Using the histological method, the percentage ± standard error of the acrosome loss was 22.0 ± 6.4, 18.0 ± 5.8, 27.0 ± 6.6 and 56.0 ± 13.5 on days 1, 2, 3 and 4, respectively. The acrosome loss in spermatozoa incubated in Tyrode's alone was 26.0 ± 3.8 (Figure 1b). The acrosome reaction on day 4 in the hamster is significantly higher ($P > 0.005$) from that observed on days 1, 2, 3 and Tyrode's, respectively.

In oestrus hamster oviducal fluid the removal rate is highest (45.0%) and declines to 4.3% in metoestrus. Thereafter tetracycline removal increases again to 42.0 and 44.0% on days 2 and 3, respectively.

Figure 2a shows ^{3}H-T-HCl removal in oestrus uterine fluid to be 51.0%. Oestrus rabbit oviducal fluid proteins and serum show 46.0 and 47.0% ^{3}H-T-HCl removal. The removal rate from spermatozoa in Tyrode's solution alone and those that were incubated without treatment, results in 50.0 and 49.0%. The staining technique reveals a remarkable difference between uterine and oviducal fluid in inducing the acrosome reaction. Oestrus rabbit uterine fluid induces an acrosome loss of 53.6 ± 3.1%, whereas the percentage for oviducal fluid is 33.3 ± 1.3%. Tyrode's solution shows an acrosome reaction of 20.5 ± 8.1% (Figure 2b). The removal of the tetracycline molecule from untreated and Tyrode's treated human spermatozoa is 46.0% (Figure 2c). The samples obtained close to the time of estimated ovulation slightly increase the removal rate of ^{3}H-T-HCl and show a radioactivity loss of 49.0% and 48.0% on day 14 and 16, respectively.

Discussion. Removal of the fluorescent label from spermatozoa has been stated to be an indication for the initial step in sperm capacitation[11]. The present results show that the rate of removal of the labelled tetracycline molecule depends on the different protein containing fluid samples (Figures 1 and 2). However, the level of significance increases highly, when a histological method is used (Figures 1b and 2b). This is due to a clear distinction of the presence or absence of the acrosome, whereas accurate determination of the number of molecules bound to a spermatozoon, is subject to estimation. The relatively high background in untreated spermatozoa and those incubated in Tyrode's may be explainable by ^{3}H-T-HCl molecules, that have not been bound, and permits the conclusion, that only about 50% of the labelled molecules will bind to the surface of spermatozoa in these species. The low level of tetracycline removal on day 1, may be due to changes in the uterine protein environment being unfavourable for spermatozoa at that time.

These data suggest, that the initial steps of capacitation can be achieved in oestrus uterine fluid proteins of the hamster in vitro, and are comparable to the acrosome reaction in hamster serum[15]. Also, higher tetracycline removal in oestrus rabbit uterine fluid may be due to either conditions being satisfactory in vitro, or the ^{3}H-T-HCl removal refers to a potential and not actual achievement of the fertilizing ability.

We conclude, that the loss of the acrosome is facilitated by exposure to female genital tract secretions. Tetracycline removal, however, is not a reliable indicator for sperm capacitation[16].

Zusammenfassung. Die Wirkung von Sekreten des Hamster- und Kaninchenreproduktionstrakts wurde auf die Akrosomreaktion in vitro getestet und festgestellt, dass sie am Tag 4 des Zyklus, sowie in uterinen Sekreten des Kaninchens höher ist als in Oviduktsekreten (wenn die Färbemethode mit der Entfernung von Tetrazyklinmolekülen verglichen wird).

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26 February 1974.

[15] C. BARROS, J. ARRAU and E. HERRERA, J. Reprod. Fert. *28*, 67 (1972).

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Deoxyguanosine, a potent Cytokinesis Inhibitor in Plant Cells

During our work on the mechanism of action of hydroxyurea[1,2], while trying to antagonize its action by addition of deoxy ribonucleosides, we have seen that deoxyguanosine induces the formation of a great number of binucleate cells, and we think it is interesting to describe this action which, as far as we know, had not yet been reported. Acetic orcein squash root meristems of *Allium sativum* L. have been prepared; 2,000 cells have been scored in each meristem and percentages of binucleate cells and mitosis given below are the mean value for 2 meristems. Late telophases devoid of phragmoplast have been set up by scoring all the telophases of 2 meristems:

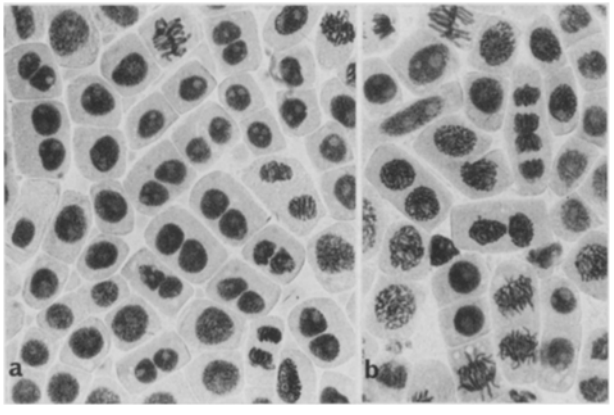
Deoxyguanosine concentration (M/ml)

		Control	3.75×10^{-7}	7.5×10^{-7}	1.5×10^{-6}	2×10^{-6}	3×10^{-6}
Binucleate cells (%)	3 h	0	scarce	scarce	scarce	scarce	scarce
	6 h	0	5.3	4	4	4.2	1.8
	24 h	0	6.6	16.3	21.3	25.7	15.7
Late telophases without phragmoplast (%)	3 h	0	4	15	28.7	37.5	43.6
	6 h	0	26.8	37.8	46	65.6	73.6
	24 h	0	24.4	42.5	36.5	62	52.5
Mitotic index (%)	3 h	9.3	4.6	7.6	7.5	5.8	5.6
	6 h	11.5	5.3	12.8	6.8	7.2	7.4
	24 h	11.6	6.6	7.8	4.9	7.1	7.4

These percentages give us a better estimate of cytokinesis inhibition, in case mitotic index should be lowered.

The concentration of 10^{-7} M/ml (2.76/100 ml) is ineffective and cytokinesis inhibition is seen only with higher concentrations. From 3.7×10^{-7} M/ml to 3×10^{-6} M/ml, the growth of roots is normal within 48 h but on the 4th day their length is about 60% of the control and the roots have swelled.

Results obtained during the first 24 h are shown in the Table. The optimal concentration inhibiting cytokinesis is about 2×10^{-6} M/ml. For all coneentrations we can observe: partial Cytokinesis, a slight chromatoclasic effect (anaphase bridges and breaks), numerous bimitosis after 48 or 72 h in deoxyguanosine. And with the highest concentration: nuclear diapedeses, distorted nuclei, and a strong mitodepressive effect after 48 h.



2′ Deoxyguanosine $7{,}5 \times 10^{-7}$ M/ml: a) 24 h, numerous binucleate cells; b) 72 h, binucleate cells enter mitosis.

A short treatment (1 h) using 1.5×10^{6} M/ml has been applied: at the end of this treatment there is no telophase without phragmoplast but, after recovery in Knop medium $^1/_2$ for 2 h, such abnormal mitosis does appear, showing a delayed effect of deoxyguanosine. Comparatively, the study of deoxyadenosine showed that it has not the same property as deoxyguanosine: the concentration of 10^{-8} M/ml is ineffective and with 2×10^{-8} M/ml there is no mitosis within 24 h.

We are continuing to study the action of deoxyguanosine (and related chemicals) on cytokinesis; Indeed, we think that, as deoxyguanosine is a physiological compound, discovering its mechanism of action could help to understand the metabolism of normal cytokinesis.

Résumé. La désoxyguanosine, à partir d'une concentration seuil de $3{,}7 \times 10^{-7}$ M/ml, inhibe fortement la cytodiérèse des cellules méristématiques d'*Allium sativum* L. L'activité mitotique n'est que faiblement touchée.

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[1] A. Brulfert and G. Deysson, Annls pharm. fr. *31*, 19 (1973).
[2] A. Brulfert, E. Clain and G. Deysson, C.r. Soc. Biol., *167*, 1740 (1973).

Electrophoretic Characterization of Melanosomal Proteins Extracted from Normal and Malignant Tissues

One approach to the study of pigment granule structure and development has been the recent development of techniques which extract the protein constituents of the melanin granules under conditions which preserve protein integrity[1,2]. In avian and mammalian melanocytes, melanin granules are ovoid and the melanin is deposited upon a filamentous matrix[3,4]; however, the matrix of premelanosomes from oocytes of the frog *Xenopus laevis* is granular and sometimes paracrystalline in appearance[5] and mature melanin granules are spherical. Further, the melanosomes of the malignant mouse melanoma are very atypical with disorganized fibres and incomplete melanization[6]. The study reported here was initiated in order to ascertain whether these fine structural variations could be demonstrated at the biochemical level by gel electrophoresis.

Materials and methods. Melanogenic tissues used in this study were obtained from: 1. 5-day-old black (C57B1) mouse eyes, 2. an actively growing S-91 mouse melanoma, 3. 15-day embryonic chick eye (White Leghorn), and 4. *Xenopus laevis* oocytes. Melanin granules were isolated and purified from the murine and avian tissues by the procedure of Hearing and Lutzner[1]; those from frog oocytes were obtained by the method of Eppig and Dumont[7]. Protein solubilization techniques are detailed in the legend to Table I. Gel electrophoresis (PAGE)[8] was carried out on these extracts in 7.5% gels at 2 mA/tube at 25 °C, using the *Tris*-glycine buffer system with either SDS or urea[9]. Proteins were stained with Fast green[10]; the relative mobilities were calculated against the migration of pyronin Y tracking dye.

Results and discussion. The relative mobility (Rm) of several protein bands extracted from melanin granules of diverse origin are very similar (Tables I and II). This similarity was especially striking with respect to extract

[1] V. J. Hearing and M. A. Lutzner, Yale J. Biol. Med. *46*, 553 (1973).
[2] P. Doezema, J. Cell Physiol. *82*, 65 (1973).
[3] M. A. Lutzner and C. T. Lowrie, in *Pigmentation: Its Genesis and Biologic Control* (Appleton-Century-Crofts, New York 1972), p. 89.
[4] V. J. Hearing and M. A. Lutzner, J. Ultrastruct. Res. *43*, 88 (1973).
[5] J. N. Dumont and J. J. Eppig jr., manuscript in preparation.
[6] F. H. Moyer, Ann. N. Y. Acad. Sci. *100*, 584 (1963).
[7] J. J. Eppig jr. and J. N. Dumont, Devel. Biol. *36*, 330 (1974).
[8] Abbreviations used: PAGE, polyacrylamide gel electrophoresis; SDS, sodium decyl sulfate; Rm, relative mobility.
[9] A. R. Ugel, A. Chrambach and D. Rodbard, Analyt. Biochem. *43*, 410 (1971).
[10] M. A. Gorovsky, K. Carlson and J. G. Rosenbaum, Analyt. Biochem. *35*, 359 (1970).