The mutagenic action of ethylene halogenhydrins to *Klebsiella pneumoniae*

	Compound	Concentration	No. of bacteria/ml after 20 h incubation	Portions without mutants	No. of mutants in median portions	Mutation rate	Increase
I	Ethylene chlorohydrin	0.15 *M*	8.4×10^8	0/100	103	6.23×10^{-9}	× 33
	Ethylene chlorohydrin	0.015 *M*	15.5×10^8	0/100	6	0.401×10^{-9}	× 3.4
	Ethylene chlorohydrin	0.0015 *M*	12.1×10^8	39/100	–	0.179×10^{-9}	–
	Control		15.3×10^8	42/100	–	0.131×10^{-9}	–
II	Ethylene chlorohydrin	0.015 *M*	13.8×10^8	0/100	10	0.648×10^{-9}	× 5.5
	Ethylene bromohydrin	0.015 *M*	8.2×10^8	0/100	272	14.8×10^{-9}	× 125
	Ethylene iodohydrin	0.015 *M*	2.3×10^8	0/100	69	17.5×10^{-9}	× 148
	Ethylene cyanohydrin	0.015 *M*	16.1×10^8	55/100	–	0.086×10^{-9}	–
	Control		18.4×10^8	53/100	–	0.080×10^{-9}	–

nutrient-agar without dihydrostreptomycin. From the number of portions without streptomycin-resistant or dependent mutants the mutation rate was calculated by the Poisson distribution. If in all portions mutants were present, the mutation rate was estimated by the number of mutants in the median portion according to LEA and COULSON[3]. The results of the experiments are shown in the Table.

The increase of the mutation rate was calculated against the average spontaneous mutation rate of the blanks of 14 experiments. It amounts to 0.1183×10^{-9} (95% confidence levels 0.051×10^{-9} to 0.186×10^{-9}).

From the results it appears that ethylene iodohydrin, ethylene bromohydrin and ethylene chlorohydrin are potent mutagenic agents. Probably the carbon halogen bond is involved. If so, the decrease of the bond dissociation energy from the C–I to the C–Cl bond is in accordance with the decrease of mutagenic action from ethylene iodohydrin to ethylene chlorohydrin.

In other experiments we found for the mutation rate induced by coffeine (2 mg/ml): 0.396×10^{-9} (increase × 3.4), and by 5-bromouracil (1 mg/ml): 1.181×10^{-9} (increase × 10).

The results of these experiments demonstrate that the substances investigated have mutagenic properties.

Zusammenfassung. Eine stark mutagene Wirkung wird durch Äthylenchlorhydrin, Äthylenbromhydrin und Äthylenjodhydrin auf *Klebsiella pneumoniae* ausgeübt.

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Cytological Effect of Gonadotropin Releasing Factor Activity from Beef Hypothalamic Extract of the Pituitary Cells in vitro

Accumulated evidence indicates that the hypothalamic-releasing factor stimulates the release of gonadotropin by acting directly at the level of the anterior pituitary gland[1]. The activity of the gonadotropin-releasing factor has been demonstrated by incubating the adenohypophysis in vitro. Extracts of hypothalamus (rat, pig, sheep, beef etc.) added to the medium may act directly on this gland and induce secretion of gonadotropins[2–4].

In the present experiment, fragments of rats anterior pituitary glands were incubated in order to study whether an extract of beef hypothalamus may modify the cytological features of pituitary cells. Preparation of crude (HEc) and semipurified (HEsp) hypothalamic extracts were obtained following the method of SCHALLY et al.[4]. The brain cortical extract (BCE) prepared in the same manner served as control. Incubation of glands was made according to MITTLER's technique[5]. The activity of both extracts in terms of the release of FSH and LH from hypophysis was evaluated in castrated testosterone pretreated male rats[6,7] and in castrated, estrogen and progesterone pretreated female rats[8,9]. For cytological studies the anterior pituitary glands of normal adult male Wistar rats (200–250 g) were used; the incubation procedure is detailed in the Table. Brain cortical extract, saline, medium TC 199 and vasopressin were assayed as controls. The hypophysis fragments were fixed in formol-Cl_2Hg, dehydrated and embedded in paraffin. Sections were stained with PAS-colloidal-iron-Kernechtrot and aldehyde-thionin to differentiate the types of basophilic cells.

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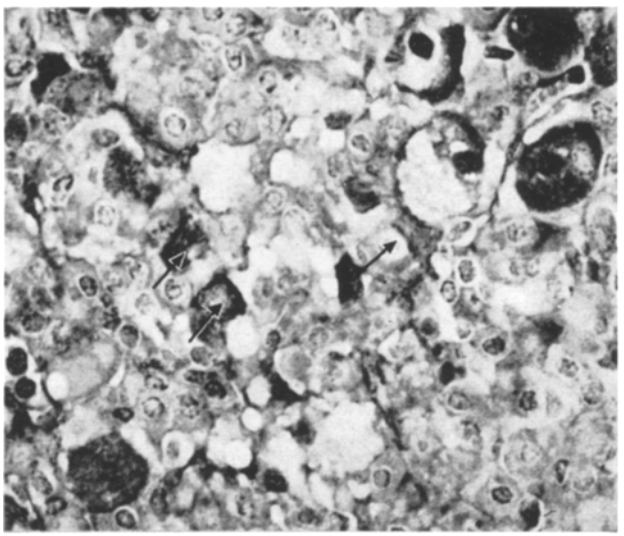
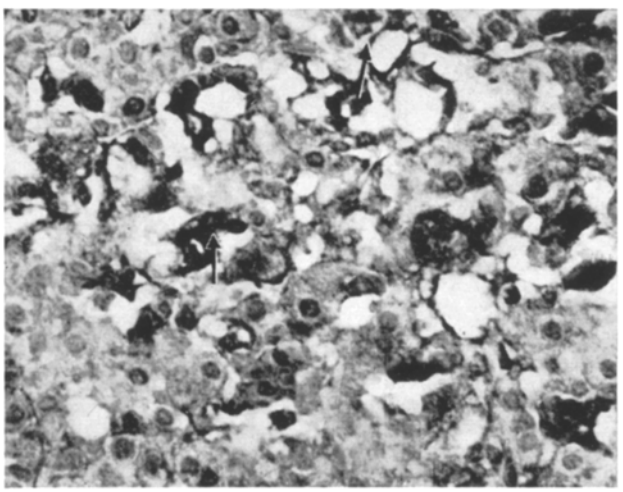


A



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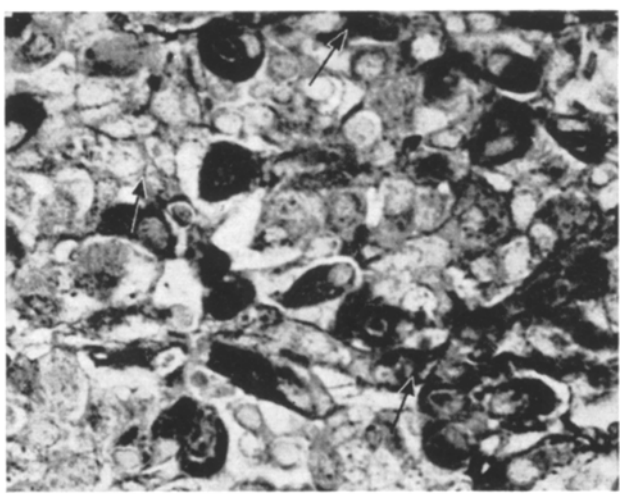

C

Cytology of sections prepared from incubated anterior pituitary fragments. Cells of central region of explants; (A) 10 mg semi-purified hypothalamic extract 1 h; (B) 10 mg HEsp 2 h; (C) 20 mg brain cortical extract 3 h. → Thyrotropic cells. × 6400.

Cytological effect of beef hypothalamic extracts on cells of anterior pituitary gland in vitro

No. of rats	Treatment (dose 10, 20 and 50 mg)	Cell type degranulation Incubation time 1 h	2 h	3 h
6	Saline	–	–	–
6	Medium TC 199	–	–	–
20	HEc + medium TC 199	Gonadotropes		Thyrotropes and acidophils
20	HEsp + medium TC 199	Gonadotropes		Thyrotropes and acidophils
15	BCE + medium TC 199	–	–	–
10	Vasopressin + medium TC 199	–	–	–

Histological examination of anterior pituitary explants taken at 1 and 2 h (Figure A and B) after incubation revealed an increased number and size of the granules from PAS and colloidal-iron positive cells (gonadotropic cells); this is followed by a gradual disappearance of same granules and the appearance of progressive vacuolization. Sections prepared from glands incubated for 3 h indicated that the cells at the periphery of the explants were more degranulated than the cells of the central region. Although less extensive, very similar changes were observed at the third hour in the aldehyde-thionin positive cells (thyrotropic cells), whereas the orange G positive cells (acidophilic cells) also appeared degranulated but no vacuolization was observed. Controls made with saline, medium TC 199 and brain cortical extracts gave no similar results (Figure C); only vasopressin may induce a slight hypertrophy in basophilic and acidophilic cells.

Concerning the effect of different doses used, the crude and purified hypothalamic extracts exerted a similar action on the cytology of the pituitary cells, but the dose of 50 mg induced faster and more extensive morphological changes than 10 mg.

The general effects of the 2 extracts were somewhat different, when injected into animals. The crude extract showed higher toxicity than the semipurified one, producing circulatory and respiratory alterations which caused, with relative frequency, death of the animals.

It should be pointed out that the hypothalamic extracts used in these experiments were tested for their ability to induce the release of pituitary tropins in vivo.

It is hard to postulate whether the effects described above of hypothalamic extract on pituitary cells in vitro are specific and direct. In spite of the similar results obtained in our in vivo experiments, further studies using more purified extracts are needed.

Zusammenfassung. Untersuchungen an spezifisch gefärbten Serienschnitten von Adenohypophysenfragmenten, die mit Rinderhypothalamusextrakten inkubiert wurden, zeigten eine deutliche Zunahme von Sekretgranula der gonadotropen Zellen, Bildung von Vakuolen und Degranulationsbeginn.

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