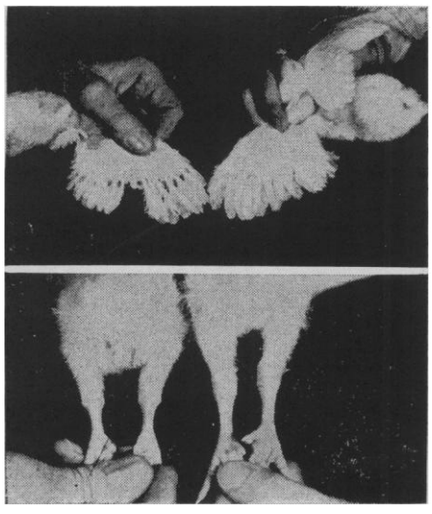

Fig. 1. Hock and feather abnormalities characteristic of zinc-deficient chicks. Wing (top left) shows abnormal thinning of wing feathers; wing (top right) is normal. Legs (bottom left) show knobby enlargement of hocks; legs (bottom right) are normally tapered. Both abnormalities, as illustrated, are relatively mild.

mium of essential zinc-dependent reactions remains to be established, but there is good basis for believing it to be considerable. Parizek (*3*) suggested interference with zinc function as the mechanism of a cadmium-induced pathology, after his observation that testicular degeneration produced in rats by the injection of a small dose of Cd^{++} was prevented if a large excess of Zn^{++} was administered simultaneously with the cadmium. Antagonistic zinc-cadmium effects in relation to testicular function also have been described by Kar *et al.* (*4*) and by Gunn and his co-workers (*5*). Cotzias *et al.*, in tracer studies with rabbits and mice, obtained sufficient evidence of interchange between zinc and cadmium (*6*) and of lack of discrimination between the two elements by certain cell fractions (*7*) to propose that cadmium may be a very subtle poison, competing with zinc and partially replacing it in some organelles and at certain cellular binding sites.

Table 1. Weight gains and incidence of hock and feather abnormalities in chicks fed cadmium or zinc, or both. Day-old Arbor Acre male chicks were used in all the tests.

Added Cd (ppm)	Added Zn (ppm)	Gain (g)	Abnormalities*	
			Hocks	Feathers
Experiment 1 (14 days)				
0	0	165	2/7	2/7
0	20	177	0/8	0/8
0	200	204	0/8	0/8
20	0	114	8/8	7/8
20	20	177	1/8	2/8
20	200	180	0/8	0/8
40	0	103	4/8	5/8
40	20	156	4/8	1/8
40	200	175	0/8	1/8
Experiment 2 (15 days)				
0	0	185	2/8	1/8
0	200	230	0/7	0/7
40	0	107	7/8	8/8
40	200	183	0/8	0/8
40	400	168	0/8	0/8
Experiment 3 (14 days)				
0	0	147	0/14	4/14
0	200	188	0/14	0/14
40	0	111	8/14	8/14
40	200	152	0/14	0/14
40	400	156	0/14	0/14
80	0	62	2/14	6/14
80	200	131	2/14	0/14
80	400	114	0/14	0/14

* Ratio of surviving birds with abnormalities to total surviving birds.

Whatever the specific mechanisms involved, the findings described in this report indicate that abnormal zinc metabolism contributes significantly to the toxicity syndrome resulting from chronic cadmium ingestion. The results suggest, also, that cadmium feeding might prove useful in studies in vivo of the physiological functions of zinc (*11*).

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2. The basal diet, in percentages, is as follows: 31, C-1 Assay Protein (Archer Daniels–Midland Co.); 57, glucose monohydrate (Cerelose); 5, corn oil (Mazola); 0.6, DL-methionine; 0.75, glycine; 0.02, antioxidant (ethoxyquin); 0.017, antibiotic (25 percent oleandomycin mix, Pfizer); 0.025, $FeSO_4 \cdot 7H_2O$; 0.003, $CuSO_4 \cdot 5H_2O$; 0.03, $MnSO_4 \cdot H_2O$; 0.0002, $CoSO_4 \cdot 7H_2O$; 0.002, KIO_3; 0.001, H_3BO_3; 0.02, $KAl(SO_4)_2 \cdot 12H_2O$; 0.001, $Na_2MoO_4 \cdot 2H_2O$; 0.005, $Na_2SiO_3 \cdot 9H_2O$; 0.002, NaBr; 0.00006, Na_2SeO_3; 0.3, NaCl; 0.77, KCl; 0.73, Na_2HPO_4; 3.21, $Ca_3(PO_4)_2$; 0.25, $MgSO_4 \cdot 7H_2O$; 0.23, $CaCO_3$; 0.48, vitamin mixture. The vitamin mixture supplies components (in milligrams per 100 g of diet) as follows: 200, choline chloride; 100, inositol; 0.6, menadione sodium bisulfite; 0.003, vitamin B_{12}; 2, ascorbic acid; 0.5, *p*-aminobenzoic acid; 1, folic acid; 0.08, biotin; 3, pyridoxine HCl; 7, niacin; 4, calcium pantothenate; 2, riboflavin; 2, thiamin HCl; 3.2, vitamin A concentrate (325,000 I.U./g), 12.5, vitamin D concentrate (15,000 U.S.P. unit/g); 140, vitamin E concentrate (44 I.U./g). The diet contains adventitious zinc in the amount of 15 parts per million. Supplementary zinc was added in the form of $ZnCO_3$; cadmium, as a 1.63-percent aqueous solution of $CdCl_2$.
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Chromosome Fibers from an Interphase Nucleus

Abstract. *Red blood cells of the newt,* Triturus, *were spread on a water surface and picked up with carbon-coated grids for examination in the electron microscope. The identifiable nuclear material consists entirely of long fibers having a diameter of approximately 400 to 600 Å. Similar fibers have been seen in human and grasshopper chromosomes prepared in the same manner.*

Conventional sectioning techniques have proved to be of limited value in nuclei studies with electron microscopy. Whole chromosome mounts and interphase nuclei have been examined but they are generally too thick for high resolution studies. Two technical problems must be overcome in developing a successful "squash" or "spread" technique for electron microscopy: (i) the nuclear material must be spread into a very thin layer, and (ii) it must be dried without undue destruction.

A solution to the first problem is suggested by the recent work of Kleinschmidt and Lang (*1*), who have obtained striking preparations of fibers by spreading bacteria and viruses on a liquid surface. Drying in air may be adequate in some cases, but the critical point method of Anderson (*2*) should be more generally useful.

A simple Langmuir trough was constructed from a glass butter dish; glass strips coated with paraffin served as barriers (*3*). The trough was filled with distilled water, and the surface was swept clean. The nucleated red blood cells of the newt, *Triturus*, were selected for test. After the tip of the newt's tail had been clipped off, a small drop of extruded blood was transferred quickly to the end of a fine glass rod. The rod was dipped lightly into the water, whereupon the blood was seen to spread over the surface. Depending upon the size of the drop of blood, the film so formed had an area of 25 to 50 cm^2. The material was picked up by touching carbon-coated grids to the surface (just as sections are picked up from a water surface). The grids were transferred, while still wet, to an ethanol series; they were finally dried either by evaporation or by the critical point method. Some grids were strained for 10 to 15 minutes with a 2 percent uranyl acetate solution before transfer to the ethanol series. Others were shadowed with platinum-palladium after they had been dried.

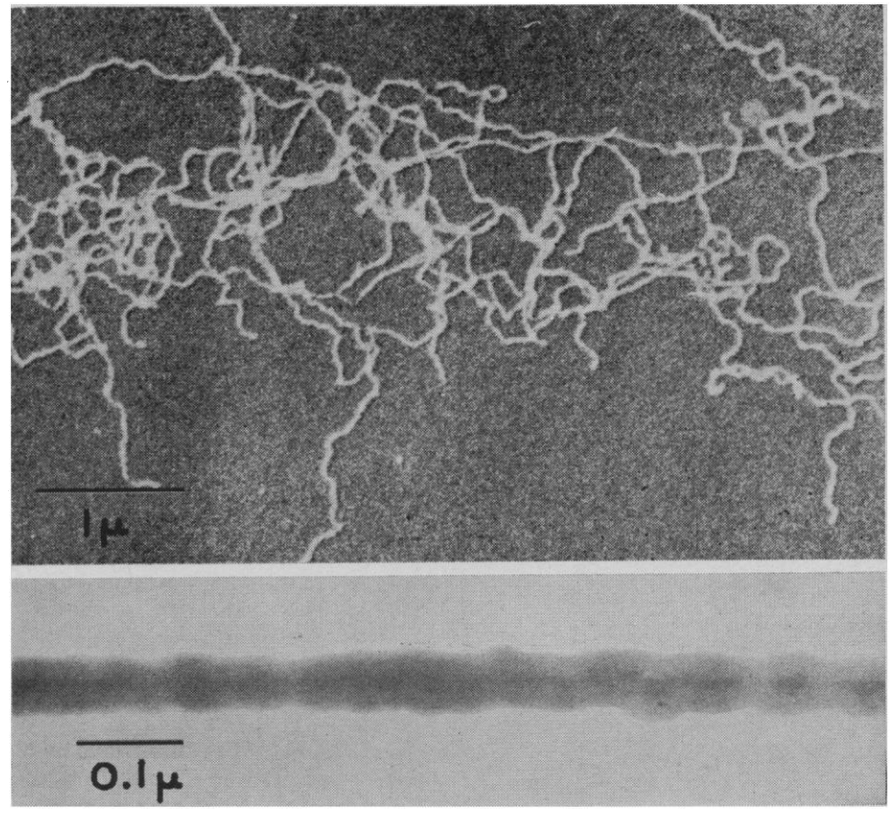

Fig. 1. (Top) Fibers from red-cell nucleus of the newt, *Triturus*, spread on water surface: Air dried, platinum-palladium shadowed (× 19,000). (Bottom) Portion of a single fiber, showing dense core: Uranyl stain, critical point drying, unshadowed (× 140,000).

In the electron microscope one can readily identify the characteristic oval nuclei, of which there are usually one to ten per grid. Individual nuclei vary considerably in the extent to which they are spread. A few retain the dimensions found in the living cell, but the majority are frayed out around the edges and some are completely disrupted. Membranes are present in the preparations but their origin is uncertain. In all cases the remaining nuclear material consists exclusively of fibers about 400 to 600 Å in diameter (Fig. 1, top). The DNA concentration of these nuclei is particularly high and approaches that of metaphase chromosomes. The fibers derived from such nuclei are, therefore, thought to represent interphase chromosomes. In the central region of the nucleus, which has presumably been subjected to least tension, the fiber diameter is remarkably uniform and there are very few free ends. Near the periphery of the nucleus the fibers are often broken into shorter segments and many show signs of stretching and thinning. It is clear from the overlapping of fibers and from variations in focus within a single micrograph that most of the fibers are not drawn completely into the surface film during the spreading process. High resolution micrographs of uranyl-stained material show a more densely stained core within the fibers. The diameter of the core is variable, depending in part on the degree of stretching; it is approximately 150 Å in the fiber shown in Fig. 1, bottom.

Davies and Spencer (*4*) have studied sections of red blood cells from the frog. They have described dense regions within the nucleus which could correspond to the fibers demonstrated here. However, it seems probable that the fibers are partially obscured in sectioned nuclei by the presence of "nuclear sap" proteins which are lost in the spreading technique.

The technique described here should be generally applicable to any cells which will spread on water. Success has already been obtained with human tissue cultures (HeLa) and testes of the grasshopper (*Melanoplus*), as well as with red blood cells of other organisms (*Necturus, Rana*). The chromosomes of dividing cells are easily recognizable on the basis of their gross morphology. Human chromosomes in metaphase and grasshopper chromosomes in meiotic prophase consist of fibers similar to those from the red-cell nuclei of the newt. The younger spermatid nuclei of the grasshopper likewise contain fibers, but the older ones display the lamellae characteristic of this stage (*5*).

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