Chiroptera) revealed two distinct types. One with a pointed anterior end and the other with a blunt club-shaped head end (Figure). According to the shape of the anterior tip, the acrosome is was pointed with a broad base or lies flat, closely attached to the nuclei in either case. The nuclei of the former type were conical, whereas in the latter they were more rectangular. On the posterior aspects of the nuclei in both the types, the 'post nuclear regions' or the 'post nuclear caps' were well pronounced. A centriole region was clearly marked in close association with the 'post nuclear cap'. The flagellum in each case seemed to develop from the centriole region, passing through the 'middle piece' and coming out as a vibratile tail. Both the sperm types were actively motile.

Uterine sections from the freshly copulated females showed the presence of both the sperm types in their lumen. Whether both are functional in fertilizing the ovum is not clear to us.

Dimorphic sperms of *Rhinopoma kinneari* as seen in the fixed smears. (1420×)

Equal numbers of both types of living sperms were measured from ten different adult individuals. The mean size was found to be 50.8 ± 12.9 $\mu$ for pointed head sperms and 52.8 ± 12.0 $\mu$ for blunt headed sperms. These size differences appear to have no statistical significance. The head lengths were also measured. There was no appreciable difference between the two types. Further the ratio of the two types was not constant in the individuals examined by us.

The outstanding differences between the two types of sperms are, (1) in their shape, and (2) in their overall lengths. There appears to be no difference in the head lengths but there is a difference in the lengths of the flagella (tail). Whether the dimorphism is due to the differences in the chromosomal complement, is not apparent in the size of the sperm heads as has been seen in the human sperms[1,2], but in their morphology[4].

*Résumé.* Les spermatozoïdes de *Rhinopoma kinneari* sont dimorphes et diffèrent les uns des autres dans leur forme et leur longeur totale, mais la longueur de leur tête reste constante. Ils peuvent être du type X et Y comme ceux des autres mammifères.

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[4] We wish to record our thanks to Prof. L. S. Ramaswami for helpful criticism and to Dr. P. N. Srivastava for helping us in statistical calculations.

## A New Puffing Pattern Induced by Temperature Shock and DNP in Drosophila

The different puffing patterns of the polytene chromosomes of Diptera show organ-specificity, developmental stage-specificity and sometimes zone-specificity[1–4]. These patterns can be explained in terms of variations of chromosome activity. It is known that puffs are due to the uncoiling of some characteristic bands[5] similar to those which have been shown to correspond to certain Mendelian loci[6,7].

It has also been shown recently[8–11] that puffs are sites of synthetic activity and that their major product is RNA. For these reasons the different puffing patterns can now be more precisely interpreted in terms of activity of genes probably due to different metabolic situations occurring in the various organs and developmental stages investigated.

Some recent investigations show that it is possible to induce directed variations in the puffing patterns. This was accomplished by Kroeger[12] by transplanting salivary gland nuclei of *D. busckii* into egg cytoplasm of *D. melanogaster*, and by Clever and Karlson by means of injections of ecdysone in *Chironomus* larvae[13].

The purpose of this paper is to report our results on the effect of temperature on the puffing patterns of the salivary glands chromosomes of *Drosophila busckii*. It will clearly appear that temperature shocks may induce well defined variations in the puffing patterns and that the variation always interests the same bands and involves specific metabolic activities.

These observations are limited to the 2L chromosomes, since the main variations are found in this region. In the

| | 2L 8 | 2L 14 | 2L 15 | 2L 20 |
|---|---|---|---|---|
| Normal at 25°C | + | - - | - - | |
| After 30 min at 30°C | | + | + | + |

[1] W. Beermann, Chromosoma *5*, 139 (1952).
[2] R. Mechelke, Chromosoma *5*, 511 (1953).
[3] M. E. Breuer and C. Pavan, Chromosoma *7*, 371 (1955).
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[8] D. J. Gross, Nature *180*, 440 (1957).
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[10] J. L. Sirlin, Exp. Cell Res. *19*, 177 (1960).
[11] F. Ritossa, Atti Assoc. Gen. Ital. *7*, 147 (1962).
[12] H. Kroeger, Chromosoma *11*, 129 (1960).
[13] U. Clever and P. Karlson, Exp. Cell Res. *20*, 623 (1960).

Table the puffing pattern is given of third instar larvae about 15 h before pupation of *D. busckii* grown at 25°C. In Figure 1 the regions 2L 14 and 2L 15 of the same larvae are shown[14].

If the larvae are subjected to a temperature shock (30°C or more) for about 30 min, a clear cut change in the puffing pattern is observed. The principal variations are shown in the Table and in Figure 2 and consist in the appearance of puffs in regions 2L 14, 2L 15 and 2L 20 and in the regression of the normal puff in 2L 8. The induction of a new puffing pattern by temperature shock has also been obtained in *D. melanogaster* and will be reported elsewhere. If the larvae that have undergone a temperature shock are transferred back to 25°C, after about 1 h the induced puffs recede while the puff in 2L 8 reappears again. When the same larvae are subjected once more to the temperature shock, the typical induced puffing pattern again makes its appearence.

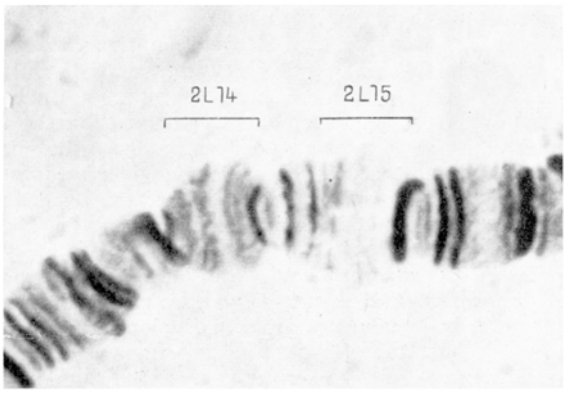


Fig. 1. The 2L 14 and 15 regions of salivary gland chromosome of *D. busckii* larvae reared at 25°C about 15 h before pupation.

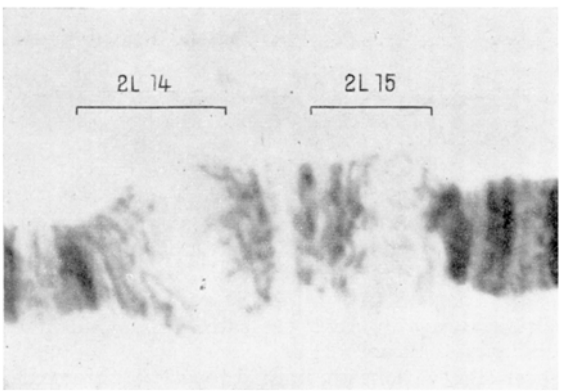


Fig. 2. The same regions as in Figure 1 after a thermal shock of 30 min at 30°C. Larvae near to pupation.

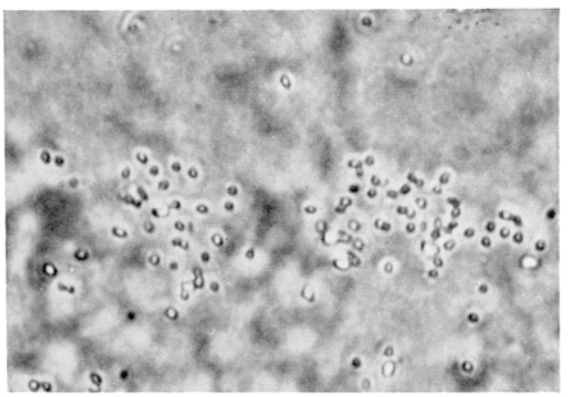

Fig. 3. The induced puffs after 10 min of incubation in 2.5 mm³ of a saline solution (0.17 mC/ml) of tritiated cytidine (Schwarz, s.a. 1 C/m*M*). 4 days exposition. Stripping films Kodak AR. 10.

It is also possible to induce the new puffs in larvae which have reached a stage very close to pupation, where puff 2L 8 has normally receded. Also in this case the induction has 100% efficiency.

When larvae grown at 19°C are brought to 25°C, some effects are sometimes noticeable, but with very low intensity and efficiency. This indicates that the puffing pattern variations may not depend exclusively on a 5–6°C jump in the temperature but on the rapid attainment of a given temperature threshold.

I have recently reported[11] a procedure for maintaining salivary glands of Drosophila metabolically active *in vitro* for about 2 h under oil drops. Under these conditions, the metabolic activity of the chromosomes can easily be investigated by means of labelled precursors which have been proved to enter the cell in few seconds. This technique has been used to show that the same effects on the puffing patterns are obtained whether the temperature shock is given to the whole larvae or to salivary glands extracted and incubated in Ringer solution. It may thus be inferred that the factors determining the phenomenon are limited to the salivary gland cells and do not involve organ interaction. The induced structural modifications can be shown to correspond to actual changes in the synthetic activity of the chromosomes bands concerned.

Tritiated cytidine was administered to salivary glands incubated *in vitro* and heated in order to induce the new puffing pattern. The presence of rather large quantities of this tracer in the puffs is apparent already after 3–4 min and it reaches high values after 10 min (Figure 3). Radioactivity is removed by RNAse[16]. This proves, as it was previously shown for the normal spontaneous puffs, that, also in the temperature-induced puffs (2L 14, 2L 15, 2L 20), a rather high rate of RNA synthesis occurs. On the other hand, this activity is found to be absent at the site of the original puff which has regressed (2L 8). Since most of the information so far available supports the idea that puffs are 'active genes', our experiments could be interpreted in the sense that function of genes (or at least of some genes) is reversible and dependent upon environmental conditions.

In an attempt to elucidate the possible mechanism of action of temperature, according to SZENT-GYÖRGYI's hypothesis[17], the effects of 2–4 dinitrophenol (DNP) and salicilate have been studied. For this experiment, salivary glands were incubated under oil in Ringer solution containing $10^{-3}M$ DNP or $10^{-2}M$ sodium salicilate for 30 min at 25°C. It was found that these substances can

[14] These positions are approximate; they have been inferred from the Sirotina's schematic maps[15]. The nomenclature is KRIVJCENKO'S

[15] M. I. SIROTINA, Mem. Gent., Acad. Sci. Urk. SSR. *2*, 61 (1938).

[16] Other evidences on the synthetic activity of puffs will be published elsewhere.

[17] A. SZENT-GYÖRGYI, *Bioenergetics* (Academic Press, New York 1957).

mimic the temperature effect for the induction of the new puffing pattern. Since a good deal of information is available on the chemical mechanisms by which these substances act, we may now study the metabolic variations that can induce the formation of these puffs[18].

*Riassunto.* Si è notato che shocks di temperatura possono indurre una variazione di «puffing pattern» in ghiandole salivari di Drosophila. Tali «puffing» sono perfettamente reversibili e rappresentano zone di intensa sintesi di RNA. Si è notato che DNP e Na salicilato portano a simili variazioni di «puffing pattern».

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[18] The author wishes to thank Dr. I. E. RASMUSSEN and Prof. G. MAGNI of the Genetics Institute of the University of Pavia for encouragement and critical discussion during the course of this work.

## Über Korrelationen zwischen der Hemmung des aktiven Na-Transportes bei Erythrocyten und der positiv-inotropen Wirkung von Digitaliskörpern nach Phenylbutazonschädigung

SCHATZMANN[1], KAHN[2,3], SOLOMON et al.[4], GLYNN[5], MACHOVÁ[6] und REPKE et al.[7] zeigten, dass Digitaliskörper in niedrigen Konzentrationen den aktiven Ionentransport kältegeschädigter menschlicher Erythrocyten hemmen und dass diese Fähigkeit sich mit einigen Struktur-Wirkungsbeziehungen, mit den Toxizitätswerten und der Hemmung der für den Ionentransport verantwortlichen ATP-ase korrelieren lässt. Unbeantwortet blieb bisher die Frage, ob auch therapeutische Konzentrationen von Digitaliskörpern, die am isolierten Herzen einen positiv inotropen Effekt hervorrufen, den aktiven Kationentransport hemmen. In früheren Untersuchungen[8–14] haben wir gezeigt, dass zwischen einer Reihe von Geninen und Glykosiden strukturabhängige qualitative Wirkungsunterschiede in dem Sinne bestehen, dass Konzentrationen, die am ungeschädigten isolierten Herzen einen äquieffektiven positiv inotropen Effekt hervorrufen, eine experimentell ausgelöste akute «Herzinsuffizienz» unterschiedlich stark kompensieren können. Als vorläufige Arbeitshypothese wurde die Vermutung ausgesprochen[11], dass eine verschieden starke Hemmwirkung auf den aktiven Ionentransport und damit auf die von SKOU nachgewiesene ATP-ase für diese spezifischen Wirkungsdifferenzen verantwortlich sein könnte. In den vorliegenden Versuchen sollte diese Arbeitshypothese überprüft werden.

Methodisch hielten wir uns an die Literaturangaben von SCHATZMANN, KAHN et al.[1–3]. Nach 4–5tägiger Aufbewahrung des Blutes bei 4°C wurde nach Reinkubation bei 37°C die Abnahme des akkumulierten Natriums flammenphotometrisch gemessen. Die Hemmwirkung auf den Na-Austausch wurde mit 15 Geninen[15], deren positiv inotroper Effekt am ungeschädigten und geschädigten isolierten Meerschweinchenvorhofpräparat festgestellt worden war[11–13], bestimmt. Die sich ergebenden Dosiswirkungskurven wurden statistisch nach der Kovarianzanalyse (BONNIER und TEDIN[16]) auf Parallelität und gemittelten mittleren Abstand überprüft. Für die in den pharmakologischen Versuchen am isolierten Vorhofpräparat gefundenen äquieffektiven Geninkonzentrationen wurde von den Dosiswirkungskurven die prozentuale Hemmwirkung auf den Na-Austausch abgelesen und eine Korrelation zwischen dieser Hemmungsstärke und der Stärke des positiv inotropen Effektes der Genine nach Chinin- und Phenylbutazonschädigung aufgestellt.

Die Strukturformeln der untersuchten 15 Genine sind in Tabelle I gegenübergestellt.

*Ergebnisse.* Entgegen den Befunden von KAHN[2,3] und MACHOVÁ[6], die für die Hemmung des aktiven *Kalium*-transportes bei allen untersuchten Digitaliskörpern parallel verlaufende Dosiswirkungskurven feststellten, fanden wir für die Hemmung des *Natrium*-Austausches signifikante strukturabhängige Unterschiede einiger Regressionskoeffizienten. Die Dosiswirkungskurven der beiden 3,14-Dihydroxygenine (Digitoxigenin und Bufalin) verlaufen flacher als die Kurven der 3,14,12- und 3,14,16-Trihydroxygenine ($P < 0{,}02$). Die Substitution einer Aldehyd- statt der Methylgruppe in $C_{10}$ (Strophanthidin: Periplogenin) vermindert ebenfalls den Anstiegswinkel. Der Einfluss des Laktonringes ist unterschiedlich: bei den 3,14,16-Trihydroxygeninen besitzen die drei Bufadienolide (Bufotalin, Desacetylbufotalin und Cinobufagin) höhere Regressionskoeffizienten als die beiden Cardenolide (Gitoxigenin und Oleandrigenin). Bei den 3,5,14-Trihydroxygeninen ist das umgekehrte Verhalten festzustellen (Figur 1). Setzt man ähnlich wie bei MACHOVÁ die $ED_{50}$-Werte der Dosiswirkungskurven zu den Toxizitätswerten bei der Katze in Beziehung, so ist der Korrelationskoeffizient hoch signifikant ($P < 0{,}001$).

In Tabelle II sind die Hemmung des aktiven Na-Austausches und die Kompensation nach Chinin- und Phenylbutazonschädigung durch die am ungeschädigten Herzen äquieffektiven Konzentrationen der Genine gegenübergestellt.

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[11] W. FÖRSTER, Habilitationsschrift, Magdeburg (1961).
[12] W. FÖRSTER, Acta biol. med. germ., im Druck (1962).
[13] W. FÖRSTER, Acta biol. med. germ., im Druck (1962).
[14] W. SZIEGOLEIT und W. FÖRSTER, Acta biol. med. germ. *7*, 614 (1961).
[15] Die verwendeten Genine wurden uns freundlicherweise zur Verfügung gestellt von Herrn Prof. K. MEYER, Basel (Desacetylbufotalin, Cinobufagin, Bufotalin, Telocinobufagin, Bufalin, Periplogenin und Oleandrigenin), Herrn Prof. TAMM, Basel (12$\beta$-Hydroxybufalin), Herrn Prof. WOLLENBERGER, Berlin (Ouabagenin), Herrn Prof. THIEN, AWD Dresden (Digoxigenin), Herrn Dr. DÜBLER, Deutsche Hoffmann-La Roche AG, Grenzach Baden, (Gitoxigenin und Digitoxigenin). Strophanthidin und Strophanthidol stammten von der Arco-Chemie, Berlin.
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