RNA, PROTEIN AND URIC ACID CONTENT OF BODY TISSUES OF PERIPLANETA AMERICANA (L.) AS INFLUENCED BY CORPORA ALLATA DURING OVARIAN DEVELOPMENT

K. K. THOMAS[1] AND J. L. NATION

Department of Zoology, University of Florida, Gainesville, Florida

Thomas and Nation (1966) showed that allatectomy results in a decrease in total haemolymph protein concentration and, more specifically, in decrease of one particular kind of protein in *Periplaneta americana* (L.) females.

L'Hélias (1953a, 1953b) observed that the corpus allatum hormone favors synthesis of proteins and RNA in *Carausius morosus*. Vanderberg (1963) more recently confirmed a positive correlation between the allatum hormone and active synthesis of RNA and protein in *Rhodnius prolixus* Stål.

These results prompted the more general study of the effects of allatectomy in *P. americana* females on total body protein, total body RNA, and of the ability of certain tissues to incorporate H^3-labelled amino acids. A determination of total body uric acid was also made following allatectomy since uric acid is one end-product of protein catabolism.

Materials and Methods

Periplaneta americana females used in this study were maintained in the laboratory on dog biscuits and water. Allatectomy was performed according to Bodenstein (1953) no later than 48 hours after imaginal molt. Sham-operated insects served as controls. Post-operative maintenance of insects has been described (Thomas and Nation, 1966).

Preparation of samples

Body proteins, uric acid and RNA were determined on tissue homogenates of roaches from which haemolymph had been collected by centrifugation. The wings, legs, and alimentary canal of the roaches were removed, and the weighed insects were ground with sand and 10.0 ml. of ice-cold distilled water in a chilled mortar. The homogenate was filtered through cheesecloth and the filtrate used for protein, RNA and uric acid determinations.

The filtered homogenate was unsuitable for direct colorimetric determinations; hence the following procedure was employed to remove the undesirable contaminants that interfered with the determinations. One milliliter of the above filtrate was diluted to 10.0 ml. with water, and, with thorough mixing, 2.0 ml. each of cold 10% sodium tungstate and $\frac{2}{3}$ N H_2SO_4 were added and the mixture kept in an ice bath for about an hour to facilitate complete precipitation of proteins. The mixture was

[1] Present address: Department of Biological Sciences, Northwestern University, Evanston, Illinois.

centrifuged in a Servall refrigerated centrifuge at 5900 *g* for 15 minutes. The clear supernatant containing the uric acid was stored for uric acid determinations. The precipitate was redissolved in 1.0 ml. of cold 10% sodium tungstate and diluted to 10.0 ml. with cold water. Protein was again precipitated by adding 1.0 ml. of $\frac{2}{3}$ N H_2SO_4. The mixture was centrifuged again after 15 minutes. The precipitate was dissolved and the precipitation procedure repeated once more to bring all uric acid from the precipitate into solution. The precipitate was finally defatted by suspending it in a solvent mixture of cold 95% ethanol, chloroform, and ether (1:2:2). The suspension was centrifuged, the protein pellet resuspended, and the process repeated once more. The defatted protein pellet was brought into solution by adding a few drops of 1.0 N NaOH solution and the volume made up to 10.0 ml. with cold water. A sample of 0.5 ml. was taken for protein determination. Protein was determined according to the method of Lowry *et al.* (1951). No uric acid could be detected in a 2.0-ml. sample with the uric acid reagent of Benedict (1922).

The supernatants left after each protein precipitation procedure were pooled, and a sample of 0.5 ml. was assayed for uric acid by the colorimetric method of Benedict (1922), using the uric acid standard of Folin (1930), and the urea-cyanide reagent of Christman and Ravwitch (1932).

A sample of 5.0 ml. from the original tissue homogenate filtrate was employed for the determination of total RNA. This volume was diluted to 9.0 ml. with ice-cold distilled water, and 1.0 ml. of cold TCA solution having a concentration of 100 g./100 ml. was added to give a final concentration of 10% TCA. The solutions were thoroughly mixed and allowed to stand in the cold for an hour to insure complete precipitation. The mixture was later centrifuged in a refrigerated centrifuge at 12,000 *g* for 15 minutes. The precipitate was suspended in 9.0 ml. of cold distilled water and then precipitated with 1.0 ml. of 100% TCA. The precipitation procedure was necessary to remove all interfering substances. The precipitate was then defatted twice by suspending in a solvent mixture of cold 95% ethanol, chloroform and ether (1:2:2). This mixture was centrifuged and the supernatant discarded.

The defatted precipitate was then washed twice with ice-cold 5% TCA to remove the defatting solvent. RNA was extracted from the defatted pellet with 2.0 ml. of 5% TCA and heated for 15 minutes in a water bath maintained at 90° C. The mixture was cooled, centrifuged at 12,000 *g* for 15 minutes, and the clear supernatant assayed for RNA. The concentration of RNA was measured by the phloroglucinol reaction for ribose according to the method of von Euler and Hahn (1946). After color development, samples were read in a Coleman Junior Spectrophotometer at 620 mμ against a 5% TCA blank and a thymus RNA standard.

Radioisotope incorporation experiments

A sample of 14 allatectomized and 14 sham-operated females were injected through the femora of the metathoracic legs with 5 μc. of H^3-labelled glycine, leucine, and histidine mixture (specific acivity 2.5 mc./m*M*) dissolved in 0.1 ml. of physiological saline. At intervals of 1.5, 3, 6, 12, 24, and 48 hours after injection, fat body tissue, ovaries and midgut were removed and washed in several changes of saline to insure complete removal of non-incorporated amino acids. Tissues were

TABLE I

Comparison of body protein concentrations of allatectomized and sham-operated control females of the American cockroach, Periplaneta americana

Days after operation*	Allatectomized		Sham-operated control	
	Number of females	Protein concentration (g./100 g. body weight) $\bar{X} \pm SD$	Number of females	Protein concentration (g./100 g. body weight) $\bar{X} \pm SD$
7**	4	0.637 ± 0.08	5	0.78 ± 0.12
14	5	0.695 ± 0.07	4	0.94 ± 0.10
21	4	0.71 ± 0.06	4	1.06 ± 0.06
28	5	0.65 ± 0.06	5	0.897 ± 0.06
35	6	0.56 ± 0.08	5	0.88 ± 0.10

* All animals operated upon 8–48 hours after imaginal molt.
** Four newly molted females had an average concentration of 0.38 g./100 g. body weight.

dried in a desiccator, weighed, and converted to a uniform condition for counting as already described (Thomas and Nation, 1966). Samples were counted on a transistorized Tricarb Liquid Scintillation Counter (Packard Instrument Co.).

RESULTS

Effects of allatectomy on body protein concentration

The concentration of body proteins in the allatectomized and the sham-operated female roaches is shown in Table I. The concentration of body proteins in the allatectomized females is much lower than that of the sham-operated females of the same age.

The concentration of RNA in the allatectomized and sham-operated female roaches, calculated on a protein basis, is shown in Table II. The concentration of RNA in the allatectomized roaches 14 days after allatectomy was only 14.18 ±

TABLE II

Comparison of RNA concentration in the body tissues of allatectomized and sham-operated control females of the american cockroach, Periplaneta americana

Days after operation*	Allatectomized			Sham-operated control		
	Number of females	RNA concentration (mg./100 mg. protein) $\bar{X} \pm S.D.$	RNA concentration (mg./100 gm. body weight) $\bar{X} \pm S.D.$	Number of females	RNA concentration (mg./100 mg. protein) $\bar{X} \pm S.D.$	RNA concentration (mg./g. body weight)
7**	3	13.96 ± 0.65	96.5 ± 5.72	3	16.99 ± 2.96	185.0 ± 8.40
14	4	14.18 ± 0.69	106.0 ± 8.62	4	22.42 ± 3.10	265.7 ± 6.80
21	3	14.46 ± 1.64	97.6 ± 10.30	3	19.21 ± 3.59	254.6 ± 15.80
28	3	14.59 ± 1.46	89.9 ± 6.40	4	18.24 ± 3.85	214.0 ± 9.90

* All insects operated upon 8–48 hours after imaginal molt.
** Three newly molted females had an average concentration of 10.39 ± 0.82 mg./100 mg. protein and 91.90 ± 6.02 mg./100 g. body weight.

TABLE III

Comparison of uric acid concentration in the body tissues of allatectomized and sham-operated control females of the american cockroach, Periplaneta americana

Days after operation*	Allatectomized		Sham-operated	
	Number of females	Uric acid concentration (g./100 g. body weight) $\bar{X} \pm SD$	Number of females	Uric acid concentration (g./100 g. body weight) $\bar{X} \pm SD$
7**	5	4.49 ± 0.19	5	6.13 ± 1.41
14	3	4.47 ± 0.21	3	8.55 ± 1.69
21	4	4.36 ± 0.32	4	8.09 ± 1.16
28	4	4.14 ± 0.22	4	9.06 ± 0.45
35	4	3.96 ± 0.17	4	8.78 ± 0.97

* All insects operated upon 8–48 hours after imaginal molt.

** Four newly molted females had an average concentration of 4.18 g. ± 0.12 g./100 g. body weight.

0.69 mg./100 mg. protein, compared to 22.42 ± 3.10 mg. in the sham-operated females.

Table III shows the concentration of uric acid in the body tissues of allatectomized and sham-operated roaches. It is interesting to observe that in allatectomized females, the uric acid concentration remained approximately constant, while

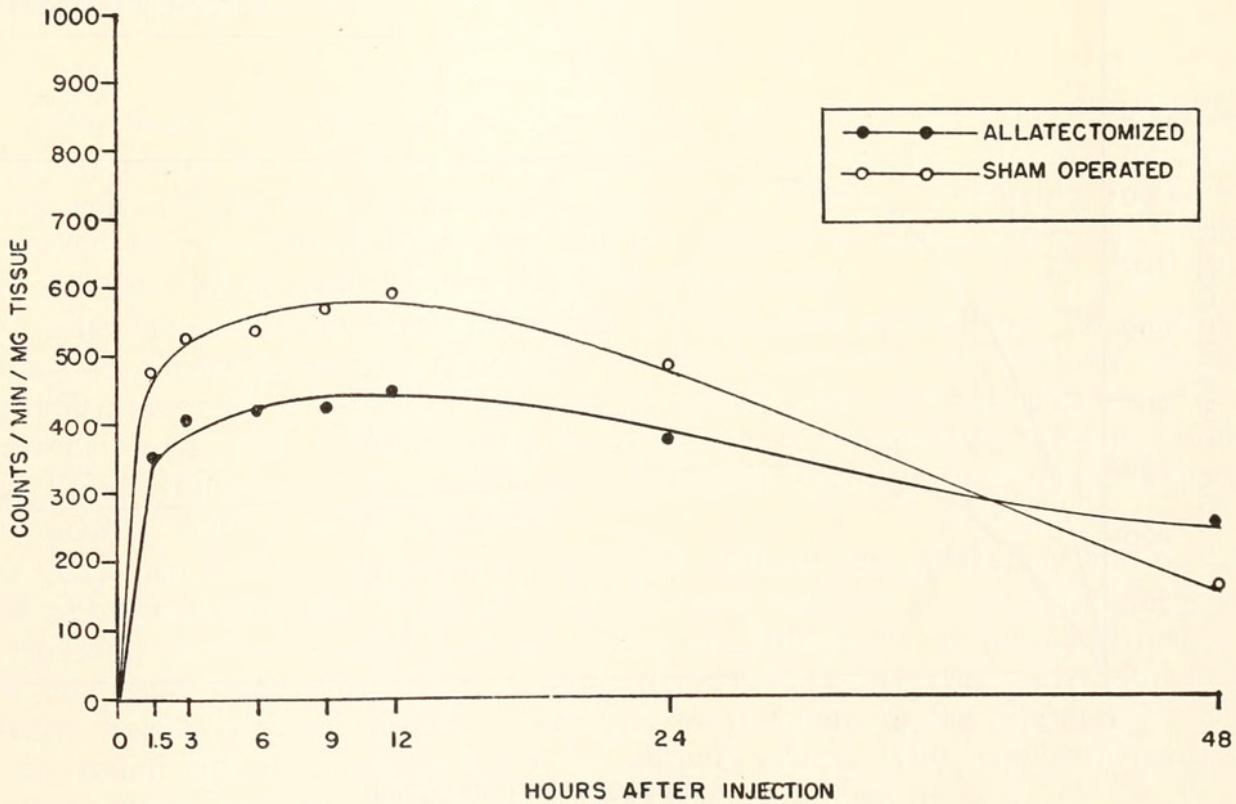

FIGURE 1. Rate of incorporation of tritium-labelled glycine, leucine, and histidine into the fat body of allatectomized and sham-allatectomized females of *P. americana* roaches (5 μc./roach). Points are averages of two experiments.

in the controls, it increased to more than double the concentration observed in the allatectomized females. However, five different analyses of fecal pellets of allatectomized and sham-operated roaches for uric acid, using the method of Wharton and Wharton (1960), failed to show any significant differences in excretion of uric acid. The lower concentration in allatectomized females is evidently the result of decreased protein catabolism.

Effects of allatectomy on the rate of incorporation of H^3-labelled amino acids

Figure 1 shows the sham-operated control females to have a greater rate of isotope incorporation and a greater total incorporation of label into fat body tissue than allatectomized females.

In allatectomized roaches, the uptake of tritiated amino acids by the ovaries was very slow compared to a very pronounced uptake by the ovaries of sham-operated females (Fig. 2). The fact that after 12 hours the specific activity in sham-operated controls was three times as great as the allatectomized females indicates marked inhibition of protein synthesis in ovaries of allatectomized females.

Incorporation of the label into midgut tissue of allatectomized and sham-operated females differed from the pattern shown in fat body and ovarian tissue, in that the total isotope incorporation was greater in allatectomized females than in control females (Fig. 3). Statistical analysis of the data (F test) showed

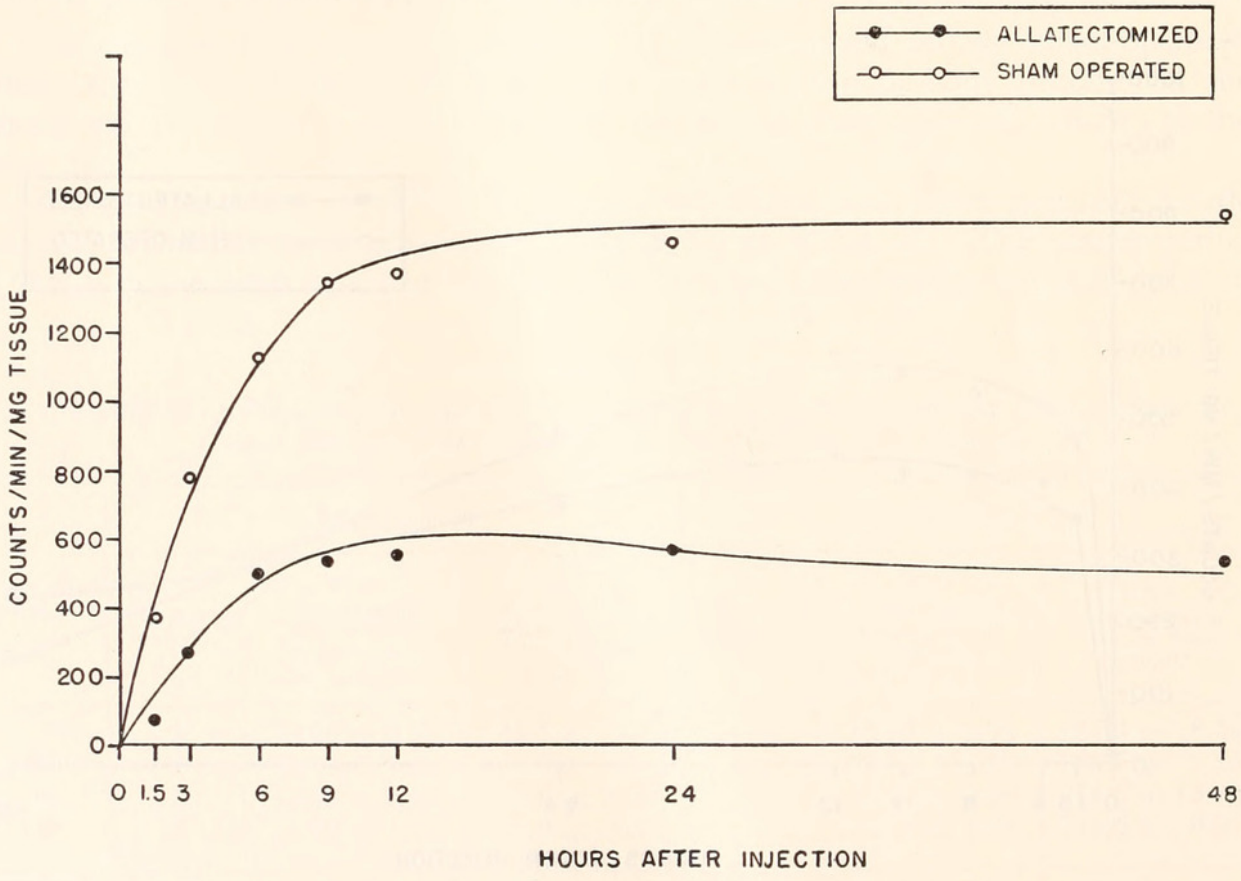

FIGURE 2. Rate of incorporation of tritium-labelled glycine, leucine, and histidine into ovarian tissue of allatectomized and sham-allatectomized females of *P. americana* roaches (5 μc./roach). Points are averages of two experiments.

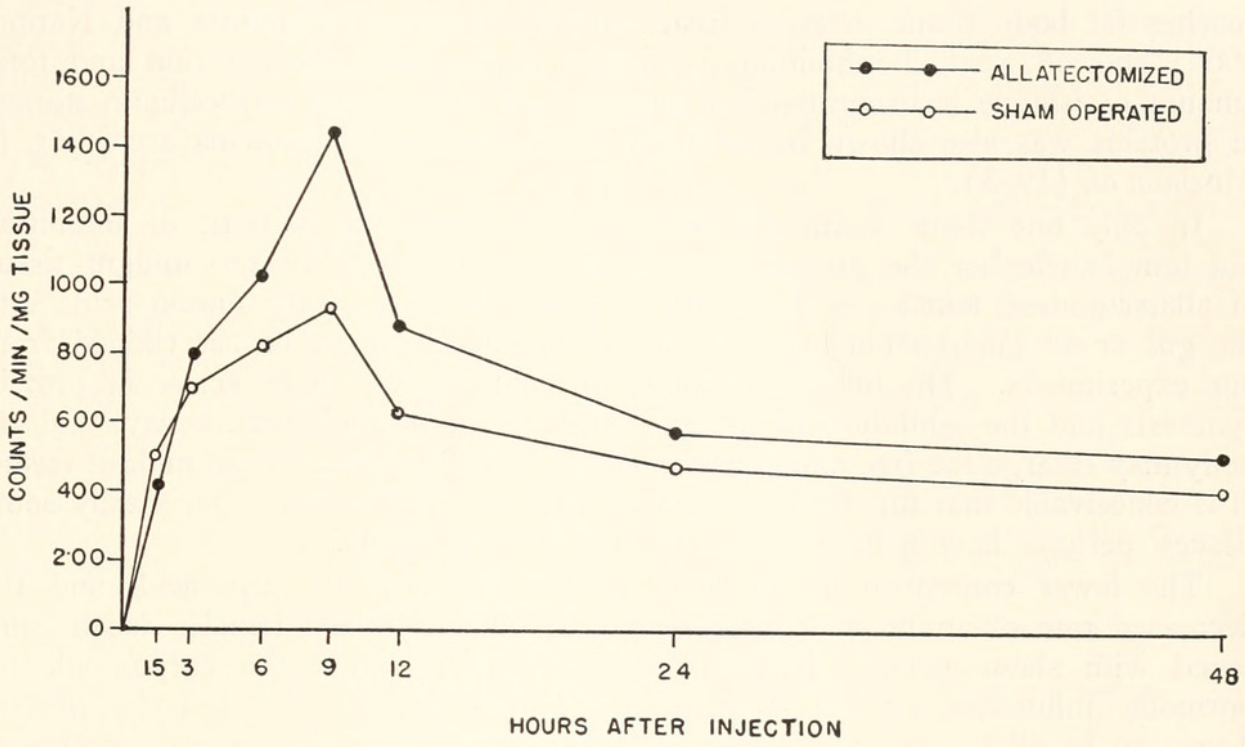

FIGURE 3. Rate of incorporation of tritium-labelled glycine, leucine, and histidine into the midgut tissue of allatectomized and sham-allatectomized females of *P. americana* roaches (5 μc./roach). Points are averages of two experiments.

that the difference is significant at 9 hours and 12 hours after injection of the radioactive amino acids.

DISCUSSION

We have shown that allatectomy during an ovarian cycle in *P. americana* females results in a lower level of total body RNA and protein, and that total body uric acid fails to increase as it does in control females. Furthermore, there is marked decrease in total incorporation of radioactive amino acids into protein of ovarian and fat body tissues following allatectomy.

The decreased body levels of protein and decreased synthesis of tissue proteins may be a consequence of a decrease in RNA synthesis. Clever and Karlson (1960) observed in larvae of *Chironomus tentans* that injected ecdysone induces chromosomal puffs, which could be demonstrated by autoradiography to be sites of RNA synthesis. It is possible that the allatum hormone functions in a similar manner to control RNA synthesis.

Uric acid in insects is formed principally from protein catabolism and the lack of an increase in urate level in allatectomized females, in the absence of any detectable increase in urate excretion, may be interpreted as the consequence of a decreased protein catabolism. Bodenstein (1953) observed a loss of urates from the fat body when corpora cardiaca were removed, but little or no effect was found upon removal of only the corpora allata. Our results support his observations that allatectomy has no influence on stored urates.

The most conclusive evidence for participation of the corpora allata in protein synthesis comes from the isotope incorporation experiments. In allatectomized

roaches fat body tissue, ovarian tissue, and haemolymph (Thomas and Nation, 1966) showed marked inhibition in rate of amino acid incorporation and total amount of isotope incorporated into protein. A decrease in intracellular storage of proteins was also shown by fat body tissue stained for proteins according to Mazia *et al.* (1953).

In only one tissue examined, midgut tissue, was this pattern of inhibition not found; whether the greater incorporation of amino acids into midgut tissue of allatectomized females is the result of active flushing of the amino acids into the gut or an initial stimulation of protein synthesis cannot be ascertained from our experiments. The midgut is known in some insects to be active in protein synthesis and the inhibition of protein synthesis in haemolymph, ovary, and fat body may enlarge the free amino acid pool and promote synthesis in midgut tissue. It is conceivable that the allatum hormone controls protein synthesis in only some tissues, perhaps having little or no effect on midgut tissue.

The lower concentrations of body proteins, RNA, and uric acid, and the decreased rate of amino acid incorporation in allatectomized females when compared with sham-operated females, support the view that the corpus allatum hormone influences protein metabolism. Not only is general body protein decreased in allatectomized females but there are changes in certain proteins in the haemolymph (Thomas and Nation, 1966). The positive correlation of these changes in protein and RNA metabolism with the gonadal cycle in female *P. americana* is consistent with the view held by some workers that the allatum hormone controls ovarian and egg development through control of protein metabolism.

We thank Dr. Berta Scharrer for helpful criticism and encouragement, and Professor K. K. Nayar for stimulating discussions and critical reading of the manuscript. This work was supported in part by NSF Grant GB-1088.

Summary

1. The concentrations of RNA, proteins, and uric acid in the body tissues of allatectomized and sham-operated female roaches were measured at intervals after the imaginal molt. The concentrations of RNA and proteins were substantially lower in allatectomized females when compared with sham-operated females. Uric acid concentration remained approximately constant in allatectomized females, while in the controls it increased to more than double the concentration observed in the allatectomized females.

2. The rate of incorporation of tritium-labelled amino acids into the fat body of allatectomized females was slow compared to that of the sham-operated roaches. A very slow rate of incorporation into ovarian tissue was observed in allatectomized females, as against a rapid rate of incorporation in the controls.

3. Midgut tissue of allatectomized roaches exhibited a significantly greater incorporation of isotope than that of sham-operated females.

4. The probable relationship between corpora allata and the synthesis of RNA, proteins, and uric acid during ovarian development has been discussed.

LITERATURE CITED

BENEDICT, S. R., 1922. The determination of uric acid in blood. *J. Biol. Chem.*, **51**: 187–208.

BODENSTEIN, D., 1953. Studies on the humoral mechanisms in growth and metamorphosis of the cockroach, *Periplaneta americana*. III. Humoral effects on metabolism. *J. Exp. Zool.*, **124**: 105–115.

CHRISTMAN, A. A., AND S. RAVWITCH, 1932. The determination of uric acid in human urine. *J. Biol. Chem.*, **95**: 115–126.

CLEVER, U., AND P. KARLSON, 1960. Induktion von Puff-Veränderungen in den Speicheldrüsenchromosomen von *Chironomus tentans* durch Ecdyson. *Exp. Cell Res.*, **20**: 623–626.

VON EULER, H. V., AND L. HAHN, 1946. A new method for the determination of ribonucleic acid in animal tissues. *Svensk. Kem. Tid.*, **58**: 251–264.

FOLIN, O., 1930. An improved method for the determination of uric acid in blood. *J. Biol. Chem.*, **86**: 179–187.

L'HÉLIAS, C., 1953a. Rôle des corpora allata dans le métabolisme des glucides, de l'azote et des lipides chez le phasme *Dixippus morosus*. *C. R. Acad. Sci. Paris*, **236**: 2164–2166.

L'HÉLIAS, C., 1953b. Étude comparée de l'azote total et de l'azote non proteinique chez le phasme *Dixippus morosus* après ablation des corpora allata. *C. R. Acad. Sci. Paris*, **236**: 2489–2491.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265–275.

MAZIA, D., P. A. BREWER AND M. ALFERT, 1953. The cytochemical staining and measurement of protein with mercuric bromphenol blue. *Biol. Bull.*, **104**: 57–67.

THOMAS, K. K., AND J. L. NATION, 1966. Control of a sex-limited haemolymph protein by corpora allata during ovarian development in *Periplaneta americana* (L.). *Biol. Bull.*, **130**: 254–264.

VANDERBERG, J. P., 1963. The role of the gonadotropic hormone in the synthesis of protein and RNA in *Rhodnius prolixus* (Hemiptera). *Biol. Bull.*, **125**: 576–581.

WHARTON, M. L., AND D. R. A. WHARTON, 1960. The determination of uric acid in biological fluids. I. A modification of the method of Bergman and Dikstein. *Anal. Biochem.*, **1**: 213–217.