

Three choice test for aggregation of the first nymphs of the German cockroach. (A) A group of the nymphs, approximately 60 individuals, was introduced into a glass pot. (B) After 19 min. A piece of filter paper conditioned by contamination with cockroach faeces was located at upper left. (C) After 43 min.

suggestive of secretory activity as previously supposed by SNODGRASS⁵.

Small pieces of filter paper conditioned by contact with male cockroaches which had been deprived of the eighth, ninth and tenth abdominal segments did not elicit the aggregation response. Nymphs did aggregate, however, in response to filter paper impregnated with a methanol extract of the rectum and posterior part of the colon.

The results indicate that material having the activity of an aggregation pheromone is possibly produced in the rectum and that it is applied to the faeces as they emerge. The activity on the surface of the abdomen is presumably from the same source, the spread of the active substances over the abdominal surface being facilitated by the fluid nature of the cuticular wax.

Cockroaches which had had their antennae amputated did not aggregate. This finding, together with the observation that aggregation occurs on conditioned filter paper even in darkness, confirms the supposition that the olfactory sense plays an important role in the aggregation behaviour.

It has recently been known that pheromones serve as attractants responsible for aggregations of *Lycus loripes* (Chevrolat)⁶, *Ips confusus* Lec.⁷, and *Calotermes flaviollis* (Fabr.)⁸. The meaning of the aggregation is, however, different in the insect species studied.

The aggregation pheromone found in the German cockroach also seems to serve as attractant for the aggregation. It is evident that this pheromone is contained in faeces excreted from the cockroach themselves without regard

to sex and nymphal stages, and that the gregarious behaviour of nymphs favours their growth and development. -A more extensive paper will be published elsewhere⁹.

Zusammenfassung. Die Larven der Schabe, *Blattella germanica* L., leben in Verbänden, wodurch ihre Wachstumsgeschwindigkeit beschleunigt wird. Der Herdeninstinkt funktioniert auch im Dunkel, nicht aber nach Abschneiden der Fühler. Eine chemische Erregungssubstanz, die für Zusammensitzen verantwortlich ist, wurde im Kot gefunden. Diese wird offenbar im Rectum produziert und im Kot ausgeschieden. Die Substanz wird als eine Art von «Pheromon» angesehen, und es wird vorgeschlagen, da sie für das Zusammensitzen der Schaben verantwortlich ist, sie als «Aggregationspheromon» zu bezeichnen.

S. ISHII and Y. KUWAHARA

Pesticide Research Institute, College of Agriculture, Kyoto University, Kyoto (Japan), 16 June 1967.

⁵ R. E. SNODGRASS, *Principles of Insect Morphology* (McGraw-Hill Book Company, New York 1935).

⁶ T. EISNER and F. C. KAFATOS, *Psyche* 69, 53 (1962).

⁷ D. L. WOOD, L. B. BROWNE, R. M. SILVERSTEIN and J. O. RODIN, *J. Insect Physiol.* 12, 523 (1966).

⁸ H. VERRON, *Insectes soc.* 10, 167 (1963).

⁹ We thank Dr. D. F. WATERHOUSE, C.S.I.R.O., Canberra, for his kind criticism.

Variations of Nucleic Acid Content in the Salivary Glands of *Drosophila hydei* during Late Larval Development

The DNA and RNA content of the salivary glands of *Drosophila* has been previously investigated by PATTERSON and DACKERMAN¹, and CHEN et al.², by biochemical methods, as well as, for DNA, by histophotometry³⁻⁵. All these works were carried out with the purpose of detecting the absolute values of the nucleic acid content in the mature gland, or in order to make a comparison between cells of different genotypes.

The only information so far available on the variations of the nucleic acids content in the salivary glands during their differentiation, comes from a recent research by

RODMAN⁶ who has been able to follow the changes in the amount of DNA with the aid of histophotometry.

The investigations reported in the present paper concern the determination by biochemical methods of the nucleic acid content in the salivary gland during the period in which the late differentiation of the gland takes place in connection with the increased nuclear politeny⁵. For this purpose, from synchronized⁷ cultures of *Drosophila hydei* St. (wild stock), we collected a consistent number of larvae every 24 h, from the late second to the late third instar. Biochemical determinations of DNA and

RNA content were carried out on the salivary glands excised from these larvae.

Extractions were made simultaneously on all the groups of glands according to the standard SCHNEIDER's procedure⁸; for the DNA determination we followed BURTON's method as improved by GILES and MYERS⁹; the RNA content was estimated by the orcinol reaction. The results are reported in Tables I and II.

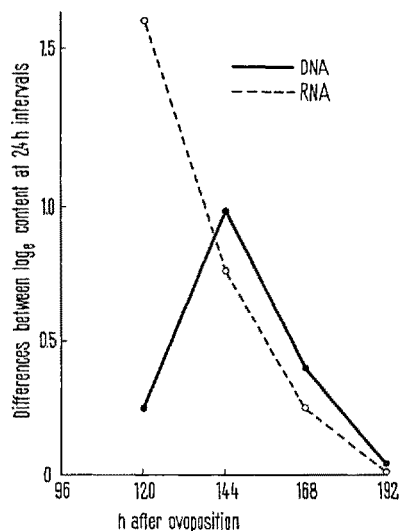
On account of the concept that in the salivary chromosomes the molecule of DNA is considered as a unit that replicates sequentially and only once in each endomitotic cycle¹⁰, the increase of 5 times in the total DNA content should correspond, on the average, to more than 2 DNA doublings in the cell nuclei. The histophotometrical

Table I. DNA content in the salivary glands of *D. hydei* during larval development

h after ovoposition	No. of glands used for the determination	Total DNA in the sample (μg)	Average DNA/gland ($\mu\text{g} \times 10^{-2}$)	Relative increase
96	436	10.90	2.50	1.00
120	155	4.96	3.20	1.28
144	335	28.77	8.58	3.43
168	251	32.19	12.82	5.12
192	251	33.83	13.47	5.38

Table II. RNA content in the salivary glands of *D. hydei* during larval development

h after ovoposition	No. of glands used for the determinations	Total RNA in the sample (μg)	Average RNA/gland increase ($\mu\text{g} \times 10^{-2}$)	Relative increase	RNA/DNA
96	436	22.86	5.24	1.00	2.09
120	274	71.44	26.07	4.97	8.14
144	335	188.60	56.29	10.74	6.56
168	251	182.35	72.64	13.86	5.66
192	251	170.73	68.01	12.97	5.04



Changes in the rate of increase of DNA and RNA content in the salivary glands of *D. hydei* during third instar, expressed as the difference in the natural logarithm of the amount of each constituent per individual, at successive 24 h intervals.

measurements by RODMAN⁶ are in very good agreement with our data.

The total RNA content increases, during the period considered, in a continuous way.

There are, however, remarkable changes of the RNA-DNA ratio during the late development and differentiation of the gland. The ratio increases rapidly in correspondence with the moult, then drops and undergoes a slow decrease. The changes of the rate of synthesis of DNA and RNA, as plotted in the Figure, give some supplementary information.

As shown by this graph, at the moment of the second moult, great amounts of RNA are synthesized in the gland; the rate of synthesis for DNA is, on the contrary, low. There is, in addition, autoradiographic evidence that just at this moment the DNA synthesis is strongly repressed, and that it resumes only after some hours¹¹. These findings are in agreement with the morphological and cytochemical data on the differentiation of the salivary glands during the larval life: after a comparatively long period spent in the synthesis and secretion of the digestive enzymes, in the first half of the third instar, the glands begin to change their function into secretion of the puparium glue⁵.

The existence of a relationship between RNA synthesis, morphogenesis and differentiation is well known¹². It seems reasonable to assume that in the gland, at the moment of the moult, the cells synthesize RNA for their further differentiation. This RNA synthesis is primed off by the pre-existing DNA and precedes the cytoplasmic enlargement and the last wave of polytenization in the nuclei.

Further experiments are in programme with the purpose of establishing the nature of this RNA and its relationship to the nucleic acid metabolism of the salivary gland cells during their late differentiation¹³.

Résumé. On a suivi la synthèse de l'ARN et de l'ADN dans les glandes salivaires chez *Drosophila hydei*. Les données biochimiques sur la teneur en acides nucléiques à différents moments du développement larval semblent indiquer que la synthèse d'une très grande quantité d'ARN a lieu dans les glandes avant la différenciation du troisième stade et aussi avant le commencement de la dernière vague de polyténisation des chromosomes.

G. A. DANIELI and E. RODINÒ

Istituto di Zoologia, Anatomia comparata e Genetica dell'Università, Padova (Italy), 18 June 1967.

- E. K. PATTERSON and M. E. DACKERMAN, *Archs Biochem. Biophys.* **36**, 97 (1952).
- P. S. CHEN, N. FARINELLA FERRUZZA and M. OELHAFEN-GANDOLLA, *Expl Cell Res.* **31**, 538 (1963).
- N. B. KURNICK and I. M. HERSKOWITZ, *J. cell comp. Physiol.* **39**, 281 (1952).
- H. SWIFT and E. M. RASCH, *J. Histochem. Cytochem.* **2** (1954).
- H. D. BERENDES, *Chromosoma* **17**, 35 (1965).
- T. C. RODMAN, *Genetics* **55**, 375 (1967).
- From eggs laid in 1 h period, hatching larvae in a 5 min interval were collected.
- W. C. SCHNEIDER, in *Methods in Enzymology* (Eds S. P. KOLLWICK and N. O. KAPLAN; Acad. Press, N.Y. 1957), vol. III, p. 680.
- K. W. GILES and A. MYERS, *Nature* **206**, 93 (1965).
- W. PLAUT and D. NASH, in *Role of Chromosomes in Development* (Ed. M. LOCKE, Acad. Press, N.Y. 1964), p. 113.
- G. A. DANIELI and E. RODINÒ, *Nature* **213**, 424 (1967).
- J. BRACHET, *The Biochemistry of Development* (Pergamon Press, London 1960).
- This investigation was supported by C.N.R. (Grant No. 115-0263-01222). We are very indebted to Prof. B. BATTAGLIA for his interest to our work.