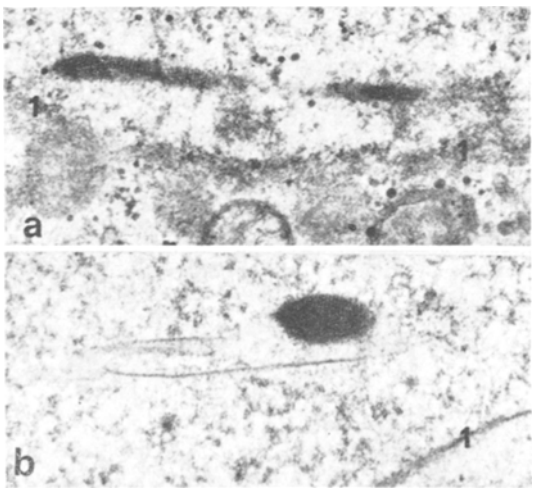
Von allen kristallartigen Einschlüssen, die ich in der Literatur abgebildet finde, ähnelt die Struktur noch am ehesten dem Externum der Einschlüsse, die LANGE und VON BREHM[2] in Epithelzellen der Glandula parathyreoidea von *Rana temporaria* abgebildet und beschrieben haben. Dort ist die Periodizität allerdings nur 70 Å. Die Autoren haben in der Nachbarschaft des kristalloiden Einschlusses ebenfalls eine fibrilläre Struktur gefunden und folgern, dass die beiden Strukturen in irgendeiner Beziehung zueinander stehen.

Filamentöse beziehungsweise tubulöse gestreckte Bündel im Karyoplasma sind in den letzten Jahren mehrfach beschrieben worden. Man hat sie in Nervenzellen[3–5] in der Adenohypophyse und im Nebenhodenepithel[6], in Oozyten[7] sowie in den β-Zellen von Pankreasinseln[8] gefunden. Für alle diese Fundorte ist es charakteristisch, dass sie nur bei bestimmten Arten vorkommen, bei nahe verwandten Formen aber fehlen. Aus den bisherigen Befunden ergeben sich keine Anzeichen eines degenerativen Geschehens. Die unsystematische Streuung der Fundorte solcher Strukturen lässt die Annahme zu, dass diese Strukturen Zeichen einer Änderung des Kernstoffwechsels sind, die zu keiner spezifischen Leistung der einzelnen Zellarten in Beziehung stehen.

Da die hier beschriebenen kristalloiden Einschlüsse mit den filamentösen Bündeln zusammen vorkommen, liegt die Vermutung nahe, dass auch sie der Ausdruck von besonderen Vorgängen im Kernstoffwechsel sind.



(a) Langgestreckter Einschluss im Karyoplasma einer Spermatide, 1,1 tangential getroffene Kernmembran, darunter Mitochondrien. (b) Gedrungener Einschluss, darunter filamentöse Struktur, 1 Kernmembran. Fix.: Glutaraldehyd, OsO_4; Kontrastierung mit Bleihydroxyd. Vergrösserung 40000fach.

Summary. In the caryoplasm of the early spermatids of a Diplopode, there is a crystalloid inclusion which is accompanied by a linear bundle of filaments.

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The Effects of Non-Thermal Radio Frequency Radiation on Human Lymphocytes in vitro

The effects of radio waves on biological material have been reported to be of both a thermal[1–3] and a non-thermal[4–6] nature. The investigation of non-thermal effects has received relatively little enthusiasm since results have often been masked by thermal effects. Nevertheless, chromosomal aberrations[7], decreased enzymatic activity[8], and alteration of polarity ('pearl chain' phenomenon)[9] have reportedly been induced in lower life forms by radio wave radiation. The heating of an organism by means of radio waves (diathermy) is the result of a condition known as a 'lossy dielectric'[10]. Investigation of non-thermal effects of radio radiation on mammalian organisms has been lacking, presumably because of this condition. Tissue culture, however, provides a system whereby heating is avoided or minimized due to the singular nature of the cell type utilized. In addition, the human lymphocyte culture technique provides a high degree of standardization and reproducibility. It was thus felt that this technique would allow for assessment of only the non-thermal effects of radio radiation on human cells in vitro.

Materials and methods. Peripheral blood lymphocytes from 18 individuals (male and female of normal karyotype) were cultured with phytohemagglutinin according to a modification of the technique of MOORHEAD et al.[11]. 3 cultures were established in each experiment and designated control 1 (C_1), control 2 (C_2) and experimental (E). C_1 was placed unshielded in the incubator, C_2 was placed within a grounded shield (Faraday Shield) in the incubator and E was placed within a tuned coil (inductive radiation source) which was housed within another Faraday Shield in the incubator. All cultures were incubated at 37 °C. The temperature of the culture media was monitored manually.

Percentages of hypotonic spreads from control and experimental cultures which demonstrated broken chromosomes

Percentage of spreads showing Broken chromosomes			
Control	0.6%	Experimental	4.0%

The tuned coil was connected to a Stancor model ST-205A mobil radio phone (Standard Transformer Co.). The transmitter, which did not possess an operative modulator section, was crystal controlled with a frequency of 27.120 MHz $\pm$ 1 kHz. The power output was 18 W to the final amplifier with an estimated effective radiating power (ERP) of 10 W.

The cultures from each experiment were harvested in groups as smears and hypotonic spread preparations[12] at given intervals from 1–84 h. 1 h prior to harvest, each culture received 0.1 µg/ml of colchicine (Eli Lilly Co.) and 1.0 µc/ml of H^3-thymidine (specific activity 6.7 c/m*M*, New England Nuclear Corp.). Radioautographs were analyzed for DNA synthetic index (percentage of labeled cells). The non-radioautographic preparations were assessed for growth (percentage of enlarged cells), mitotic index (percentage of cells in mitosis) and for chromosomal aberrations.

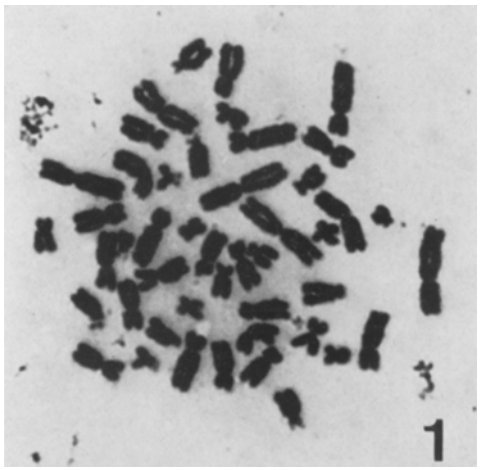

Fig. 1. Hypotonic spread of a human lymphocyte from a control culture which shows normal chromosome morphology. × 1150.

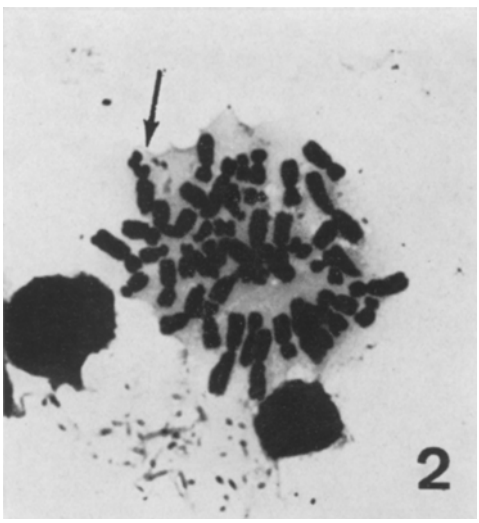

Fig. 2. Hypotonic spread of a human lymphocyte from an experimental culture which shows a broken chromosome indicated by the arrow. × 1150.

Results. No substantial differences were noted between the various cultures of each experiment with regard to DNA synthetic index, growth, and mitotic index. However, analysis of chromosome morphology performed on hypotonic spreads from 72 h cultures, revealed almost 7 times more spreads with broken chromosomes in the experimental cultures than in the control cultures (Table). The 2 control cultures were summed and the percentages of broken chromosomes were compared to the experimental cultures, based on 300 counts per group. Spreads which contained at least 42 chromosomes were used for analysis. A normal chromosome spread is shown in Figure 1 and a spread from an experimental culture showing a broken chromosome in Figure 2.

Numerous readings of the temperature of the experimental cultures revealed variations of less than 1 °C from the control cultures. This was taken as evidence that the radio waves were not producing thermal effects which could alter the results.

Discussion. Non-thermal radio radiation of human lymphocytes in vitro demonstrated no significant difference in growth, DNA synthesis or mitosis between radiated and control cultures. The data obtained for these 3 aspects of human lymphocyte response in culture agree well with those of other investigators[14–17]. On the other hand, cultures radiated continuously for 72 h demonstrated almost 7 times more chromosome breaks than did control cultures. Chromosomal aberrations (bridges) have also been observed in garlic meristematic root cells as well as increased mutation rates in *Drosophila melanogaster*[7] when subjected to radio waves of similar frequency to those employed in the present investigation. The potential danger of radio waves has been inferred provided a sufficiently high field intensity is present[18,19].

It is interesting to note in the present study that chromosomal breaks occurred without apparently affecting DNA synthesis. However, the techniques employed to measure DNA synthesis were not designed to detect small differences in the intensity of the label which might occur if a single chromosome was affected. Similarly, it might be difficult to quantitatively compare the

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observed chromosomal aberrations with slight variations in mitotic index or cellular enlargement. Nevertheless, according to the designated parameters of this investigation, radio waves at 27.120 MHz and 10 W of power (ERP) apparently cause an increased incidence of chromosomal breaks compared to controls without significantly altering cellular response in culture.[20]

Zusammenfassung. Hypotonische Streuungen, die in radiobestrahlten Kulturen menschlicher Lymphozyten Chromosomenaberrationen erkennen liessen, traten dort siebenmal häufiger auf als in unbestrahlten Kulturen; offenbar das Ergebnis einer wärmefreien Bestrahlung. Trotz des Fehlens bedeutender Unterschiede in der DNA-Synthese, der Zellvergrösserung und im mitotischen Index zwischen bestrahlten und unbestrahlten Kulturen kam es zu Aberrationen.

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[20] This investigation was supported by the United States Department of Health, Education, and Welfare National Defense Education Fellowship No. 66-7808.

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Highly Purified Noradrenaline Storage Vesicles from Bovine Splenic Nerve Trunk: Preliminary Electron Microscopy

An improved method for the purification of noradrenaline (NA) storage vesicles from bovine splenic nerve trunk was recently described[1]. Sucrose-heavy water density gradients are used to take advantage of a differential increase in NA vesicle density relative to that of microsomal contaminants. The average concentration of sedimentable NA in the purest gradient fraction, representing $^1/_3$ of the total sedimentable NA in the original nerve trunk, is about 3.5 µg/mg protein. This is a 4–7 fold improvement in purification over values previously reported in the literature[2–6].

From quantitative analyses of marker enzymes to estimate contamination by other subcellular components relative to NA content, it could be calculated that in the purest fraction a minimum of 20% of the total sedimentable protein is associated with the NA storage vesicles. This improvement in vesicle purity enhanced the chances for meaningful electron microscopic examination of the fraction.

Materials and methods. Fraction FIII of purified NA storage vesicles from bovine splenic nerve trunk was prepared as previously described[1]. Aliquots were diluted to isotonicity in 0.25 *M* sucrose containing 20 m*M* potassium phosphate buffer at pH 7.2–7.4. Samples were incubated for 15 min at 37 °C in a medium containing 0.5 µg/ml L-NA, 3 m*M* $MgCl_2$ and 3 m*M* *tris*$_4$-ATP (adenosine triphosphate). This medium is known to prevent loss of NA from isolated vesicles and also to promote uptake of NA into partially depleted vesicles[7]. The incubated samples were chilled immediately to 0–4 °C and layered over an equal volume of 6% glutaraldehyde containing 0.154 *M* potassium phosphate buffer at pH 7.2 to 7.4. The suspension was then centrifuged at 226,000 g_{max}-30 min in a refrigerated Beckman Ultracentrifuge L2-65B. Thus, the vesicles are fixed in suspension while being sedimented into a pellet. The pellets were post-fixed for 90 min in 2% OsO_4 containing the same buffer. Grey to silver-grey sections were cut through the entire pellet from top to bottom, both vertically and parallel to the surface, with a diamond knife on an LKB Ultrotome. The pellets were thin enough to allow the entire depth to be examined in a single section. Sections were stained for 15 min in 4% uranyl acetate and 5–10 sec in lead citrate. Micrographs were taken with a Zeiss EM9A.

Results and discussion. When the vesicle fraction is fixed with glutaraldehyde (or OsO_4) in suspension during centrifugation, the result is superior to fixation of the pellet after sedimentation, particularly in terms of vesicle distribution in the pellet. In addition, incubation of the vesicle suspension with Mg^{++} and ATP before fixation yields a more homogeneous appearing population of vesicles.

A layer of essentially pure vesicles accounting for about 25% of the total pellet depth occurs at the upper surface

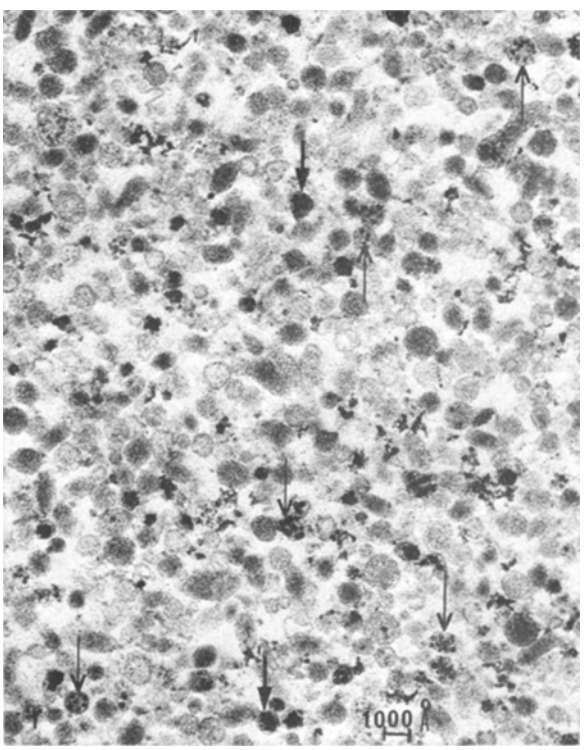

Fig. 1. Purified NA storage vesicles from fraction FIII of sucrose-heavy water density gradient. Thin arrows indicate 200 Å intravesicular granules; thick arrows indicate clusters of these granules giving the appearance of a dense 'core'. × 35,000.