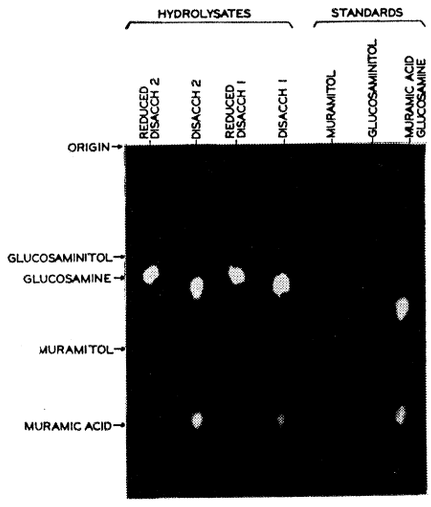


Fig. 2. Chromatogram of hydrolyzed disaccharides (40 mμmole) and reduced disaccharides (40 mμmole) in a mixture of butanol, acetic acid, and water (3:1:1); the products were detected with ninhydrin. Glucosaminitol ($R_{glucosamine}$ 0.9) and muramitol ($R_{glucosamine}$ 1.55) gave faint spots, invisible in the photograph.

No further liberation of reducing groups occurred during the remainder of the incubation, and no peptide bonds were hydrolyzed (measured as increase of free amino groups reactive with dinitrofluorobenzene). The products of lysis were separated by filtration with Sephadex G-50 and by paper electrophoresis (*3*) into two fractions: the glycopeptide (140 mg), and a teichoic acid-glycopeptide complex (124 mg). These products yielded analyses identical with those of fractions obtained from cell walls of *Staphylococcus aureus* by the action of the "32 enzyme" and similar fractionation (*3, 4*).

The liberated reducing group was shown to be muramic acid by reduction with $NaBH_4$ by two methods. Glycopeptide and a reduced sample of glycopeptide were subjected to acid hydrolysis, and the amount of amino sugars present was determined colorimetrically (*4*). The glucosamine content was unchanged by reduction, but more than 95 percent of the muramic acid disappeared. Two more portions were subjected to two-dimensional paper chromatography in (i) a mixture of pyridine and water (4 : 1) and in (ii) a mixture of *n*-butanol, acetic acid, and water (3 : 1 : 1). Glucosamine and muramic acid (detected with ninhydrin) were present in the glycopeptide in about equal amounts, but only glucosamine was found in the hydrolyzate of the reduced glycopeptide. The glycopeptide was further treated with acetylmuramyl-L-alanine amidase, and then chromatographed on carboxymethyl cellulose. A disaccharide fraction (which came through in the water eluate and which represented 75 percent of the theoretical yield from the original cell wall) was separated from the polypeptide which was absorbed onto the column. The disaccharide fraction was then separated into disaccharide No. 1 and *O*-acetylated disaccharide No. 2 by paper chromatography. After purification of the eluted disaccharides by chromatography on Sephadex, a sample of each was reduced with $NaBH_4$, hydrolyzed, and subjected to paper chromatography in a mixture of butanol, acetic acid, and water (3 : 1 : 1). The products were detected with ninhydrin. In both cases the muramic acid was totally reduced while the glucosamine was unchanged, indicating that muramic acid was the reducing end group (Fig. 2).

About 40 percent of the muramic acid residues in the isolated cell wall of *S. aureus* strain Copenhagen are *N*-acetylmuramic acid residues; the remainder are *N,O*-diacetylmuramic acid residues (*4*). Since all the aldehyde groups of muramic acid can be reduced with $NaBH_4$ after hydrolysis of cell walls with the B enzyme from *Chalaropsis*, it is apparent that, like the "32 enzyme" and unlike egg-white lysozyme whose lytic action is inhibited by the presence of *O*-acetyl groups (*6*), the B enzyme is an acetylmuramidase which can catalyze the hydrolysis of linkages of *N,O*-diacetylmuramic acid as well as those of *N*-acetylmuramic acid.

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25 August 1964

## Uric Acid in the Reproductive System of Males of the Cockroach Blattella germanica

Abstract. *Uric acid is stored in the utriculi majores of the accessory sex glands of the German cockroach. Most of the uric acid is eliminated during copulation by being poured over the spermatophore. Mating appears to be an important means of excretion in this cockroach.*

The spermatophore of *Blattella germanica* (Linnaeus) is formed from a mixture of secretions (which give positive protein reactions) from three distinct groups of accessory gland tubules. A ventral group of four to six tubules contains a "milky secretion." A dorsal group of tubules produces a transparent, water-soluble secretion, and a third group, located between the other two, secretes a translucent material. Three secretions make up the body of the spermatophore. A clear transparent material covers the ventral surface; a milky white mass contains the two sperm sacs; and a translucent lamellated mass forms the dorsal wall of the spermatophore and is in close contact with the female sclerites after copulation. Masses of a "milky granular secretion" are scattered over the spermatophore and adjacent sclerites of the female (*1*). A chalk-white secretion hardens over the spermatophore (*2*), and when a mating pair has separated the white material sometimes adheres to the subgenital plate of the male; this usually occurs when males mate after they have been isolated from females for more than a week. The material hardens on drying and flakes off when the male rubs his terminal segments with his hind legs. The white compound which adheres to the male is the same as that which covers the spermatophore in the female, and it is the milky secretion of the ventral accessory sex glands mentioned by Khalifa (*1*). Brehm (*3*) called these glands the "utriculi majores."

In *B. germanica*, the utriculi majores of the recently emerged male contain little or no secretion but become filled with white granules in about 1 or 2 days. When the male does not have access to females, these glands become greatly enlarged and fill up a large part of the abdominal cavity. When a male with such distended glands mates, the glands empty almost completely, and

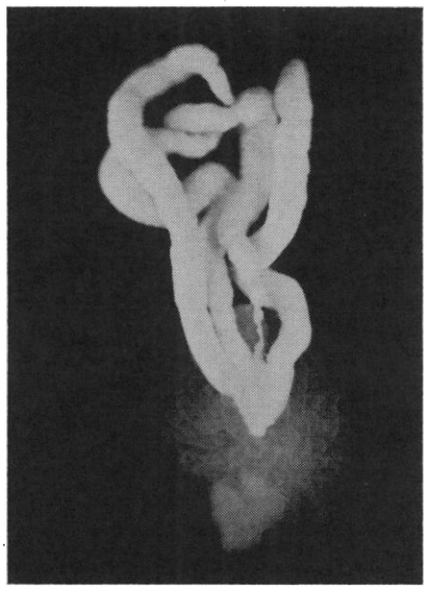

Fig. 1. Male accessory sex glands of *Blattella germanica*. The utriculi majores are filled with white uric acid (× 15).

the excess material flows out over the spermatophore (see *2*, Figs. 12 to 16).

This note reports the identification of the white compound present in the male's utriculi majores (Fig. 1). In *B. germanica* the utriculi majores contain spherical granules with radial striations, variable in diameter. A sample taken from the tubules was dried over silica gel. The infrared spectrum of the material in a potassium bromide disc was essentially that of the uric acid. The ultraviolet absorption spectrum was similar to that of uric acid ($\lambda_{max}$ in 0.1*N* NaOH, 296 m$\mu$; $\lambda_{max}$ in 0.1*N* HCl, 230 and 287 m$\mu$). The water washed contents of the gland assayed (ultraviolet) 89 percent uric acid (dry wt.) Paper chromatography in three systems (*4*) confirmed the presence of uric acid. Identification was completed by isolation of the uric acid as follows: Pulverized, dried glands from several males were extracted with two portions of 0.05*N* LiOH and centrifuged, and the supernatant was acidified with 5*N* $H_2SO_4$. The precipitated uric acid was washed with water and dried over silica gel. The ultraviolet spectrum in 0.01*N* NaOH showed maximum absorption at 296 m$\mu$ (log $\epsilon$ 4.09). Its infrared spectrum (Fig. 2) was identical with that of authentic uric acid.

According to Khalifa (*1*), during the first stage in the formation of the spermatophore while the pair is *in copula*, some of the "milky substance" (that is, uric acid) secreted by the utriculi majores flows into the ejaculatory pouch and is then surrounded by secretion from the other two groups of tubules. Roth and Willis (*2*) could attribute no function to the chalky material and concluded that, when extruded at the end of copulation, it did not form an intimate part of the spermatophore. The spermatophores of *B. germanica* are sometimes formed without a coating of uric acid (*2*); this probably occurs if males mate frequently. Four spermatophores of *B. germanica* taken from females just after copulating were examined. The uric acid adhering to the outer surface of the spermatophores was removed before testing them chemically, and no uric acid was detectable. Also, spermatophores were removed from the ejaculatory pouches of seven males that had been separated from females while *in copula* and therefore had no uric acid adhering to their outer surfaces. These specimens were ground with 0.4 ml of 0.01*N* LiOH, and 0.4 ml of 6 percent $HClO_4$ was added to precipitate protein. A 0.5-ml sample of the supernatant was diluted with 0.25 ml of $H_2O$ and 2 percent $HClO_4$ to 5 ml, and uric acid was determined from the absorption at 284 m$\mu$. No uric acid was detected *inside* these spermatophores. If uric acid is actually introduced into the spermatophore, it amounts to less than 1 $\mu$g per sperm capsule.

The uric acid content of extracts of whole bodies of *B. germanica* (developmental stage and sex not indicated) is reported to be 15 mg/g live weight (*5*). We estimated the amount of uric acid in the utriculi majores only as follows: Live weights of five males were determined individually, and their glands were removed and dried over silica gel. The glands were pooled and pulverized, and a weighed portion was dissolved in 25 ml of 0.01*N* NaOH. The uric acid was estimated by measurement of the absorption at 296 m$\mu$. Average weight of the males was 51.5 ± 1.3 mg, and the average dry weight of the gland was 0.8 ± 0.05 mg. The uric acid amounted to 820 mg/g of gland (dry wt.) or 13 mg/g of male (live wt.).

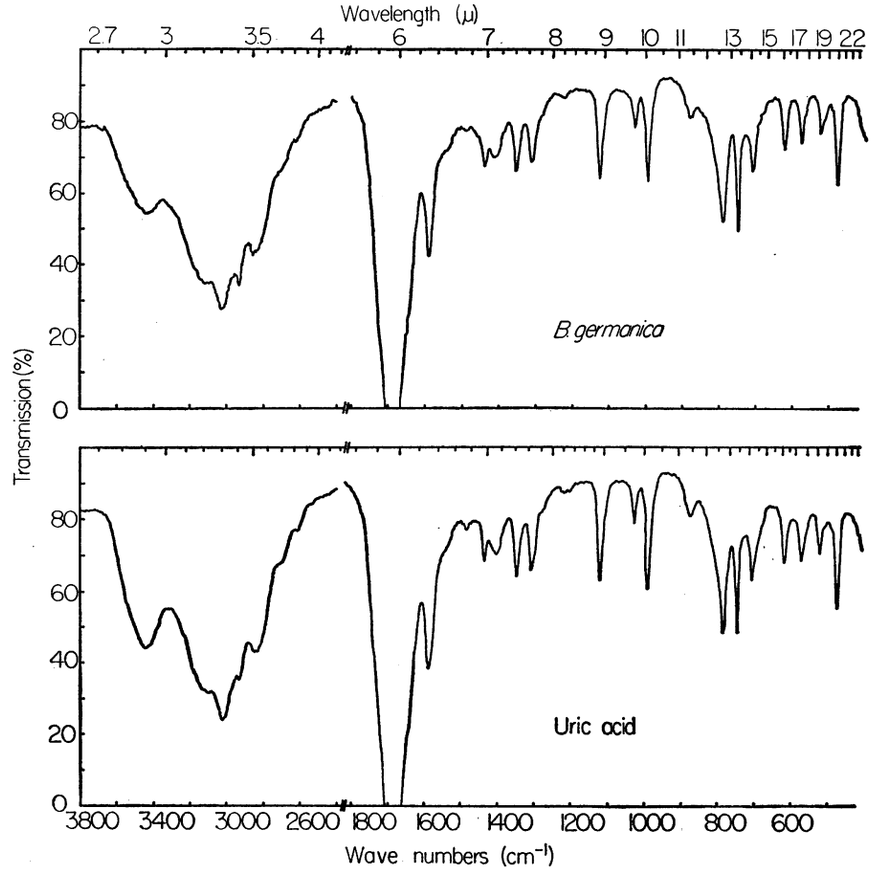


Fig. 2. Comparison of infrared spectrum of uric acid from the utriculi majores of *B. germanica* with that of authentic uric acid.

Although the utriculi majores of the males were filled with uric acid, they were not stretched to their fullest extent. The amount of uric acid in the gland depends on the frequency of mating, since these glands are virtually emptied during copulation.

Mating appears to be an important means of excreting uric acid in males of *B. germanica*. However, not all male cockroaches possess these uricose (from uric + -ose, full of) glands. Only males of 7 of 39 species examined had uric acid in their accessory sex glands (*6*).

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4. System 1: 3 percent $NH_4Cl$ (aq.). System 2: *n*-propanol and 1 percent $NH_3$ (aq.) (2 : 1). System 3: *n*-butanol and 5*N* acetic acid (2 : 1) [W. Pfleiderer, in *Ciba Foundation Symposium on the Chemistry and Biology of Purines*, G. E. W. Wolstenholme and C. M. O'Connor, Eds. (Little, Brown, Boston, 1957), p. 77]. When the chromatograms are examined under ultraviolet light (2570 Å), the filter paper fluoresces, and uric acid, if present, absorbs light and appears as a dark spot. Uric acid spots were also made visible by (i) spraying with 0.25 percent mercuric acetate in 95 percent ethyl alcohol with addition of a few drops of glacial acetic acid, (ii) drying in air, and (iii) applying a solution of 0.05 percent Diphenylcarbazone in 95 percent ethanol [F. Bergmann and S. Dikstein, *Methods Biochem. Analy.* **6**, 79 (1958)]. As little as 1 μg was detectable.
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28 August 1964

## Reptilian Thermoregulation: Evaluation of Field Studies

Abstract. *Inanimate objects may exhibit distributions of temperature similar to those of reptiles in the field. The uncontrolled field methods of collecting body temperatures of reptiles have resulted in the accumulation of much inconclusive data. A return to more comprehensive study is called for.*

In 1944 Cowles and Bogert showed that many reptiles regulate their body temperature by behavioral means (*1*). This demonstration stimulated both the accumulation of records of body temperature from many reptiles and discussion of the interpretation of the records. Since that time the method of Cowles and Bogert has been progressively simplified (*2*).

Many workers have discarded the several categories of thermal responses proposed by Cowles and Bogert in favor of determining the body temperatures of reptiles surprised in the field. These records are often presented without interpretation and only to indicate the body temperatures that occur during the normal life of an animal. A problem arises when the field records are used to try to elucidate thermoregulatory mechanisms when no true regulation has been observed.

The method now in use by many workers is quite simple. Reptiles are shot, noosed, or run down, and their body temperatures are recorded with a small, sensitive thermometer. The air temperature is usually taken, but there is no standardization in the way this is done. In some cases body temperatures below an arbitrary level are ignored because they lie in the so-called "basking range" of the animal. The activity of the animal prior to measurement is rarely known, but we are usually assured that temperature regulation is occurring by reference to the work of Cowles and Bogert. Concern about this method led to the development of a simple demonstration that data on reptile temperatures must be collected and interpreted with great care.

In order to determine whether the distribution of temperatures of inanimate objects would be distinguishable from those obtained from reptiles under similar conditions, I substituted water-filled metal cans for reptiles. Thirteen cylindrical metal containers (beer cans), 11.5 by 6.8 cm in diameter, with colored patterns on the outside, were filled with tap water at air temperature. Eleven were placed so as to receive direct sunlight throughout a full day. Two were kept in full shade. Temperatures 1 cm from the top of the can were recorded hourly from 1030 until 1830 P.S.T. with a hypodermic thermistor probe 0.3 mm in diameter. Accurate temperatures can be recorded in 0.2 second with this small probe. Air temperature next to the cans was recorded with a shaded probe. The recording process could not substantially alter the temperature of the can. Evaporation was too low to change the can's temperature measurably. It was sunny throughout the day.

Cans in the shade remained within 1°C of the air temperature. The distribution of temperature recorded from cans in direct sunlight is remarkably

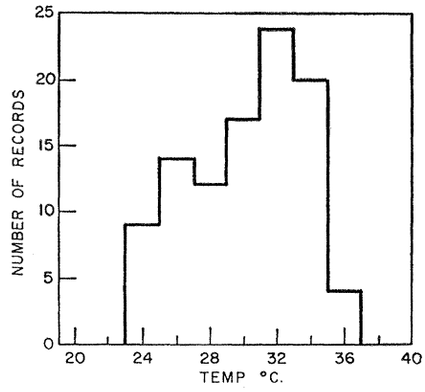


Fig. 1. Temperature distribution of cans in direct sunlight (July 1963). The skew reflects both the skew in ambient temperature and the relatively constant radiative heat input from the sun through the middle of the day (mean 30.3°C; standard deviation 3.14; $N = 97$).

like that of a heliothermic reptile (Fig. 1). A sharp break, in this case at 35°C, is typical of records obtained from reptiles. The break has been interpreted to mean that an animal actively seeks shade at that temperature. In view of the data obtained from cans, such an interpretation would need to be justified.

Arranging the data into a scatter diagram is informative (Fig. 2). The records were treated two ways. The lower diagram shows all the points obtained. Can temperature is loosely correlated with air temperature ($r = +.41$;

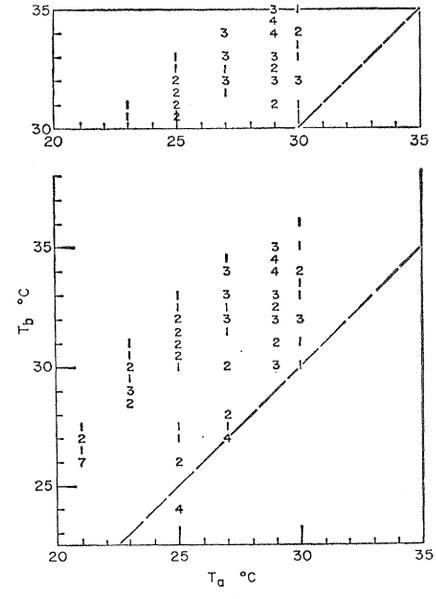


Fig. 2. Scatter diagram showing the relation of can temperature ($T_b$) to air temperature ($T_a$) in intervals of 0.5°C. In the upper graph all can temperatures below 30°C are eliminated. Dashed line, isothermal line; numerals, number of records at each interval.