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Ecological and Nutritional Studies on *Coleomegilla maculata* (Coleoptera: Coccinellidae) IV. Amino Acid Requirements of the Adults Determined by the Use of C^{14}-Labeled Acetate[1]

YOUSEF H. ATALLAH AND ROSAMOND KILLEBREW

Department of Entomology and Nuclear Science Center, Louisiana State University, Baton Rouge

ABSTRACT

Fifty adult *Coleomegilla maculata* (De Geer) were fed on 250 ml of an aqueous solution of sodium acetate-1-C^{14} which contained 600 micrograms of the salt; total C^{14} was 10 microcuries. After 4 hr, the beetles were homogenized, the amino acids were extracted and separated by thin-layer chromatography, and the activity was measured, using a scintillation counter. Glycine, serine, aspartic acid, glutamic acid, proline, and lysine showed high activity, and are considered to have been synthesized in vivo. They are apparently nonessential, in contrast to threonine, phenylalanine, isoleucine, and valine, which are essential or derived exclusively from essential dietary constituents. The low activity shown by alanine, leucine, arginine, and histidine indicated a low level of C^{14} incorporation. Three unknown ninhydrin-positive compounds were isolated, in addition to the 19 amino acids that were identified.

The lady beetle *Coleomegilla maculata* (De Geer) is an important predator of several pests in Louisiana. In previous work, Atallah and Newsom (1966, unpublished) developed an artificial diet and a satisfactory rearing technique for this species.

During the course of the work on artificial diets, it became obvious that a better understanding was needed of the amino acid requirements of *C. maculata*. This study was undertaken to determine the amino acid composition of this beetle, and its biosynthesis of these acids in vivo.

Animals cannot synthesize all amino acids; some must be supplied in the diet (Albritton 1955, Hinton 1956, House 1962). Amino acid requirements of several animals have been studied by eliminating the acid under investigation from the animal's diet (House 1961). Chemically defined diets were necessary for such studies. The lack of availability of chemically defined media and the possibilities of their contamination were 2 major problems which made results from this type of work less reliable. Also, by elimination of 1 or more amino acids from the diet, the carbon-nitrogen ratio and the ratios of amino acids to each other are changed. The use of radioactive isotopes in biological research has overcome these weaknesses, and has proved to be of great advantage in studying the biosynthesis of several materials in animals (Calvin et al. 1949).

Steel (1952) reported that ingested carbohydrate should supply some carbon to all of the carbon-containing compounds of the body, with the possible exception of the essential dietary constituents and whatever other compounds are derived exclusively from them. He also stated, "However, it is theoretically possible that metabolic routes exist whereby one of the carbon atoms of an essential amino acid exchanges with a carbon atom derived ultimately from sucrose. This would be analogous to the known replaceability of the α-amino nitrogen of most of the essential amino acids (Schoenheimer 1942), and might imply the existence of a compound not normally present in the diet, which could serve as a metabolic precursor for the essential amino acid in question." Steel applied these principles in studying the essential amino acids for the mouse.

Rafelson et al. (1951) studied the effect of Theiler's G C VII mouse encephalitis virus on the uptake of C^{14} from glucose in vitro by amino acids in mouse brain. They stated that in the mouse brain incubated with uniformly labelled C^{14} glucose, radioactive peaks of roughly comparable specific activity were found to be associated with all the nonessential and essential amino acids except proline and threonine; the presence of the virus stimulated the incorporation of radioactive carbon from glucose into most of the amino acids, but inhibited the incorporation of glucose fragments into lysine and histidine. They concluded that virus propagation in this system is intimately associated with effects on lysine and histidine metabolism.

Kasting and McGinnis (1958, 1960, 1962), Kasting et al. (1962), and Schaefer (1964) applied more or less similar techniques in studying the biosynthesis of amino acids in insects. Nicholas et al. (1960) studied the incorporation of C^{14} from sodium acetate-2-C^{14} into the amino acids of the soil-inhabiting nematode

[1] Accepted for publication April 15, 1966.

Caenorhabditis briggsae and associated the incorporation of C^{14} with whether or not an amino acid is essential.

MATERIALS AND METHODS

Adult beetles were reared on an artificial diet in the laboratory under nonaseptic conditions (Atallah and Newsom 1966, unpublished). The diet contained about 7 g protein, 5 g carbohydrates, 0.6 g Wesson's salt, 2 g fats, 0.2 g antibiotics, cotton leaf extract (carotenoids and sterols), and vitamins per 100 ml of distilled water, plus 2 g of agar to solidify the medium. Adult beetles also were maintained on this diet prior to use in the experiments (the adult can live up to 1 year). The experimental animals were 15 days old.

Fifty adult beetles were starved for 12 hr (overnight), then fed on 250 μl of aqueous solution of sodium acetate-1-C^{14}, containing 600 μg of the salt. Total C^{14} was 10 μc. The solution was offered to the beetles on a cover slip; they consumed it in 1 hr, and then they were fed on the synthetic diet for 3 more hours. After this, they were homogenized in 80% ethanol, heated to 60°–65°C, and centrifuged. The supernatant was collected and the residue was extracted 2 additional times, once with 80% ethanol and once with water. The residue, which contained the proteins, was refluxed with 6 M HCl for 6 hr. The acid was removed under vacuum and the sample was redissolved in water, then filtered. The filtrate and the supernatant from the first step were combined and evaporated under vacuum to 5 ml. It is worth mentioning that tryptophan might be destroyed by the acid hydrolysis. However, if it was present as a free amino acid, it should appear in the extracts of either the 80% ethanol or water.

The thin-layer chromatography procedures described by Brenner and Niederwieser (1960), Fahmy et al. (1961), and Schaefer (1964) were largely followed. Glass plates, 20×20 cm, were coated with a layer of silica gel-G about 250μ thick, and were air-dried for 1 hr. Samples were applied in about 1 μl aliquots, and dried between applications. Two solvent systems were used: the first, *n*-butanol, acetic acid, and water (60:20:20 by weight); the second, phenol and water (75:25 by weight). Only reagent-grade solvents were used; in the case of phenol, only colorless crystals were used.

A running distance of about 16.5–17.5 cm was used for each dimension, and plates were dried overnight before running the second dimension. The developed plates were sprayed with 0.5% ninhydrin in 95% ethanol. Resulting spots were identified, then carefully removed and placed in vials.

Fifteen milliliters of scintillator fluid (3 g 2,5-diphenyloxazole, 100 mg 2,2-paraphenylene *bis* 5-phenyloxazole, and 1000 ml toluene) and 0.6 g Carb-O-Sil® were added to each vial containing 1 amino acid. The vials were placed in a Tri-Carb® liquid scintillation spectrometer operated on H. V. of 970 volts (tap 4) and with discriminators set on 10, 50, and 100 volts. The experiment was repeated twice, and triplicate analyses were run on each extraction. Amino acids extracted from untreated beetles and separated were used as checks.

RESULTS

The 19 amino acids that were separated by these procedures were glycine, valine, leucine, cystine, alanine, methionine, threonine, phenylalanine, tryptophan, lysine, aspartic acid, glutamic acid, serine, proline, histidine, tyrosine, isoleucine, arginine, and cysteine. Alanine and threonine ran as 1 elongated spot; however, the 2 poles of the spot were each represented by 1 amino acid only, as was confirmed by a pyridine system. The other 17 amino acids were well resolved.

Table 1.—Activity (cpm above background) of amino acids extracted from *C. maculata* adults fed C^{14} sodium acetate.

Compound	Checks (avg)	Replicates					
		1	2	3	4	5	6
Glycine	2	202	212	184	216	193	101
Alanine	4	39	31	46	62	55	34
Valine	3	23	23	18	20	15	22
Leucine	7	73	65	43	51	36	77
Isoleucine	1	18	20	19	11	13	17
Phenylalanine	2	8	9	7	4	7	6
Serine	5	176	179	162	145	171	112
Threonine	3	11	6	19	18	16	7
Aspartic acid	4	107	108	32	96	113	81
Glutamic acid	4	74	73	69	73	65	87
Lysine	6	90	91	91	118	98	85
Arginine	3	75	76	76	39	58	60
Histidine	2	39	40	35	8	37	29
Proline	3	1519	1522	520	971	1069	505
Unknown I	1	97	85	81	79	93	86
Unknown II	0	12	4	7	9	6	5
Unknown III	5	374	362	373	298	292	306

R_f values for the first and second solvent systems, respectively, were: .068 and 0.315 for Unknown I; 0.181 and 0.315 for Unknown II; and 0.484 and 0.250 for Unknown III.

Table 1 shows the average counts per minute for each amino acid on the "lower" scaler (10–50 volt pulses). The degree of quenching was constant.

DISCUSSION

Glycine, serine, aspartic acid, glutamic acid, lysine, proline, Unknown I, and Unknown III had high levels of C^{14}, which indicated that they are synthesized in vivo and are apparently nonessential.

Threonine, phenylalanine, isoleucine, valine, and Unknown II did not contain C^{14}. This result indicated that if they are required in the insect's metabolism, they are either essential or derived exclusively from the essential dietary constituents. Alanine, leucine, arginine, and histidine had little activity, which indicated a low level of C^{14} incorporation. The low level of C^{14} incorporation indicates a limited biosynthesis, exchange of carbon atoms between the amino acid and acetate, a fast rate of utilization of the amino acid, or that the position of C^{14} in the amino acid molecule is characterized by low activity. Lysine has always been found to be essential; however, it appears to be nonessential for *C. maculata* under nonaseptic rearing conditions. In spite of a low activity level in leucine and histidine, they are most probably essential, and the C^{14} incorporation is due to exchange of carbon atoms.

Arginine can be synthesized slowly in rats, though the amount synthesized is not sufficient for normal growth (Steel 1952), and it has been found essential for insects (House 1961). Limited biosynthesis of this compound might be the case in this predator.

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Studies on Black Flies of Panama (Diptera: Simuliidae). I. Two New Species of *Simulium* of the Subgenus *Notolepria*[1]

GORDON FIELD[2]

Preventive Medicine Division, Womack Army Hospital, Fort Bragg, North Carolina

ABSTRACT

Simulium (Notolepria) **subexiguum** is described from males and females reared from pupae, and both sexes and certain pupal structures are illustrated. *S. (N.)* **blantoni** is based on females taken in a New Jersey-type light trap, and structural details of this sex are figured. These are the first species of the subgenus *Notolepria* Enderlein reported from Panama. They bear strong generic resemblances to other species in the *exiguum*-complex, such as *S. (N.) exiguum* Roubaud, 1906; *S. (N.) incrustatum* Lutz, 1910; *S. (N.) gonzalezi* Vargas and Díaz Nájera, 1953; and to descriptions and figures of *S. (N.) exiguum* of authors, but not of Lane and Vulcano, 1943.

Prior to 1940, little was known of the simuliid fauna of the Republic of Panama or the Canal Zone. Malloch (1914) reported *Simulium quadrivittatum* Loew as occurring in Panama, as did Dunn (1934). Jennings (1915) described *S. samboni* from Empire, Canal Zone. Fairchild (1940) raised the known num-

[1] Accepted for publication March 23, 1966.

[2] Lt. Colonel, USA, MSC.