and confirmed the findings. They point out that it is not known at present whether insulin promotes synthesis of tissue proteins from blood amino acids or inhibits its breakdown.

From the standpoint of the possible mechanisms involved it is of interest to analyze the changes observed in the level of the various amino acids in the blood as a result of insulin and glucose administration. It will be observed that, with the exception of arginine, and histidine, the amino acids thus far studied fall into the group of so-called essential amino acids. These amino acids may be synthesized in the body to only a very limited extent or not at all.[8] This should simplify, somewhat, the analysis of the changes observed. The figure for total amino acid nitrogen represents both essential and nonessential amino acids in the blood plasma.

It will be seen from Table I that in the controls the level of the amino acids shows little change during the 3½ hours of observation in the postabsorptive state. Following the administration of insulin or glucose (Tables I and II) there is a significant drop, but to a variable degree, in the levels of the various amino acids.

As the controls indicate, the patients, during the 3½ hours of observation, are approximately in a state of dynamic equilibrium as far as the level of the various amino acids in the blood are concerned. That is, the amino acids supplied to the blood as a result of protein hydrolysis or amino acid synthesis is about balanced by those removed from the blood for deamination and oxidation or protein or polypeptide synthesis.

Since insulin or glucose tends to spare protein metabolism one should expect a decrease in deamination and oxidation of amino acids. This is in keeping with the findings of Mirsky[21] and Bouckaert, and his co-workers[20] in their animal experiments. However, a decrease in deamination and oxidation of amino acids should tend to raise the level of amino acids in the blood. Since an appreciable drop in the level of amino acids is observed following glucose or insulin, an inhibition of hydrolysis of tissue proteins or an increase in the synthesis of polypeptides or proteins probably occurs to account for the findings.

If, in the various patients, insulin or glucose inhibited the hydrolysis of the same type of storage protein or increased the synthesis of some polypeptide or protein of similar composition, then the ratios of the amounts of the various amino acids disappearing from the blood stream should be similar in the different patients. This tendency occurs for some of the amino acids in some of the patients. However, there are many significant deviations. Thus, for example, patient (J.B., No. 1) in Table I shows a drop of 37 $\gamma$ of leucine and 18 $\gamma$ of lysine per ml of plasma. This is a ratio of leucine to lysine of approximately 2. In patient (A.J., No. 5), leucine dropped 41 $\gamma$ and lysine only 10.5 $\gamma$ which gives a ratio of leucine to lysine of approximately 4. Thus the mechanisms which depress the level of the amino acids, thrown into action as a result of the insulin hypoglycemia, may not be identical in the 2 patients. This marked difference in the handling of essential amino acids may be highly significant.

In the case of the nonessential amino acids, arginine and histidine, it will be seen that there are marked differences in the ratios of the amounts of these amino acids which disappear from the blood plasma. Thus, for example, patient (J.M., No. 2) showed a drop

[20] Bouckaert, J. B., and de Duve, Chr., *Physiol. Rev.*, 1947, **27**, 39.

[21] Mirsky, I. A., *Am. J. Physiol.*, 1938, **124**, 569.

[22] Frame, E. G., and Russell, J. A., *Endocr.*, 1946, **39**, 420.

of 8.6 γ of arginine and 9.8 γ of histidine per ml of plasma while in patient (A.M., No. 6) 18.8 γ of arginine and 6.2 γ of histidine disappeared from the plasma. Thus, while more than twice the amount of arginine disappeared in the latter as compared with the former patient, as regards histidine the situation was reversed. Thus the extent of the relative effect of insulin hypoglycemia on arginine and histidine formation and utilization must be significantly different in these patients. The studies of previous investigators regarding the effect on the level of total amino acids in the blood failed to reveal the complexity of the metabolic processes which require consideration. General speculation as to hydrolysis and synthesis of tissue protein to account for the marked effects of insulin and glucose administration would appear to be a rather crude approach to complex metabolic processes.

In some patients the oral administration of glucose produced as marked a depression in the level of valine and arginine as that seen in insulin hypoglycemic coma. (See patient J.F., No. 10, Table II). However, the effect on the other amino acids was not comparable. This points to some special effects of insulin aside from the supply and utilization of glucose on the metabolism of some of the amino acids. This seems to be especially marked as regards leucine and lysine of the amino acids thus far investigated. In this connection it may be of interest to point out that Steele and his co-workers[23] recently have found that leucine is one of a few amino acids which is not excreted in human urine, either free or combined, except under very special conditions. This occurs in spite of the comparatively high level of leucine in the blood. These findings may point to some special or unique function of leucine in the nitrogen metabolism of man. This will be investigated further.

The possible relation of the amino acids to clinical conditions will be the subject of another paper.

*Summary.* 1. The effect of insulin hypoglycemic coma and of the oral administration of glucose on the level of various amino acids in the blood have been investigated in 15 mental patients.

2. The level in the blood of all the amino acids, thus far investigated, decreases to a variable extent during insulin hypoglycemia and also after glucose administration.

3. Insulin hypoglycemia produced a more marked effect on the level of blood amino acids than did the administration of glucose. Valine appeared to be an exception in some patients.

4. Leucine and lysine showed the most marked changes of the amino acids thus far investigated.

5. The total amino acids does not reflect the extent of the changes in the blood of the level of the individual amino acids.

6. The possible mechanisms which may account for the changes are discussed.

[23] Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., *J. Nutrition*, 1947, **33**, 209.

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## Studies on Cigarette Smoke Irritation. I. Determination of Edema-Producing Properties of Certain Types of Cigarette Tobaccos.

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We described[1] a method for the quantitative evaluation of cigarette smoke irritation, as judged by its edema-producing properties, based on gravimetric determination of the increase in moisture content of the upper

[1] Finnegan, J. K., Fordham, Doris, Larson, P. S., and Haag, H. B., *J. Pharm. and Exp. Therap.*, 1947, **89**, 115.