Estimation of Nitrogenous Compounds in the Feces of Boll Weevils, *Anthonomus grandis*, Fed Different Diets[1]

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ABSTRACT

The effects on the nitrogenous end products of the metabolism of the boll weevil, *Anthonomus grandis* Boheman, fed on artificial diet, cotton squares, or bolls were determined. Fecal analyses for amino nitrogen, ammonia, creatine, creatinine, guanine, urea, and uric acid showed that presence of these materials varied with the type of diet fed the insects. The purine guanine was found in relatively large quantities in the feces of weevils fed all 3 diets. A metabolic block in its deamination was hypothesized.

In an earlier paper we reported the amounts of nonprotein amino acids in the feces of the boll weevil, *Anthonomus grandis* Boheman (Mitlin et al. 1964). As a result of that investigation we became interested in determining how much of the total nitrogen could be identified and quantitated. Studies of nitrogenous end products have been made with other insects as an aid to the study of metabolism (Brown 1936; Nation and Patton 1961; Powning 1953). It was also of interest to determine whether the kind of food fed to the insect influenced the type and quantities of the nitrogenous end products, particularly since earlier work had uncovered the purine guanine as a component of this insect's feces (Mitlin and Vickers 1964). The analytical methods described in this paper were utilized because in mammals essentially 100% of the nitrogen can be so accounted for (Hedin and Schultze 1961).

BIOLOGICAL MATERIALS

The feces were collected at 24-hr intervals from laboratory-reared boll weevils of both sexes fed on diets of cotton squares (flower buds), cotton bolls (fruit), and an artificial medium modified from that of Vanderzant et al. (1959) in which acetone-extracted cotton square powder and soy bean protein were substituted for casein. The weevils were derived from a stock originally collected in Mexico. Thorough sifting of the fecal pellets through a 40-mesh sieve served to screen out any extraneous material such as plant parts. Presence of only feces was confirmed by microscopic examination. The feces were kept at 4°C until used.

ANALYTICAL PROCEDURES

Total nitrogen was determined by the Kjeldahl method, for which Sher's 2-step indicator was utilized (1955).

Amino nitrogen analyses were made according to the method of Frame et al. (1943) fecal samples were hydrolyzed with 6N hydrochloric acid in sealed ampoules in which the air had been replaced with nitrogen. After being heated at 105°–110°C for 48 hr, the ampoules were chilled in a Dry Ice®-acetone bath, opened, and the residue filtered through a sintered glass funnel of medium porosity. Determinations were made on measured aliquots after adjusting the hydrolysate to pH 9.2 and aerating for the removal of ammonia. At least 6 analyses were run and the results corrected for uric acid.

Ammonia was determined by a modified procedure of Van Slyke and Cullen (1914) from aqueous extracts of finely ground feces. Creatine and creatinine were determined from similar extracts by the method of Folin (1914), as detailed by Hawk et al. (1954). Measurements were made spectrophotometrically on a Beckman® DU spectrophotometer. At least 6 determinations were made for each material.

Guanine, first reported in boll weevil excreta by Mitlin and Vickers (1964), was extracted by the method therein reported and quantitatively determined by the use of 2-dimensional paper chromatography and spectrophotometry. *Sec*-butanol saturated with water (Fink et al. 1963) and adjusted to pH 10 was used as a first-dimension solvent and 65% isopropanol-2N HCl (Wyatt 1951) as the second. Spots were located under short-wave ultraviolet light, and eluted with 0.1 N hydrochloric acid. Absorption of the eluates was read on a Backman® DK2A spectrophotometer and quantitative determinations were made by employing differential extinction values (Vischer and Chargaff 1948). At least 4 determinations were made for each type of feces.

For urea a measured quantity of feces finely ground in a hammer mill was extracted at least 3 times with ethanol (to eliminate interference from protein). The ethanol was then evaporated and the residue taken up in a measured quantity of water. Original identification was made by paper chromatography in 65% isopropanol:2 N HCl (Wyatt 1951) with Ehrlich's reagent utilized as a detection agent (Smith 1960). Quantitation was achieved by Archibald's method (1945). Although this method was shown by Halvorsen and Schultze (1950) to give higher results than the urease method of Van Slyke and Cullen (1914), with us it gave decidedly more consistent results than the urease method.

The method of Benedict and Franke (1922) was used to analyze for uric acid. Samples of finely ground feces were leached with about 100 ml of 0.05 sodium hydroxide. These samples were made up to 500 ml and suitable aliquots taken for analysis. At least 4 extractions were made for each fecal sample and each analysis was replicated at least 8 times.

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Examinations were made for other nitrogen-containing compounds. These included the purines adenine, xanthine, and hypoxanthine; the pyrimidines cytosine and uracil; and the carboxamide allantoin. Paper chromatographic systems included: butanol: formic acid:water (120:30:50), ethanol:acetic acid: water (85:5:10), and 65% isopropanol: 2 N HCl.

RESULTS AND DISCUSSION

The results of the analyses are shown in Table 1. Feces derived from square-fed weevils contained nitrogen far in excess of those derived from boll-fed or artificially-fed weevils. The percentages of amino nitrogen were essentially the same in the feces of boll-fed and artificially-fed weevils, but were significantly less in those of square-fed insects. Creatine and creatinine were found in the highest percentages in the feces of boll-fed weevils, whereas the differences between the insects fed the other diets were borderline. Parenthetically, there is some doubt that creatine occurs in insect excreta (Patton 1963). Therefore the identification of the compounds should be accepted with some reservation. With all 3 diets, ammonia and urea appeared as minor constituents of the feces, and although the amounts of each differed statistically, they contributed relatively little to the total amounts of nitrogen. The purines, uric acid and guanine, accounted for the greatest percentages of defecated nitrogen in all 3 diet groups.

We hesitate to attach any biological significance to differences in total nitrogen. Analyses based solely on weights of feces tend to be misleading, since fecal material may be made up of materials which do not reflect metabolic activity. For instance, it was shown earlier (Mitlin et al. 1964) that about 11% of the feces from artificially-fed weevils was paraffin (the coating of the artificial diet).

The greater part of the total nitrogen could be accounted for in the feces derived from boll-fed weevils only. We can speculate that the unidentified nitrogen in feces from the other 2 groups of weevils might be accounted for by undigested cotton plant pigments such as gossyfulvin and gossypurpurin. Some might be endogenous, possibly derived from pteridines. Craig (1960) pointed out that these compounds are present in the excreta of many insects.

The differences found seemed to form no pattern. It was difficult to relate the amounts of these end products to the type of diet fed the weevil. The artificial diet is not yet completely defined and the literature reveals no complete analysis of the nitrogenous constituents of bolls and squares, although Burks and Earle (unpublished data) determined the free amino acids of the artificial diet. Although most of the nitrogenous products are results of metabolic activity, some of those we observed were due to undigested food passing through the gut, particularly in the feces of boll- and square-fed weevils. In these feces microscopic examination showed what appeared to be partially digested food, such as fragmented pollen grains and other plant parts. In contrast, feces of insects fed the artificial diet were all of uniform texture, size, and color and may have more nearly represented true metabolic products. This diet was a semiliquid, completely homogeneous mixture, so the possibility of absorption through the gut was greater. In all instances the results were valid insofar as they represented actual elimination products.

Part of the amino nitrogen in the feces from weevils fed an artificial diet was previously identified. Mitlin et al. (1964) showed that free and bound nonprotein amino acids accounted for 3.23% of the nitrogen. Presumably a great part of the remainder may be ascribed to protein.

Particularly striking were the large percentages of guanine (Table 1). This purine was second only to uric acid in amount for a single compound. As was reported earlier (Mitlin and Vickers 1964) such a condition appears to be unique in this insect. It is possible, however, that investigation may uncover its presence in the feces of other species.

Some metabolic block in the deamination of guanine is indicated (such as a deficiency in the enzyme guanase), as well as some other pathway of uric acid synthesis in addition to nucleic acid catabolism. The pathway may be similar to that of the American cockroach, *Periplaneta americana* (L.) (McEnroe and Forgash 1957), and to the pigeon as Heller and Jezewska (1959) concluded from their work with *Antheraea perniyi*.

One point must be made. In any series of analyses of the excreta of insects that have been reared by

Table 1.—Amounts of nitrogenous components of the feces of the boll weevil fed on the indicated diet per gram of feces ± standard error, and percent of nitrogen each constitutes.

Component	Artificial		Bolls		Squares	
	mg N	%	mg N	%	mg N	%
Amino nitrogen[a]	13.50±.77	45.06	13.71±.62	41.04	11.61±.32	25.57
Ammonia	.45±.01	1.50	.16±.03	.48	.17±.01	.37
Creatine	.91±.01	3.04	2.48±.05	7.42	1.75±.03	3.85
Creatinine	1.08±.05	3.60	1.81±.04	5.41	.90±.03	1.98
Guanine	2.25±.07	7.51	3.78±.20	11.31	5.83±.13	12.84
Urea	.01±.002	.03	.06±.01	.18	.11±.05	.24
Uric acid	7.29±.01	24.33	9.51±.05	28.46	11.84±.04	26.07
Totals	25.49	85.07	31.51	94.30	32.21	70.92
Total nitrogen	29.96		33.41		45.42	

[a] Corrected for uric acid.

other than aseptic methods the results are, of necessity, biased by end products of the microflora of the gut. The results of this study are no exception. This bias will continue to exist until the problems of maintaining adult boll weevil cultures aseptically are overcome.

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REFERENCES CITED

Archibald, R. M. 1945. Colorimetric determination of urea. J. Biol. Chem. 157: 507–8.

Benedict, S., and **E. Franke.** 1922. A method for direct determination of uric acid in urea. J. Biol. Chem. 52: 387–91.

Brown, A. W. A. 1936. Studies on the excreta of a grasshopper (*Melanoplus bivittatus* Say). Biochem. J. 14: 87–94.

Craig, R. 1960. The physiology of excretion in the insect. Ann. Rev. Entomol. 5: 53–68.

Fink, K., R. E. Cline, and **R. M. Fink.** 1963. Paper chromatography of several classes of compounds; correlated R_f values in a variety of solvent systems. Analyt. Chem. 35: 389–98.

Folin, O. 1914. On the determination of creatinine and creatine in urine. J. Biol. Chem. 17: 469–73.

Frame, E., J. A. Russell, and **A. E. Wilhelmi.** 1943. The colorimetric determinations of amino nitrogen in the blood. J. Biol. Chem. 149: 255–70.

Halvorsen, H. O., and **M. O. Schultze.** 1950. The colorimetric determinations of urea in the blood of normal and uremic rats. J. Biol. Chem. 186: 471–6.

Hawk, P. B., B. L. Oser, and **W. H. Summerson.** 1954. Practical Physiological Chemistry. McGraw-Hill Book Co. New York. 1439 p.

Hedin, P. A., and **M. P. Schultze.** 1961. Amino acids in rats. J. Nutr. 74: 131–8.

Heller, J., and **M. M. Jezewska.** 1959. The synthesis of uric acid in the Chinese tussur moth (*Antheraea pernyi*). Bull. L. Polon. Acad. Sci., Cl. II, –7: 1–4.

McEnroe, W., and **A. Forgash.** 1957. The in-vivo incorporation of C^{14} formate in the ureide groups of uric acid in the American cockroach, *Periplaneta americana* L. Ann. Entomol. Soc. Amer. 50: 429–31.

Mitlin, N., and **D. H. Vickers.** 1964. Guanine in the excreta of the boll weevil, *Anthonomus grandis* Boheman. Nature (in press).

Mitlin, N., D. H. Vickers, and **P. A. Hedin.** 1964. End products of metabolism in the boll weevil, *Anthonomus grandis* Boheman: Nonprotein amino acids in the feces. J. Insect Physiol. 10: 393–7.

Nation, J. L., and **R. L. Patton.** 1961. A study of nitrogen excretion in insects. J. Insect Physiol. 6: 299–308.

Patton, R. L. 1963. Introductory Insect Physiology. W. B. Saunders Co. Philadelphia and London: 246 p.

Powning, R. F. 1953. Studies on the digestion of wool in insects. VIII. Significance of certain excretory products of the clothes moth, *Tineola bisselliella,* and the carpet beetle, *Attagenus piceus.* Australian J. Biol. Sci. 6: 109–17.

Sher, I. H. 1955. Two-step mixed indicator for Kjeldahl nitrogen titration. Analyt. Chem. 27: 831–2.

Smith, I. 1960. Chromatographic and Electrophoretic Techniques. Vol. I. Wm. Heinemann Medical Books, Ltd. London. 617 p.

Vanderzant, E., C. D. Richardson, and **T. B. Davich.** 1959. Feeding and oviposition by the boll weevil on artificial diets. J. Econ. Entomol. 52: 1138–43.

Van Slyke, D. D., and **G. E. Cullen.** 1914. A permanent preparation of urease and its use in the determination of urea. J. Biol. Chem. 14: 211–25.

Vischer, E., and **E. Chargaff.** 1948. The separation and quantitative estimation of purines and pyrimidines in minute amounts. J. Biol. Chem. 176: 703–14.

Wyatt, G. R. 1951. Recognition and estimation of 5-methylcytosines in nucleic acids. Biochem. J. 48: 581–4.

The Taxonomic Significance of the Spider Trochanter[1]

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ABSTRACT

The following parts of the trochanter were named for this study: the circulus, a sclerotized ring; the setal carina, a thickened rim bearing a row of stout setae at the distal edge of the circulus; the cuneus, a ventral, wedge-shaped, glabrous and lightly sclerotized part of the circulus; the cuneal disk, a circular, swollen, membranous area of the cuneus which is characteristic of the family Pholcidae; and the limulus, the heavily sclerotized, raised border of the cuneus which is the "notched trochanter" of many authors. The distal border of the limulus differs in shape among spiders. In most of them (22 out of 39 families, and part of the genera of 7 others) it is rounded to broadly truncate. It is deeply notched in the Pisauridae, Lycosidae, and Heteropodidae; less so in the Oxyopidae, Zoropsidae, and Homalonychidae; and much less in the Zodariidae. The notch occurs in 1 or more genera of Linyphidae, Clubionidae, Thomisidae, Agelenidae, Argiopidae, and Dictynidae. Although Petrunkevitch, in writing of the Salticidae, referred to "notched trochanters," no limuli were found notched in this family. Notched limuli occur occasionally in the Agelenidae, on the fourth or the third and fourth pairs, but never on all 4 pairs. As the genus *Barrisca* Chamberlin and Ivie has all limuli deeply notched and the tarsal trichobothria irregularly placed, this necessitates its placement in the subfamily Rhoicininae of the Pisauridae.

This investigation was stimulated by a need to determine the taxonomic significance of the notched trochanter in spiders, and more specifically in the family Agelenidae. The need arose when I attempted to determine the familial affiliation of several South American spiders related to the family Agelenidae.

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A search of the literature indicated that this segment of the spider leg has been ignored almost totally by previous taxonomists. A review of the spider leg discussed in the following general works by Comstock

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