concentration of 1.0 *M*. Applying the ninhydrin reaction after alkaline hydrolysis at 100 °C for 2.5 h, 5 peptide peaks, A, B, C, D and E, were found in the eluate. The yields of peptides isolated were as follows; A, 10 mg; B, 37.5 mg; C, 9 mg; D, 4 mg; and E, 13 mg. On high voltage paper electrophoresis at pH 3.5, each peptide gave a single spot staining with the peptide reagent *tert*-butylhypochlorite-*o*-tolidine-KI[5]. Peptide E gave a positive Ehrlich's reaction and peptides A, B and D gave positive reactions with Sakaguchi's reagent. Moreover, peptides A, C and D did not react with ninhydrin reagent, suggesting the absence of free amino groups in their N-terminals. The action of bradykinin on isolated guinea-pig ileum was potentiated twofold by 5–20 µg of any of these peptides. The Table shows the amino acid compositions of the peptides. All contained 1 mole of glutamic acid and 4 moles of proline and a total of 8–10 amino acid residues. From their amino acid compositions, it is assumed that parts of the amino acid sequences of peptides A, B, C and D are homologous. Investigations on the amino acid sequences of these peptides are now in progress. One characteristic of these materials is their high proline content. Bradykinin also has a high proline content, therefore its structural relationship with bradykinin potentiating peptides seems interesting.

Zusammenfassung. Aus dem Gift von *Agkistrodon halys blomhoffii* konnten durch Säulenchromatographie an Sephadex G-100, G-25 und CM-Sephadex C-50 fünf Peptidkomponenten isoliert werden, welche die kontrahierende Wirkung von Bradykinin auf den isolierten Meerschweinchendarm signifikant erhöhen.

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[5] R. H. MAZUR, B. W. ELLIS and P. S. CAMMARATO, J. biol. Chem. *237*, 1619 (1962).
[6] T. W. GOODWIN and R. A. MORTON, Biochem. J. *40*, 626 (1946).

Inhibition of Reduction in Female Mouse Meiosis*

The mitotic spindle inhibition effect of Colchicine and Vincristine is well known[1,2], as is the effect in mammalian embryonic heteroploidy[3] and pronuclear formation[4]. Earlier stages in female oogenesis have not been studied, although experiments with mammalian spermatogenesis[5] and annelid oogenesis exist[6–8]. Since inhibited reduction of meiosis could lead to pentaploidy in zygotes[9], the effects of spindle inhibition on early mouse oogenesis in vivo and in vitro have been studied in the experiments described herein.

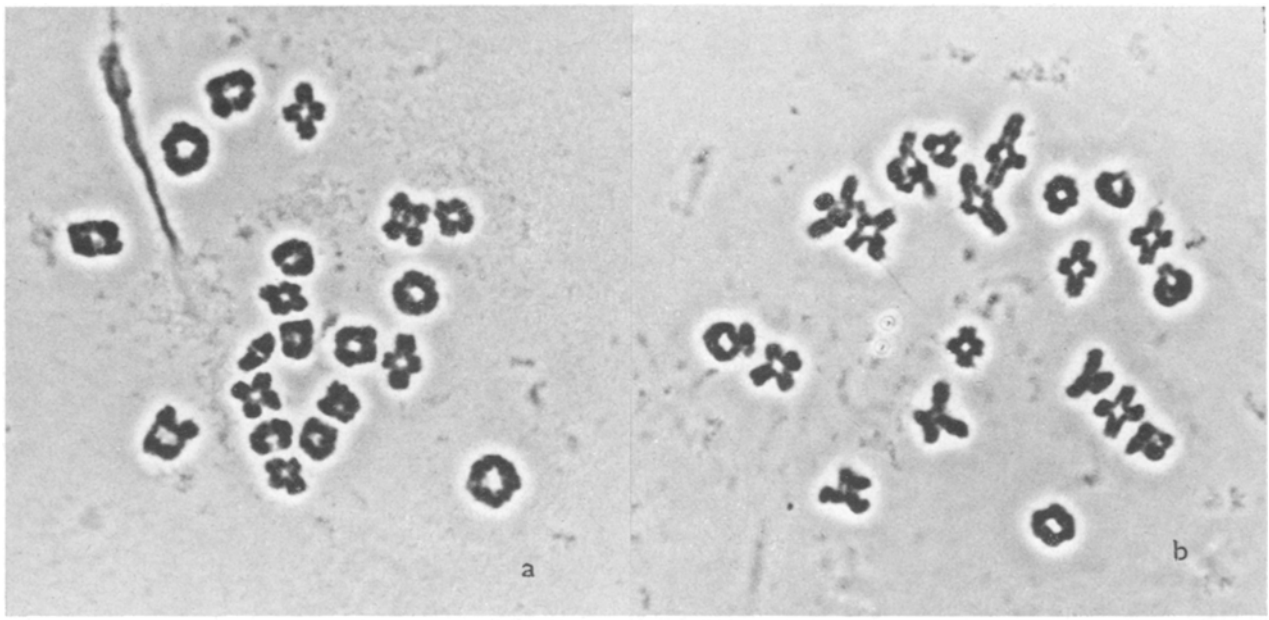
Materials and methods. Groups of twelve 21-day-old Strong-A derived female mice weighing 10–12 g were injected s.c. with doses of the colchicine derivative Colcemid from 0.1–50 µg/g body wt. or Vincristine from 0.01 to 5.0 µg/g body wt. at the same time that 2.5 IU of pregnant mare serum (PMS) were given and on the subsequent 2 days, or the total minimum effective dose (MED) of Colcemid was given with the human chorionic gonadotrophin (HCG) and Vincristine with PMS or up to 10 h after HCG. 48 h after PMS, 1.0 IU of HCG were given and 14–16 h thereafter the oviducts were examined for ova. Ova thus recovered were prepared for cytogenetic examination by a modified TARKOWSKI method[10] and assessed as to number and morphology of chromosomes in the second metaphase (M^{II}) and first polar body (PB^{I}). If ovulation had not occurred, it was considered that the agent had blocked spindle formation and intrafollicular ova were prepared in a similar fashion. The presence of a first meiotic metaphase (M^{I}) configuration in these ova was considered evidence that the agent had inhibited spindle formation. In vitro studies were carried out with ova from adult dioestrous females. Approximately 20 ova were added to each culture flask which contained 4×10^{-1} to 10^{-8} (stepwise) µg of Colcemid or 4×10^{-1} to 10^{-9} (stepwise) µg of Vincristine/ml of media. These were harvested after 14–16 h of incubation and prepared for cytogenetic examination. Criteria for spindle block were the same as in vivo.

In vivo recovery was studied, by giving a single dose of the total MED of Vincristine coincident with the PMS or Colcemid with HCG to groups of 12 female mice and checking at hourly intervals for ovulation beginning 12 h after the HCG, the time when ovulation normally occurs. When it had occurred, half of the recovered ova were observed with phase microscopy for spindle constitution, particularly for bi- and tripolar orientations and preparations were made for chromosome analysis to determine distribution in M^{II} with PB^{I}. Actinomycin D (AMD), freshly prepared in saline, was given in a dose of 1.0 µg/g body wt. s.c. with the HCG or at 1, 3, 4, 5, etc. hours after HCG. This dose was selected on the basis of preliminary experiments as the maximum dose which did not produce any microscopically observable effects on the chromosome configurations of ova. Observations for recovery were made as above.

For study of in vitro recovery, eggs were incubated in standard fashion using the same concentrations as in the inhibition studies. After 14 h of incubation the ova

* Part of this data was presented in abstract in Clinical Research, vol. XV, No. 2, April 1967, p. 268.

[1] O. J. EIGSTI and P. DUSTIN JR., *Colchicine* (Iowa State College Press, Ames, Iowa 1955).
[2] E. FREI, J. WHANG, R. B. SCOGGINS, E. J. VAN SCOTT, D. P. RALL and M. BEN, Cancer Res. *24*, 1918 (1964).
[3] R. G. EDWARDS, J. Expl Zool. *137*, 317 (1958).
[4] R. G. EDWARDS, Expl Cell Res. *24*, 615 (1961).
[5] K. W. PETERSEN, M. S. LEGATOR and C. B. JACOBSON, Mammalian Chromosomes Newsletter *9*, 421 (1968).
[6] S. INOUE, H. SATO and M. ASCHER, Biol. Bull. *129*, 409 (1965).
[7] S. INOUE and H. SATO, J. gen. Physiol. *50*, 259 (1967).
[8] S. E. MALAWISTA, H. SATO and K. G. BENSCH, Science *160*, 770 (1968).
[9] R. A. BEATTY, Cambridge monographs in Expl. Biology No. 7 (Cambridge University Press 1957).
[10] A. K. TARKOWSKI, Cytogenetics *5*, 394 (1966).



Inhibited first meiotic metaphase from mouse ova (a) in vivo from animal treated with 1 µg of Vincristine and (b) in vitro incubation with 4×10^{-3} µg/ml of Colcemid.

were checked for the presence of PB^I and M^{II}. If these were *not* present and M^I figures present the eggs were washed 3 times in fresh media and replaced in a new aliquot for further incubation without inhibitor. At 1, 2, 3, 4, 8, 12 and 24 h thereafter observations were made of the meiotic stages. Demonstration of the presence of M^I in sample ova was considered evidence that inhibition had persisted and the presence of M^{II}/PB^I that escape had occurred. The recovery from inhibition was studied in the presence of AMD added to the fresh media, after washing 3 times, at a concentration of 0.1 µg/ml of media, selected as the maximum amount which did not damage chromosomes. Sampling was carried out as above.

Results. In vivo, the MED was 10 µg/g body wt. for Colcemid and 1 µg/g body wt. for Vincristine. When the total MED was given as a single injection with the HCG or PMS respectively and recovery assessed, it was found that with Colcemid PB^I and M^{II} formation and ovulation had occurred by 14 h after the dose. However, with Vincristine, the block persisted for 67.5 h or 19.5 h after HCG. AMD did not prolong or enhance recovery from either Vincristine or Colcemid.

In vitro, the minimum effective dose of Colcemid studied was 4×10^{-3} µg/ml of media with recovery at all concentrations attained by 2 h. The MED of Vincristine was 4×10^{-7} µg/ml and recovery was partially complete with the formation of some M^{II}/PB^Is by 8 h after removal from inhibitor containing media. At a concentration of 4×10^{-6} µg/ml a few M^{II}/PB^Is were detected at 24 h. At concentrations of 4×10^{-5} µg/ml and greater, recovery had not been achieved at 36 h when M^I was the only configuration observed in all ova. Spiralization changes consistent with such long incubation were seen. Some segmentation was also visible at 24 h and 36 h in the 4×10^{-6} and 10^{-7} concentrations. The addition of AMD with or after inhibitor did not affect inhibition or recovery.

Discussion. In this mammalian system spindle inhibition at first meiotic metaphase was induced in vivo and in vitro (Figure). In sharp contrast to marine annelid oocytes[8] Vincristine was much more potent than Colcemid in terms of effective concentration and duration of action. The comparison may not be an exact one, since the spindle was already formed in the annelid oocyte when the inhibitor was added and was in the process of formation in both mouse oocyte systems. Since the first metaphase mouse oocyte can be inhibited in vivo as described in the experiments reported here, one could reasonably expect heteroploidy with fertilization, although examination of ova which had recovered from inhibition revealed normal spindle orientation and disposition of chromosomes. This is difficult to reconcile with the report of Bomsel-Helmreich[11] of triploid rabbit eggs fertilized in Colcemid which developed only to mid-gestation with subsequent death, or the production of multiple pronuclei in fertilized mouse eggs treated during *second* maturation division[4]. It may be that mammalian 1st and 2nd spindle formations differ intrinsically[12].

Zusammenfassung. Es wurde der Hemmungseffekt von Colcemid und Vincristin auf die Meiose bei Mäuseeiern in vitro und in vivo untersucht. Die in vitro wirksame minimale Konzentration für Vincristin wurde bei 4×10^{-7} µg/ml, für Colcemid bei 4×10^{-3} µg/ml gefunden. Die in vivo wirksame Minimaldosis von Vincristin war 1 µg/g Körpergewicht, von Colcemid 10 µg/g Körpergewicht.

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[11] O. Bomsel-Helmreich, *Preimplantation Stages of Pregnancy* (Eds. G. E. W. Wolstenholme and M. O'Connor; Little Brown & Co., London 1965), p. 246.

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