GUANINE, THE PRINCIPAL NITROGENOUS COMPONENT OF THE EXCREMENTS OF CERTAIN SPIDERS*

by

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Shortly after the discovery of guanine by UNGER[1], GORUP-BESANEZ AND WILL[2] found in 1849 that spider excrements contained considerable amounts of a purine base which they considered as identical with guanine on the basis of qualitative tests. These findings, which were confirmed in several other laboratories (for references of the older literature, see[3]) were interpreted by FÜRTH[3] in 1903 as evidence supporting the concept that guanine represents the main end product of nitrogen metabolism in spiders. The only recent investigation of this question is a paper by VAIROPALLA[4] who identified guanine in the excrements of the spider *Epeira diademata* by means of the specific enzymic method of SCHMIDT[5]. According to his determinations, this purine base accounted for up to 12% of the dry weight of the excrements of this species. In view of the sensitive methods now available for purine analysis, a reinvestigation of the problem was desirable, not only because of its obvious importance in comparative biochemistry, but also because of the possibility that the elucidation of the biosynthesis of guanine might be facilitated by studies of an organism which is especially adapted to the formation of this amino purine.

EXPERIMENTAL

44 mg of air-dry excrements were collected within two days from 10 specimens of silk spiders (*Nephila claviceps*) (purchased from the Carolina Biological Supply Company, Elon College, North Carolina). The powdered material was suspended in 2 ml of water and dissolved by adding a few drops of an *N* sodium hydroxide solution. On neutralization of the solution with *N* sulfuric acid, a copious amorphous precipitate appeared immediately. It dissolved on further dropwise addition of *N* sulfuric acid except for a small amount of insoluble material. The suspension was brought to a volume of 10 ml, and an aliquot of 3 ml was pipetted off under shaking, and set aside for the determination of the total nitrogen. The insoluble material was centrifuged off and a 4 ml aliquot of the clear supernatant fluid was pipetted into a conical centrifuge tube. The purines of the solution were precipitated by addition of 2 ml of *N* silver nitrate solution. The copious precipitate was centrifuged after standing in the refrigerator overnight, washed five times with water, and decomposed with 5 ml of *N* hydrochloric acid on a boiling

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water bath. The suspension was brought to a volume of 25 ml and centrifuged. The supernatant solution was used for the chromatographic and spectrophotometric analyses.

A paper chromatogram prepared according to VISCHER AND CHARGAFF[6] showed only one UV-absorbing spot whose R_F value was identical with that obtained with a solution of guanine chloride.

The absorption spectra of the supernatant solution in 0.1 *N* hydrochloric acid and in 0.1 *N* sodium hydroxide were practically identical with those of guanine. Table I shows some quotients of extinction coefficients obtained in 0.1 *N* sodium hydroxide for certain pairs of wave lengths with the purine fraction of the spider excrements and with solutions of guanine, hypoxanthine, xanthine and uric acid. It can be seen that none of the three latter compounds could have been present in more than negligible amounts. The absence of adenine can be shown by the application of the binary equations of LORING *et al.*[7] for mixtures of adenine and guanine to the quotients of the extinctions at 240, 262 and 280, in 0.1 *N* hydrochloric acid. The values of these extinctions are reported below.

TABLE I

SPECTROPHOTOMETRIC COMPARISON OF THE PURINE FRACTION OF SPIDER EXCREMENTS WITH KNOWN PURINES

Quotients of molar extinctions	*Spider fraction*	*Guanine*	*Xanthine*	*Uric acid*	*Hypoxanthine*
E_{240}/E_{270} (0.1 *N* NaOH)	0.70	0.69	0.85	1.75	0.53
E_{270}/E_{290} (0.1 *N* NaOH)	2.29	2.28	0.83	0.31	< 25

The amount of guanine was calculated from the UV absorption spectrum of a solution of the purine fraction of the excrements. The molar extinction coefficients of guanine used for the calculation were obtained from the spectrum of a solution of twice recrystallized guanine chloride. Both spectra were recorded on the same spectrophotometer on the same morning. The amounts of guanine nitrogen in 44 mg of excrement, calculated from the extinctions at 240, (e = 0.380), 252 (e = 0.440), 262 (e = 0.310), 276 (e = 0.275), and 280 (e = 0.268) mμ were 8.82, 8.79, 8.75 and 8.70 mg respectively (average 8.77 mg). The purine fraction was dissolved in 1250 ml for the spectrophotometric readings. The amount of total nitrogen in 44 mg of the excrements was 9.9 mg. The guanine accounted for 88.8% of the nitrogen of the excrements.

The concentration of guanine in the spider excrements is so high that 6 mg of pure guanine could be isolated from 18 mg of excrements by neutralization of the extract and by two reprecipitations of the washed precipitate.

Determinations of the total nitrogen and of guanine on excrements of the garden spider (*Argiope aurantia*) gave results very similar to those obtained with silk spiders.

SUMMARY

Guanine accounts for more than 85% of the total nitrogen of the excrements of two species of spiders.

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RÉSUMÉ

Plus du 85 % de l'azote total des excréments de deux espèces d'araignées se trouve sous forme de guanine.

ZUSAMMENFASSUNG

Mehr als 85 % des Gesamtstickstoffes der Exkremente von zwei Spinnenarten findet sich als Guanin vor.

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