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Environmental Research Laboratories

CHROMOSOME MUTAGENESIS IN DEVELOPING MACKEREL EGGS SAMPLED FROM THE NEW YORK BIGHT

A. Crosby Longwell

Marine Ecosystems Analysis Program Office
Boulder, Colorado
April 1976

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Chromosome Mutagenesis in Developing Mackerel Eggs Sampled from the New York Bight

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Introduction

In the last 30 or so years a diversity of chemicals has been released into the environment that individually, collectively or synergistically have powerful mutagenic effects, just as does radiation (Sanders, 1969a,b; Fishbein et al., 1970; Legator, 1970; Bridges, 1971; DuBois, 1971; Hollaender, 1971a,b, 1973; Malling, 1972; Ramel, 1973; Committee 17, Environmental Mutagen Society, 1957). Almost all readily recognized mutations, whether induced or spontaneous, are harmful. Importantly, mutagens can cause genetic damage at sub-toxic concentrations.

It is a fact of hitherto mostly unconsidered implications to the fisheries that, in addition to radionuclides, such major classes of marine pollutants as heavy metals and pesticides are among such recognized environmental mutagens (Longwell, 1975). These substances accumulate in the cells of marine species generally, and in fish (Waldichuk, 1974; NOAA First Interim Report on Microconstituent Resource Survey, 1975). Herring eggs incubated in seawater with a cadmium concentration of 1.0 ppm yielded a very low percentage of viable larvae relative to controls (Rosenthal and Sperling, 1974; Westernhagen et al., 1975). In laboratory experiments Rosenthal and Sperling (1974) and Westernhagen et al. (1974) were able to show that fish eggs incubated in cadmium-polluted water accumulate the metal. Recently, elevated concentrations of cadmium were reported in plankton off Baja, California (Martin and Broenkow, 1975).

On the basis of numerous tests on non-aquatic organisms, pesticides are known to have effects on chromosomes and their normal division process, which range from no observable effect to one greater than that for the most powerful mutagens. See the following: D'Amato, 1952; Unrau and Larter, 1952; Amer, 1965;

Legator et al., 1969; Umeda et al., 1969; Hoopingarner and Bloomer, 1970; Epstein and Legator, 1971; Ahmed and Grant, 1972; Tomkins and Grant, 1972; Dikshith and Datta, 1973; Parry, 1973.

Genetic effects of heavy metal exposure are generally the same type as those which result from ionizing radiation and from treatment with well recognized mutagenic chemicals, as mustard gas. See the following: Levan, 1945; Oehlkers, 1953; Gläss, 1956; Potter, 1961; Ramel, 1967; Muro and Goyer, 1969; Shaw, 1970; Paton and Allison, 1972; Shiraishi and Yosida, 1972; Shiraishi et al., 1972; Teisinger et al., 1973; Friberg et al., 1974. Heavy metals are known, in addition, to have embryopathic effects in mammals (Ferm and Carpenter, 1967; Gale and Ferm, 1971), and also in the American oyster (Calabrese et al., 1973).

Fish are the most radiosensitive form of marine life, and the radiosensitivity of fish eggs approaches that of mammalian zygotes (Donaldson and Foster, 1957; Polikarpov, 1966; Purdom and Woodhead, 1973). Recent study of a freshwater mud minnow, Umbra limi, unusually well suited to cyto-genetic study for a fish because of its low number of large chromosomes, showed the fish to have an incidence of chromosome breakage on radiation similar to that of other vertebrates (Kligerman et al., 1975). Eggs as those of the Atlantic mackerel must be undergoing highly sensitive meiotic divisions as the fish move into polluted coastal waters and swim up to surface water layers to spawn (for spawning of mackerel see Sette, 1950a; Macer, 1974). Moreover, fish eggs are spawned while they are still in the second half of meiosis. They must so complete meiosis, be fertilized, and undergo cleavage and embryo mitosis protected only by their membranes. The well-known special susceptibility of cells in gametogenesis to induced mutation is further increased as the egg enters early cleavage (Solberg, 1938; Mueller, 1959; Murakami, 1971). Contaminants accumulated in

fish eggs from the body of the female would have increased opportunity of effecting genetic damage as the egg moved into this most genetic-sensitive stage at cleavage. At this same sensitive stage the egg would also be under exposure to whatever other mutagens could enter through its membranes from polluted waters in which the developing eggs are suspended. Chromosome abnormalities carried by the sperm would, in addition, contribute to the genetic disturbances observed in the developing egg. The germ-line primordial cells of a successfully hatched juvenile fish are formed as early as gastrulation and are so subject to irreversible genetic damage at this early stage of embryogenesis along with somatic cells.

Similar to radiation, chemical mutagens can lower the rate of cell division (Vogel and Röhrborn, 1970). Fish embryos with incorporated radionuclides have a reduced mitotic index (AEC-TR-6940, 1968). As inhibitors of the spindle apparatus, methyl and phenyl mercury compounds are more potent than any other substance known, including colchicine commonly used in experimental inhibition of the spindle apparatus (Fiskesjo, 1969; Rep. Intern. Committee, 1969; Ramel, 1972). A lowering of the rate of cleavage mitosis alone could result in developmental and genetic errors as the fish eggs age. Recently, chlorinated hydrocarbons, DDT, and polychlorinated biphenyls, ubiquitous pollutants of the marine environment, have been reported to reduce the rate of cell division of marine phytoplankton (Fisher, 1975).

The pelagic eggs of sea fish develop in the uppermost layer of the ocean. Not only is the oceanic microlayer exposed to all the pollutants in the atmosphere, but it absorbs the brunt of oil spills. It avidly concentrates heavy metals and long-lasting chlorinated hydrocarbons (MacIntyre, 1974).

From studies of mammals and insects it is known that most dominant lethal mutations which kill at early stages of development are associated with gross chromosome alterations leading to chromosomal, hence genic imbalance (Sonnenblick, 1940; Whiting, 1945; Mueller, 1954a; Cave and Brown, 1957; von Borstel and Rekemeyer, 1959; von Borstel, 1960; Epstein et al., 1970; Bateman and Epstein, 1971). In man practically all spontaneous abortions are probably due to chromosome alterations (Hertig et al., 1956; Warburton and Fraser, 1964; Carr, 1967, 1969). Up to 24% of early dead chicken embryos have abnormal chromosomes (Bloom, 1972). Chromosome mutations induced during the time of major organogenesis can lead to developmental abnormalities (Solberg, 1938; Mueller, 1954a,b; Russell, 1956; Allen and Mulkay, 1960; Moore, 1963; AEC-TR-6940, 1968; Austin, 1969; Carr, 1969; Hirschhorn and Cohen, 1969).

In spite of the acknowledged importance of direct studies on the neuston, the richest biosphere in the world (Polikarpov, 1966), all development studies of fish eggs have employed the spawn of experimental, laboratory-treated fish. Only a very limited amount of cyto-genetic work has been conducted even on experimentally treated fish eggs. Most of this has been done by Russian workers studying the effects of radiation on developing fish eggs (AEC-TR-6940, 1968; AEC-TR-7299, 1972). Roberts (1967) reviewed the status of chromosome cytology of the Osteichthyes. Although certainly not so convenient as working with experimentally spawned and experimentally treated fish eggs, there is no technical reason why the chromosomes and division apparatus cannot be studied in field-spawned eggs sampled directly out of polluted waters. Such would show the cyto-genetic stage of the developing eggs actually in the fishery on their post-spawning exposure to waters where they were spawned naturally in the presence of pollutant combinations hardly duplicable experimentally.

A cruise of the Westward*, May 7-18, 1974, sponsored by the Marine Ecosystems Analysis (MESA) of the U. S. Department of Commerce, National Oceanic and Atmospheric Administration, and assisted by the Middle Atlantic Coastal, and Northeast Fisheries Centers, provided fish eggs from the neuston of the New York Bight for cyto-genetic study. The New York Bight is one of the most heavily polluted coastal areas in the United States and, at the same time, the spawning grounds for large numbers of commercial fish of most value to the U. S. fishermen (NOAA/PA 74012, 1974; Hess and Stanford, 1975). Almost 80% of the ocean dumping of municipal and industrial wastes occurs in this area (Dewling et al., 1975). There is, in addition, heavy atmospheric fallout to the Bight (Duce et al., 1975). While pollutant input from other sources is below the surface of the water, atmospheric input is through the water surface and neuston where pollution may have its most direct serious effects on the entire ecosystem. Compared to background mass loads, heavy metals are the largest class of man-made inputs directly into the Bight (Mueller and Jeris, 1975). There is a special purpose, deep water chemical dump site, located in close proximity to the Bight, used for disposal of industrial wastes deemed too toxic to put directly into the estuaries. Just south of this deep water chemical dumpsite is an old, now unused radioactive waste dump. It is very likely that even the general degradation of the environment in the Bight could contribute to the increase in the rate of chromosome aberrations in developing fish zygotes. There is evidence that environmental stresses, such as hypoxia shortly after fertilization, may induce detectable chromosome changes in developing eggs (Huang and Clark, 1967; Endo and Ingalls, 1968). Increased nitrite levels in

*Westward of SEA (Sailing Education Association).

the water column resulting from denitrification of organic matter, which occurs at reduced oxygen levels, could increase the natural mutation rate. Stress appears to accentuate the genetic-damaging effects of radiation (Evans, 1962) as it probably also does for other mutagens.

Sample Collection and Methodologic Procedures Developed

Part of the egg samples was collected in neuston nets (0.947 mm mesh) and part in bongo nets (0.333 and 0.505 mm mesh). Tows of the nets were made at 1-3 knots per hour. Towing time was from 1.5 to 7.5 minutes for the neuston samples, and 15 minutes for the bongo samples. The neuston nets sampled only the upper 6 inches of the water. Bongo samples were taken in an oblique tow starting at the surface and averaging 5 to 10 meters off the bottom. Bongo samples would so have included eggs at the surface, as sampled with the neuston nets. Because of the species identification of the eggs and spawning behavior of adults of that species, both bongo and neuston samples consisted of eggs from the uppermost portion of the water columns.

Sea surface temperatures recorded at Westward sampling stations in the New York Bight at the time eggs were sampled ranged from 10.4°C to 12.2°C. At 10 feet recorded temperatures were from 9.2° to 12.1°; 20 feet, 7.0° to 11.1°; 30 feet, 7.1° to 9.6°; 40 feet, 7.1° to 9.6°. Sampling was done at a time of the year when the thermocline would just be developing.

At most of the approximately 40 sample stations where fish eggs were found, aliquots of eggs were fixed in both Carnoy 3:1 solution for cyto-genetic study, and in a 1:10 dilution of neutralized commercial formaldehyde. The fixing fluid never had more than an approximate 15% carry-over of seawater, and usually much less. Formalin-fixed eggs were originally intended only for species identification of the eggs.

There was no evidence from gross morphology that the eggs were damaged in the sampling process. As specifically requested, fixation was done rapidly on removal of eggs from the water.

Eggs collected from the 15 sample stations chosen for initial cyto-genetic study were identified by W. G. Smith of the Sandy Hook laboratory of the National Marine Fisheries Service as being almost exclusively those of Scomber scombrus, the Atlantic mackerel. Of 100 randomly selected eggs of 14 stations reported here only two stations showed eggs of other fish. One station had 1 egg of a Urophycis (hake) species, and 14 eggs of 3 unidentified species. Another had 4 eggs tentatively identified as those of Scophthalmus aquosus (windowpane flounder).

Extraneous material and small organisms which would interfere with preparation of the microscope slides were easily removed from the field samples by screening.

With exception of the first eggs examined, the embryos were dissected off the eggs prior to their staining and preparation of the microscope slides for cyto-genetic study. Dissection is accomplished with ordinary sewing needles while eggs are viewed under a low-power dissecting microscope. To reduce movement of the egg about the dish an egg to be dissected is first moved into a groove etched onto the edge of a glass microscope slide glued onto the dish used to hold the eggs. About 60 embryos can be removed per hour from the approximately 1 mm diameter Scomber scombrus eggs. If the embryo is not cut off the eggs, it sometimes conveniently pops from the egg membranes in the process of slide preparation. Plates 1 and 2 show intact eggs alongside dissected embryos, of the early cleavage stage of the embryo, the morula, blastula, gastrula, early embryo, the tail-bud, and tail-free stage.

After storage for a few weeks in the laboratory during which time methodologic procedures were being worked out, the Carnoy-fixed samples intended for cyto-genetic study lost their stainability with stains used for chromosome studies employing the now almost exclusively used squash technique. The fish eggs or dissected embryos were rendered stainable again for squash preparations in 1% or 2% aceto-orcein in 45% or 60% acetic acid by treating them with 1% NaOH for 2 minutes at room temperature. They were carried through 35%, 25%, 15% and 5% acetic acid to the dilute NaOH, then back up the series to the stain. Staining was from several hours to overnight.

It has since been found that the NaOH treatment of formalin-fixed samples can be eliminated if the standard aceto-orcein stain is diluted before use with 19 parts of stain to 1 part proprionic acid just before the embryos are put in the stain on the microscope slide, as described below. This procedure is now being used exclusively since staining intensity does not vary from embryo to embryo as with the NaOH method.

It was determined that chromosome preparations equally good to those obtained from the Carnoy-fixed samples could be obtained from the formalin-fixed samples. (This opens up the possibility of using already collected plankton samples for such work.) Eggs are post-fixed overnight in 45% acetic acid. Embryos are either treated with NaOH, or proprionic acid added to the orcein stain, as formalin-fixed eggs also lose stainability of their chromosomes on storage.*

*Pre-treatments and staining procedures are discussed fully in a methods paper in preparation.

Dissected embryos are flattened onto the microscope slide in a few drops of aceto-orcein stain with pressure from the thumb on the coverslip as usual in chromosome squash techniques (Darlington and La Cour, 1966). Usually, 4 embryos can be squashed onto one slide in the aceto-orcein stain without monolayer pieces of different embryos becoming mixed. See Plate 3. Both embryos and earlier blastodiscs remain largely intact in the flattening process. Large portions of tail-bud embryos can be squashed into a monolayer, and at early stages entire embryos or blastodiscs. At the late-tail-free embryo stage monolayers become more difficult to achieve.

Entire sections of monolayer cells are viewed for general presence of mitotic figures in the 16 X phase-contrast objective. For scoring abnormalities of the chromosomes and their division figures a 100 X phase-contrast neofluar lens is used. Plate 4 (Figs. 1 and 2) shows low-power, phase-contrast images of monolayers of cells from the early embryo stage. Different non-dividing cell types are clearly seen, as well as some mitotic figures of the type studied under higher power resolution.

Results

Eggs of the Atlantic mackerel, Scomber scombrus, from 14 different sample stations in the New York Bight were studied cyto-genetically. These were collected and fixed on the May 7-18 cruise of the Westward into the Bight, as described above.

Embryos of the Atlantic mackerel, Scomber scombrus, examined ranged from early cleavage (1-64 cells) to the tail-bud stage (stages according to Mansueti and Hardy, 1967). However, the largest portion of all data recorded from all stations on individual cells within embryos came from the early embryo stage,

which follows gastrulation and precedes the tail-bud stage. Grossly, almost all embryos appeared morphologically normal on dissection from their eggs.

A grand total of 470 eggs from the several stations was scored simply for complete absence of chromosome and mitotic abnormalities. Less than 20% of these eggs had all their scorable chromosome and mitotic figures normal.

Mitosing chromosome figures were scored for chromosome abnormalities and division errors in 30,689 embryo cells in 452 eggs from the 14 different sample stations. About one-third of all mitoses scored showed some abnormality of the chromosomes or their division. The mitotic figures of some embryos were almost all abnormal. The cells of other embryos were nearly all normal. In one embryo a translocation bridge was clearly observed in a number of mitoses in one embryo sector, while mitoses over the remainder of the embryo were normal.

Abnormalities extended the entire range of radiomimetic effects on the chromosomes and their division apparatus. They included severe stickiness of metaphase chromosome figures in embryos also displaying such stickiness of anaphase chromosomes that mitosis was inhibited. Sometimes the extremely sticky mitotic figures were merely cleaved by dividing cytoplasm. Some metaphases showed the chromosomes only as partitioned masses. Commonly observed were laggard chromosomes at anaphase and chromosomes outside of the spindle field at metaphase. There were single and multiple translocation bridges and also sticky bridges. There were acentric fragments of broken chromosomes, and micronuclei formed from broken, unoriented or lagging chromosomes. Some of the chromosomes were so far outside the spindle field they must have developed from micronuclei developed in a prior division. There were a few instances of severe pulverization of the chromosomes.

Plates 5 through 10 (Figs. 3-14) show normal division figures in the Atlantic mackerel embryos; plates 11 through 15 (Figs. 15-31) show the various types of abnormal chromosome figures.

As work progressed, methodology was greatly improved, as noted above. Thus, the first stations studied have far fewer mitotic figures scored for about equivalent numbers of eggs (see Table 1). This was due to the obscuring by the egg membranes of large portions of the embryos which might otherwise have been studied. Subsequent to study of the first stations, once embryos were removed from the egg and its membranes, large numbers of mitoses became available for study in most eggs. These were scored in all portions of the eggs suitably flattened and stained. Even in eggs of the last of the stations examined here, however, embryo staining was variable. Wide variation in numbers of cells that could be examined in various embryos does not mean that any single score was less reliable. (Improved methodology now makes it possible to score mitotic figures in nearly all mitosing cells of an embryo.)

For the 14 Westward stations studied over the New York Bight the mean percent of cells with at least one chromosome or mitotic abnormality varied from 12.7 to a high of 78.6 (see Table 1). For location of samples in the Bight see map with mean percent abnormalities marked next to the sample stations (Plate 16). Station 8 had an overnight re-run sample, which was treated separately.

Two stations (Nos. 6 and 7), less than 5 miles apart along the Long Island coast north of Sewage Sludge, were among those with the lowest incidences of chromosome aberrations and mitotic abnormalities (24.4% and 21.7%, respectively). A third station, No. 28, south of the Long Island coast halfway to Montauk Point, was somewhat higher (34.2%).

Station 9, about 5 miles southwest of the two coast stations in the direction of Sewage Sludge and Dump Sites, hardly differed from them at all (28.2%). Station 8, though, also southwest of the coastline and 5 miles from Station 9, showed a higher incidence (55.3%) in one of two samples. The second sample taken, an overnight re-run, was somewhat lower (41.1%).

Station 37, near Acid Waste, showed an appreciably higher incidence of abnormalities (60.6%). Station 10, to the southwest, and Station 11, to the south of Acid Waste, also showed somewhat increased numbers of abnormalities (38.6% and 52.1%, respectively).

Away from the dump sites and coast about halfway to the edge of the continental shelf 4 stations showed relatively low rates of abnormalities akin to those of the two Long Island stations. The mean % of early embryo cells with chromosome aberrations and mitotic abnormalities at Station 35 was only 12.7. For Station 18, this was 35.1; Station 16, 30.2; Station 15, 15.8.

Another outermost station, No. 19, the furthest point from Sewage Sludge and Dump Sites and approximately 55 miles off the New Jersey shore, had the highest incidence of abnormalities (78.6%). This station was closer (approximately 67 miles) to the Toxic Chemicals Disposal Area and Radioactive Waste Disposal Area than other samples. A station in relatively close proximity (20 miles) to this one, No. 21, about 45 miles off the New Jersey shore and 79 miles from the Toxic Chemicals Disposal Area, had the second highest incidence of abnormalities (63.1%).

This Station 19 was the only one with any significant egg mortality. On the basis of cell contrast and deterioration of the nuclei, 20 of 76 eggs (26%) were cellularly dead. There was not yet any gross deterioration of the embryos

or eggs. Moreover, a considerable portion of the cells scored for Stations 19 and 21 had multiple abnormalities, in contrast to the pattern of single abnormalities usually observed in cells of other stations.

In an attempt to determine if old collections of field-sampled fish eggs could be studied to provide a baseline for current studies, using the same or somewhat modified methods, a combination of old samples of mixed planktonic species was processed. The combined sample consisted of a mixture of 2 species from Long Island Sound collected in 1944 by S. Richards of Yale University, and a mixture of a 1966 collection from the New York Bight obtained from W. Smith of the Sandy Hook laboratory (MACFC). Species were mixed since a unique staining response of some one species could give an incorrect impression of the ease or difficulty of working with old plankton samples. At the time these older eggs were processed staining was more variable with the older samples than with the Westward eggs. (Present methodology works equally well on samples several years old as on relatively new samples only a few weeks old.) In 13 eggs of this mixed sample 20,875 mitosing figures were scored. Embryos of these old eggs had a lower incidence (4.8%) of cyto-genetic abnormalities than any sampled in the New York Bight in 1974. The low figure is probably of some biological significance in relation to Westward samples. However, some embryos at later developmental stages in this old collection contributed a significantly large portion of cells scored. Data may not then be directly comparable to those of the Westward samples on mostly earlier embryo stages. Information on these old eggs is presented here merely as an indication of how eggs might be compared cyto-genetically even over decades.

Statistical analysis of the data was done using both the "t" test comparing each station in all combinations and Duncan's new multiple range test. The 0.05 level of significance was used. Results of the two tests agreed generally and revealed a similar pattern of statistically significant differences. See Table 2 for results of "t" test pairs and Tables 3 and 4 for results of the multiple range test.

With the "t" test pairs the combined species of old eggs from 1966 and 1944 differed significantly from all but the two Westward samples with least abnormalities, both outermost stations halfway to the edge of the continental shelf (Nos. 35 and 15). In the multiple range test the combined old samples, in addition, failed to differ from the three Long Island coast stations of low means (Nos. 7, 6, 9).

The peripheral Westward station with least abnormalities (No. 35) differed in the "t" test from all others, except the peripheral station of second lowest mean (No. 15) and the three near Long Island shore (Nos. 7, 6, 9). The second best peripheral station (No. 15) showed a near identical pattern of differences. Unlike Station 35 it did not differ from one of the other peripheral stations of somewhat higher mean (No. 16). In the multiple range test the two best peripheral samples did not differ from Long Island or stations of intermediate means. Significant differences were only with the near dumpsite stations and the two southernmost stations off the New Jersey coast.

Both Long Island Stations 6 and 7 showed an identical set of differences which did not vary in the two statistical tests. They differed significantly from one of the two samples of Station 8 southwest of the coast in the direction of Sewage Sludge and Dump Sites. These shore stations also differed from

Station 11 about 15 miles south of Acid Waste. There were differences also with Station 37, near Acid Waste, and from the two southernmost stations off the New Jersey coast (Nos. 19 and 21).

In both tests Station 9, about 5 miles from Station 8, differed significantly only from Station 19, the one with the highest incidence of abnormals.

A peripheral station (No. 16) of higher mean than the two best ones, and also No. 28, south of the Long Island shore halfway to Montauk Point, differed in the "t" test pairs from Station 11, south of Acid Waste, Station 37, near Acid Waste, and from the two southernmost stations. In the multiple range test they did not differ from Station 11, but otherwise showed the same differences.

In both tests the peripheral station with highest mean (No. 18) differed only from the station with the highest incidence of abnormals (No. 19) off the New Jersey coast. Station 10, to the southwest of Acid Waste, and the overnight re-run sample of Station 8, about 5 miles southwest of the Long Island stations, similarly differed in both tests only from the worst southernmost station.

Station 11, to the south of Acid Waste, differed in both tests only from the station with the highest mean off the New Jersey coast (No. 19).

In neither statistical test were there any significances among the 4 stations with highest means; that is, there were no significant differences among the regular (daytime) sample of Station 8 to the southwest of the Long Island stations, No. 37, near Acid Waste, and Stations 21 and 19, off the New Jersey coast.

Discussion

In a continuation of this study mackerel eggs collected at all 40 Westward stations are being studied in depth with comparisons of cyto-genetic abnormalities over the developmental stages from early cleavage to the tail-free embryo stage.

A rapid scoring system provides data on chromosome breakage based on chromatin bridges observed between telophase nuclei of dividing cells and on laggard and misoriented telophase chromosomes. At the same time data on the mitotic index are collected and a record made of eggs that are moribund at the cell level.

Fortunate sampling of almost entirely one species of identifiable eggs makes it possible to rule out at least some variable pre-spawning exposure of parents to pollutants that would result in different contaminant loads of eggs on spawning. The Scomber eggs studied here were presumably spawned by the southern contingent of the two Atlantic mackerel populations (Sette, 1950b). In the mackerel, gametogenesis must utilize tissue supplies of food reserves stored during the summer while the fish feed in relatively unpolluted waters. The southern contingent of Atlantic mackerel spend the summer feeding in the Gulf of Maine, and overwinter off the continental shelf (Sette, 1950b). The only major impact Bight pollution may have on mackerel surviving their juvenile period in the Bight is on their spawned, developing eggs and perhaps on their nearly ripe pre-spawned eggs. Yet, the incidences of abnormalities recorded for some stations near the Acid Waste Dump Site, and the two stations off the New Jersey coast halfway to the edge of the continental shelf, are significantly higher than those of other stations in the Bight. Particularly with the cytogenetic scoring system used in the study of the mackerel eggs from the Bight, not all chromosome and division irregularities are microscopically detectable. The collection of this Westward data on largely the early embryo stage means that earlier stage zygotes, so chromosomally abnormal that they could not gastrulate, are not represented. As dead planktonic eggs deteriorate and their oil

globules are dispersed, they must eventually sink and no longer be sampled in the neuston. Differences between best and worst stations must so have been minimized in the study reported here.

The two central regions of high heavy metal concentration in the New York Bight around disposal sites have already been shown to correlate well with areas which show a marked impoverishment of benthic fauna (Pearce, 1970). Areas around disposal sites are populated by benthic communities of low diversity and high dominance of taxa apparently resistant to the stress conditions associated with dredge spoil and sewer sludge. Lower crustaceans exposed to sewer sludge and dredge spoil develop distinct pathologies.

Pollution input from Long Island and New Jersey coastal zones is small, contributing less than 6% of the total Bight input (Mueller and Jeris, 1975). This appears to be reflected generally in the Westward data. The high incidences of abnormals in the two southernmost samples occur at stations about 50 miles off the New Jersey coast. These stations have closer proximity to the Toxic Chemical Disposal Area (NOAA Report 75-1, 1975) than others reported here, but are yet about 75 miles distant from it. Additional work on other Westward sample stations is further revealing a trend towards a high aberration rate west of the entire Hudson Canyon along a line to these two southernmost stations found to have such high aberration rates.

These Stations 19 and 21 off the New Jersey coast were the only two studied at which eggs other than Atlantic mackerel were present in the sample. At Station 19, eggs other than mackerel constituted 15% of a random sub-sample. At Station 21, eggs other than mackerel were 4%. Embryos were of necessity (at that time) studied cyto-genetically before eggs of the sample were identified and sorted. In one sample a few embryos clearly had a lower species chromosome number than in all other embryos at this and other stations. There is though no basis to assume eggs of these other species at Stations 19 and 21 were selectively picked out for microscopic study.

Natural environmental factors, as too low a temperature, may have teratological effects linked with chromosome abnormalities in cold-blooded animals and in plants (Blakeslee and Cartledge, 1927; Darlington and La Cour, 1940). A temperature of about 0°C appears to inhibit the spindle apparatus in fish (Svardson, 1945; Swarup, 1959) and also in the newt (Barber and Callan, 1943). In the fish, Fundulus, teratological effects of low temperature are accompanied by irregular distribution of the chromosome material (Kellicott, 1916).

Temperature effects probably did not contribute significantly to the induction of cyto-genetic abnormalities reported here for the Scomber scombrus eggs. Recorded temperatures in the New York Bight (sea surface temperatures 10.4°-12.2°C) at the time these eggs were sampled appear to be compatible with normal development of the Atlantic mackerel embryos. According to Sette (1950a) prevailing temperatures on the spawning grounds at the height of the spawning season of this fish are between 9° and 12°C. The higher maximum temperature for development of Scomber scombrus eggs reported by Worley (1933) is attributed by Sette (1950a) as most likely due to the fact that mackerel used in the experiment were taken from seawater at a higher prevailing temperature.

Abnormalities of fertilization, meiosis and cleavage and heteroploidy averaged 30% for over 1600 eggs from mass-spawned groups of 853 wild oysters of Crassostrea virginica collected largely from Long Island Sound (Stiles and Longwell, 1973). In an experimental cyto-genetic study on the effects of exposure of spawned eggs to silver and cadmium (Longwell et al., to be published), more data were collected on background rate of chromosome abnormalities in unexposed control oysters. Of 528 eggs scored from 10 spawnings 34% were abnormal. Of 12,728 mitosing oyster cells scored in their early stage cleavages only 3.2% were abnormal. These data are from a total of only about 50 oysters but are in agreement with those collected from the mass spawning experiments with a total of 853 spawning oysters. A primitive lower invertebrate as the oyster, one female of which can produce as many as 65 million eggs in one spawning season, should be able to tolerate a higher incidence of zygotic loss than a less fecund fish. The Atlantic mackerel is a moderately fecund fish producing 360,000 to 450,000 eggs (Sette, 1950b; Bigelow and Schroeder, 1953). Yet, an overall average for the Bight of a third of all its mitosing embryo cells had some cyto-genetic abnormality at a considerably later embryo stage than studied in the oyster.

In somatic, adult body, not embryo cells, the central mud minnow has a low incidence of background chromosome breakage of only about 0.03%, as seen in colchicine-treated cells (Kligerman et al., 1975).

In an experimental study on the cyto-genetic effects of incorporated radionuclides on eggs of their turbot and ruff, Russian workers reported background levels of chromosome damage for controls (AEC-TR-7299, 1972). Using experimental eggs at gastrulation and recording percentage of anaphases and telophases with chromosome breakage, they found background of ruff to be approximately 12%, 16%,

and 14%. The ruff, Scorpaena porcus, is viviparous. In the turbot control 10% of the cells were chromosomally abnormal. In the freshwater loach, Misgurnus fossilis L., however, the mean percentage of such aberrations was lower, being only 1.5% in blastula and gastrula cells, and 3.3% in larval epithelium (Romashov and Belyayeva, 1966). The figures for the loach early embryo cells are similar to that obtained for the oyster. In the sturgeon, Acipenser guldenstadt Brandt, and sterlet, Acipenser ruthenus L., measured chromosome aberrations in the mitoses of the epithelium of the caudal ridge were similarly low, 2.8% in the sturgeon and 2.0% in the sterlet. The incidences reported for early embryo cells of the ruff and turbot were approached only by the somatic cell divisions of late stages of larvae held in unsuccessful laboratory cultures. Though obtained for different species of fish, at a different developmental stage with a slightly different scoring method, background figures for the turbot and ruff are very close to the percentages of chromosome aberrations and abnormal mitoses recorded for the two Westward stations, with least abnormal mitoses towards the Bight periphery halfway to the edge of the continental shelf, 13% and 16%.

Sufficient general information is available to recognize that just as in insects and mammals there must be some real correlation between incidence of microscopically detectable chromosome aberrations and mitotic errors in fish embryos at any stage and successful development. The significance of appreciable levels of chromosome aberrations and mitotic errors in post-gastrula early embryos of mackerel eggs from the New York Bight ought then be considered in regard to recruitment. Mackerel probably spawn near the surface of the water. Spawning occurs both close to shore and as far as 80 miles to sea, but mostly 10 to 30 miles from shore. Mackerel eggs develop mostly near the water surface and all above the thermocline (Sette, 1950a; Bigelow and Schroeder, 1953). The oceanic bight between Chesapeake Bay and southern New England was indicated by Sette (1950a) as the most productive spawning area.

Fish embryos with most severe injuries to chromosomes resulting from experimental radiation die precisely during the transition from late blastula to gastrulation (Romashov and Belyayeva, 1966). However, in surviving embryos the percentage injured chromosomes remains at a rather constant level for the duration of gastrulation.

In their experimental study on incorporated radionuclides on the turbot and ruff, Russian workers examined the significance of chromosomal breakage in the further development of the embryos (AEC-TR-6940, 1968; AEC-TR-7299, 1972). Although cells with most severe chromosome breakage must die, it was noted that abnormal chromosomes could be preserved, in the form of a stabilized cycle of chromosome bridges, for many cell generations. They found that even externally normal prolarvae with no external morphological deformities have considerably more statistically significant chromosome breakage than do control specimens. A cytological analysis of the tissues in the prolarvae with varying morphological anomalies revealed a still greater degree of chromosome aberrations than observed in morphologically normal prolarvae. The same was observed in the study of the freshwater loach (Romashov and Belyayeva, 1966).

Deformed embryos had a high incidence of death just prior to hatching. The low viability of normal-appearing embryos with incorporated radionuclides that do hatch was attributed to the increased incidences of chromosomal aberrations contained in these externally normal embryos. The later mortality of morphologically normal larvae may be partly a manifestation of differentiation problems encountered by genetically abnormal cells in the post-hatching juvenile phase of differentiation which occurs in fish. Incorporated radionuclides in fish cause largest mortalities at hatching in grossly normal and grossly abnormal prolarvae alike (AEC-TR-6940, 1968).

On the basis of radiation and hybrid studies the blood and hematopoietic organs, the eye, and central nervous system of fish may be particularly susceptible to malformation resulting from chromosome imbalance (Newman, 1915; Reagan and Thorington, 1915; Allen and Mulkay, 1960). Hematopoietic tissue may be the most sensitive of all to induced mutation (Allen and Mulkay, 1960). The dominant lethality of a chromosome mutation affecting this system need not be expressed until after hatching of the fish embryo when circulation would fail to be established (Thorington, 1915; Kellicott, 1916).

Summary

In addition to the radionuclides, such major classes of marine pollutants as heavy metals and pesticides are recognized mutagens and, as such, may have important implications in survival of fish populations. Mutagens can cause genetic damage at sub-toxic levels. Many marine contaminants accumulate in the body tissues of fish and other marine species. Additionally, cadmium has been shown to be absorbed from seawater by post-spawned fish eggs.

Cells in the meiotic divisions of gametogenesis, still in process as fish migrate in towards polluted coastal waters to spawn, are particularly sensitive to the damaging effects of mutagens. Early cleavage mitoses of the fertilized egg are even more sensitive. When fish eggs, often already carrying significant contaminant loads, are spawned in polluted waters they have only halfway completed these sensitive meiotic divisions with their intricate chromosome maneuvers. As components of the neuston in surface waters, fertilized fish eggs must then undergo repeated divisions of their chromosomes during the even more sensitive stages of early cleavage.

From studies of mammals, insects and plants we know that most dominant lethal mutations which kill at early stages of development are associated with gross chromosome alterations. In an experimental study of incorporated radionuclides in eggs of commercial turbot (Rhombus maeoticus) and ruff (Scorpaena porcus), Russian workers found increased incidences of chromosome aberrations in normal and abnormal larvae associated with poor hatchability and reduced vigor of hatched larvae.

Standard cyto-genetic methodology has been modified to make it suitable for studying embryos of fish eggs collected in plankton samples. The chromosomes of developing fish eggs can now provide a sensitive test for genetically active substances, both experimentally and in polluted natural waters.

Altogether 30,689 embryo cells were scored in 452 eggs from the 14 different stations in the New York Bight collected from surface waters during the May 7-18, 1974 cruise of the Westward. Less than 20% of all the eggs had all their dividing cells free from chromosome and division abnormalities. One-third of the 30,689 division figures scored for all the embryos were abnormal. All but an insignificant number of eggs sampled were those of Scomber scombrus, the Atlantic mackerel.

Abnormalities of the chromosomes extended through the entire range of radiomimetic effects on the chromosomes and their division apparatus, including extreme stickiness of chromosomes having obvious division difficulties and irregularities, failure of chromosomes to orient on spindles, with consequent loss of chromosomes, and chromosome breakage.

Cells with at least one chromosome or mitotic irregularity for the eggs at any one Westward station varied from a mean low of about 13% to a mean high of about 79%. Interestingly, this low of 13% is close to the frequency of abnormalities reported by Russian workers as background aberrations in the

turbot and ruff in an experimental study, using a different scoring system and slightly earlier embryos still in gastrulation.

Stations in close proximity to each other did not differ significantly, nor did the day and night samples taken at one station. Stations with lowest means, such as the two Long Island shore stations and two of the four northern outermost stations halfway to the edge of the continental shelf, showed few significant differences with one another. They did differ significantly from stations near Sewage Sludge and Acid Waste Dump Sites, and the southernmost peripheral stations with highest means. Stations with intermediate means, such as two of the four northern outermost stations and the one south of Long Island halfway to Montauk Point, differed in general only from stations with highest and lowest means. There were no significant differences among the four samples with highest mean aberration rates; that is, the two southernmost stations, the one near Acid Waste, and one of the two samples taken at the station southwest of the Long Island shore stations in the direction of Sewage Sludge and Dump Sites.

Not all chromosome or division irregularities are microscopically detectable even with the most refined methods. Only a portion of observable abnormalities would be expected to be recorded with the cyto-genetic scoring system used in the study of the mackerel eggs reported here. The collection of the Westward data on largely the early embryo stage means that the earlier stage zygotes, so chromosomally abnormal that they could not gastrulate, are not represented. Differences between best and worst stations are so minimized.

Some portion of the abnormalities must be attributable to natural environmental factors and, as such, may be referred to as background.

Sufficient general information is available to recognize that there must be some real correlation between incidence of microscopically detectable chromosome aberrations and mitotic errors in fish embryos at any stage and successful development of a natural spawn to its final period of differentiation after hatching.

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Table 1

Chromosome Aberrations and Mitotic Abnormalities in Developing Mackerel Eggs at Sample Stations Over the New York Bight - May 7-18, 1974 Westward Cruise

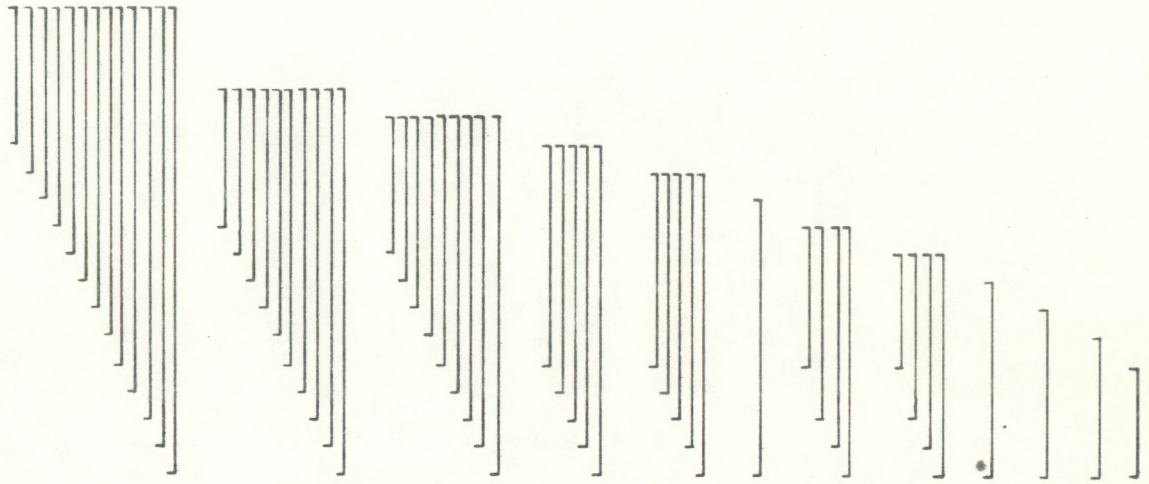
Sample station no.	Lat. (north)	Long. (west)	General station description	Total no. eggs studied	Total no. cells scored in all embryos studied	Mean % cells of all embryos with at least 1 chromosome or mitotic abnormality
6	40°34'	73°32'	Long Island Shore north of Sewage Sludge	16	3,096	24.4
7	40°35'	73°31'	" " " "	30	4,113	21.7
8	40°32'	73°36'	About 5 miles to southwest of Long Island Shore Stations 6 & 7 in direction of Sewage Sludge and Dump Sites	84	352	55.3
8-OV-N			Overnight re-run sample of same Station 8	45	351	41.1
9	40°32'	73°37'	About 5 miles to southwest of Long Island Shore Stations 6 & 7 in direction of Sewage Sludge and Dump Sites and about 5 miles from Station 8	31	240	28.2
10	40°22'	73°43'	Same general area as Station 37 near Acid Waste but more distant than 37 from Acid Waste in southwesterly direction	32	324	38.6
11	40°13'	73°26'	" " " "	25	2,701	52.1
15	40°26'	72°23'	One of far-most stations from Sewage Sludge, Acid Waste and Coast about half-way to edge of continental shelf	22	5,845	15.8
16	40°14'	72°47'	" " " "	16	2,316	30.2
18	40°05'	72°59'	" " " "	7	1,312	35.1
19	39°25'	73°20'	One of far-most stations from Sewage Sludge & Acid Waste; closer to Toxic Chemical & Radioactive Waste Disposal than others, ± 50 miles off N.J.Coast	38	306	78.6*
21	39°35'	73°22'	" " " "	32	119	63.1*
28	40°38'	72°59'	South of Long Island Shore halfway to Montauk Point	24	5,927	34.2
35	40°12'	73°03'	One of far-most stations from Sewage Sludge, Acid Waste & Coast about halfway to edge of continental shelf	31	2,212	12.7
37	40°20'	70°31'	Near Acid Waste, Dredge Spoil	19	1,475	60.6

*Multiple abnormalities in an increased portion of the abnormal cells of eggs from Stations 19 and 21.

Table 2

Statistically Significant (0.05 Level) Station Differences in Mean % Chromosome Aberrations and Mitotic Abnormalities - "t" Tests of Each Pair of Stations

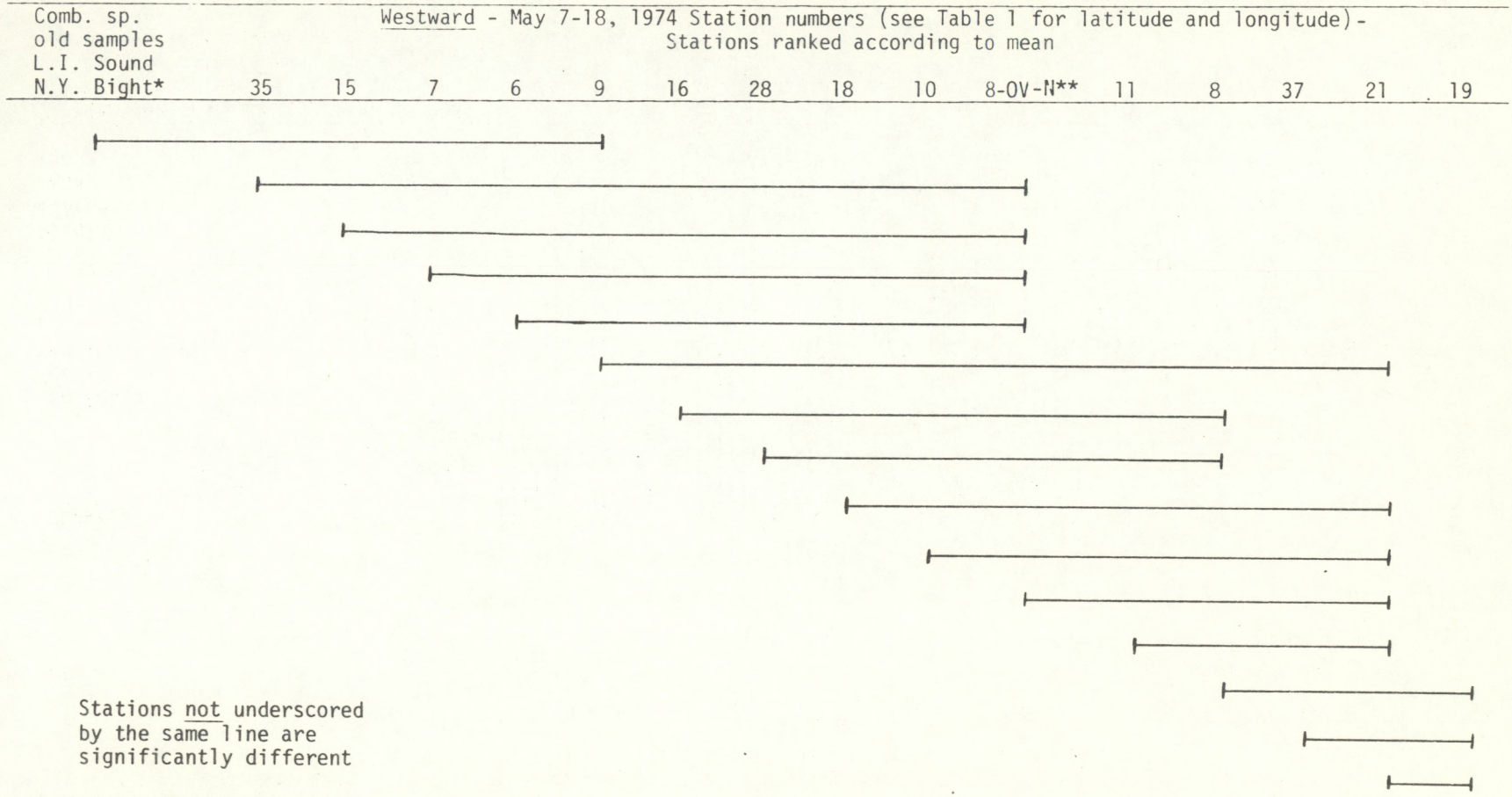
Station*		Mean %
Comb. sp. old samples L.I. Sound N.Y. Bight**		4.8
Westward-May 7-18, 1974 Station	#35	12.7
	15	15.8
	7	21.7
	6	24.4
See Table 1	9	28.2
for latitude	16	30.2
and longitude	28	34.2
	18	35.1
	10	38.6
	8-OV-N***	41.1
	11	52.1
No significant	(8	55.3
differences	(37	60.6
among	(21	63.1
these	(19	78.6



*Stations ranked according to mean.
**These samples collected in 1944 (from Yale University Collection) and in 1966 (Middle Atlantic Coastal Fisheries Center Collection).
***8 overnight re-run sample.

Table 3

Statistically Significant (0.05 Level) Station Differences in Mean % Chromosome Aberrations and Mitotic Abnormalities - Duncan's New Multiple Range Test



*These samples collected in 1944 (from Yale University Collection) and in 1966 (Middle Atlantic Coastal Fisheries Center Collection).

**8 overnight re-run sample.

Table 4

Multiple Range Tests

Analysis of Variance

	Sum of squares	DF	Mean square	F ratio
Between groups	76005.2500	15	5067.0156	7.7418*
Within groups	142026.1875	217	654.4985	
Total	218031.4375	232		

*Statistically significant at 0.05 level.

Legends to Photographs - Scomber scombrus eggs, embryos, and cells

Plate 1 - Viewed under 20 X magnification of a dissecting microscope -

Upper left - two- and four-cell stage cleavage embryos on their eggs (oil droplet to left of each egg).

Upper right - early cleavage embryos dissected off their eggs.

Lower left - intact egg with morula stage embryo and 2 morula embryos dissected free.

Lower right - 4 intact eggs with blastula embryos and free blastula embryos.

Plate 2 - Viewed under 20 X magnification of a dissecting microscope -

Upper left - 5 intact eggs with gastrula embryos and 3 invaginating gastrula embryos dissected off their eggs.

Upper right - 5 intact eggs with early embryos and 4 free early embryos.

Lower left - 4 intact eggs with tail-bud embryos and 2 free tail-bud embryos.

Lower right - 6 intact eggs with tail-free embryos and 3 tail-free embryos dissected off their eggs.

Plate 3 - Three microscope slides with mackerel (Scomber scombrus) embryos stained and flattened for cyto-genetic study. Uppermost slide has 4 embryos; middle slide, 3; and bottom slide, 4 embryos.

Plate 4 - Figures 1, 2 - Lower-power microscopic views of monolayers of embryo cells prepared for cyto-genetic study. Arrows point to cells with dividing chromosomes of the sort studied.

Plate 5 - Figures 3, 4 - 100 X phase contrast - Very early cleavage cells. Subdivision of the nucleus into separate micronuclei seen here is classically characteristic of the first few cleavage divisions of some fish.

Plate 6 - Figure 5 - 100 X phase contrast - Early cleavage cell in normal anaphase division of its chromosomes.

Plate 7 - Figures 6, 7 - 100 X phase contrast - Two early cleavage cells in normal metaphase and one in normal anaphase division of the chromosomes.

Plate 8 - Figures 8, 9, 10 - 100 X phase contrast - Each of 3 photomicrographs shows a normal telophase stage of chromosome division.

Plate 9 - Figures 11, 12, 13 - 100 X phase contrast - Cells of early embryos in telophase of cell division.

Plate 10 - Figure 14 - 100 X phase contrast - Sector of embryo showing different stages of normal chromosome division.

Plate 11 - 100 X phase contrast -

Figure 15 - Normal telophase.

Figure 16 - Abnormal telophase with several chromosomes in a thick bridge and one abnormal laggard chromosome at arrow.

Figure 17 - Abnormal telophase with laggard chromosomes at curved arrow and other chromosomes probably broken, outside spindle field at straight arrow.

Plate 12 - 100 X phase contrast -

Figure 18 - Upper arrow points to a laggard telophase chromosome and lower arrow to an abnormal partitioning of the lowermost of the two developing telophase nuclei.

Figure 19 - Telophase with 2 chromosome bridges and an abnormal chromatin piece above uppermost telophase nucleus at arrow.

Figure 20 - Thick telophase bridge in an abnormal division.

Figure 21 - Telophase with 2 micronuclei (at arrows) developed from laggard chromosomes.

Plate 13 - 100 X phase contrast -

Figure 22 - Abnormally partitioned, abnormally sticky prometaphase group of chromosomes with two separate chromatin clumps, and 1 distinct chromosome (at arrow) separate from the rest.

Figure 23 - Sticky prometaphase with one chromosome (at arrow) quite apart from the group.

Figure 24 - Extremely sticky metaphase chromosomes with one chromosome (at arrow) prematurely at one spindle pole.

Plate 14 - 100 X phase contrast -

Figure 25 - Telophase group with evidence of old chromosome bridge.

Figure 26 - Telophase with 2 thick chromosome bridges.

Plate 15 - 100 X phase contrast -

Figure 27 - Late telophase with trace of old bridge.

Figure 28 - Abnormally sticky prometaphase with a projection, probably a remnant of an old telophase bridge.

Figure 29 - Sticky telophase division with 2 bridges.

Figures 30-31 - Extremely sticky telophase divisions with probably all the chromosomes in thick sticky bridges.

MAP

Plate 16 - Map of the New York Bight and its Waste Disposal Sites showing mean % early embryo cells with chromosome aberrations and mitotic abnormalities for 14 sample stations. Westward, May 7-18, 1974, cruise. (Figures marked next to sample site. One station has two figures, one an overnight re-run sample.)

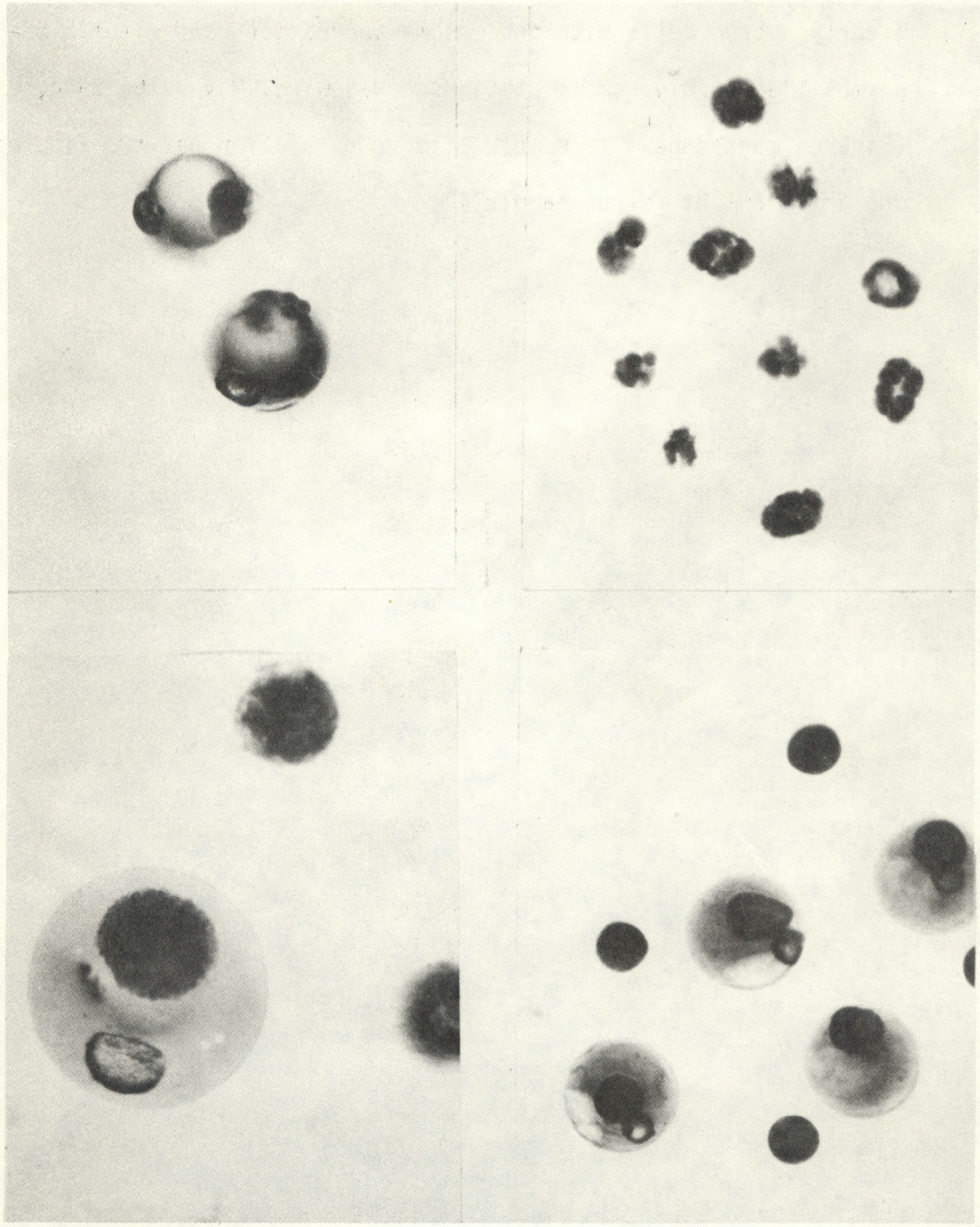


Plate 1

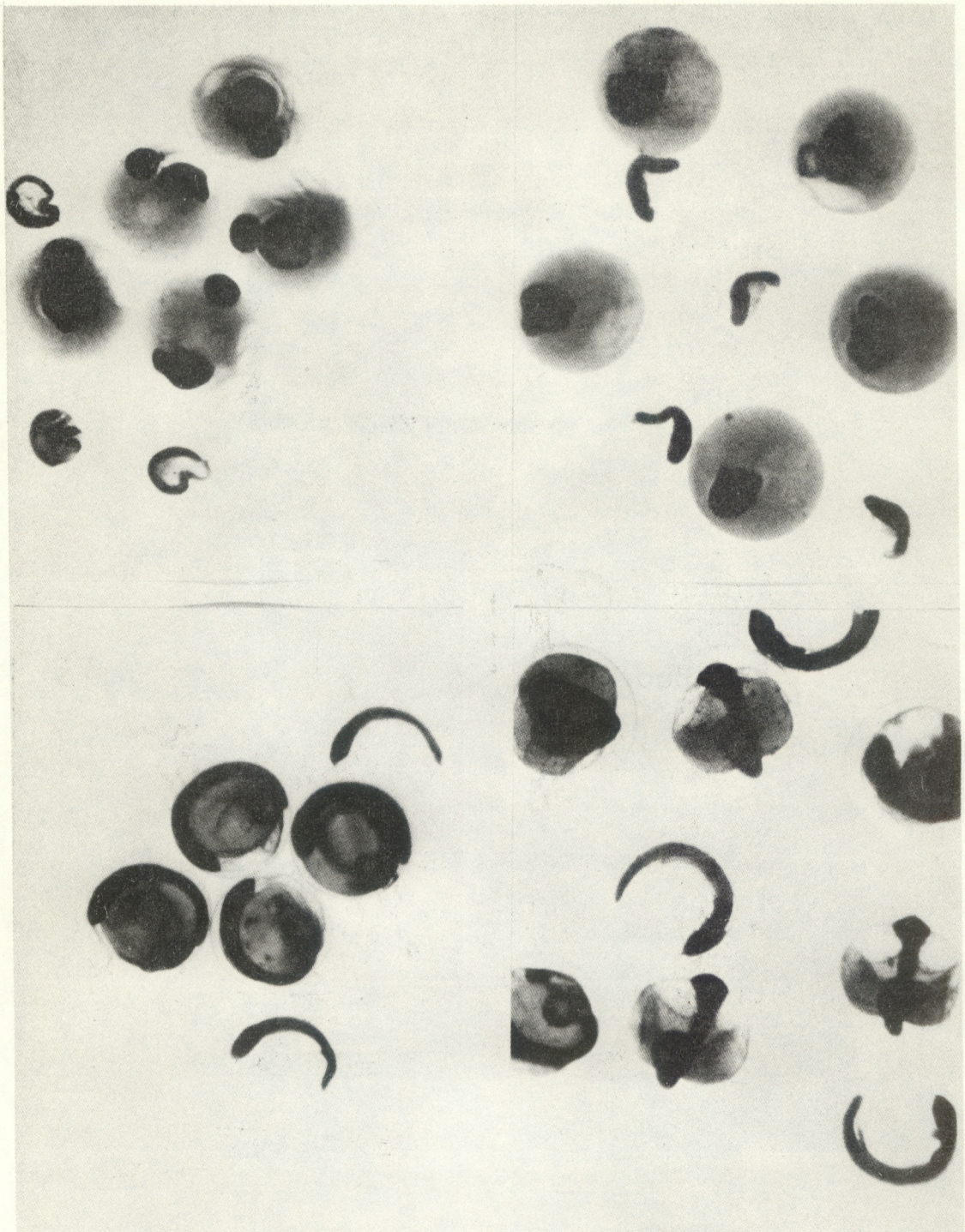


Plate 2

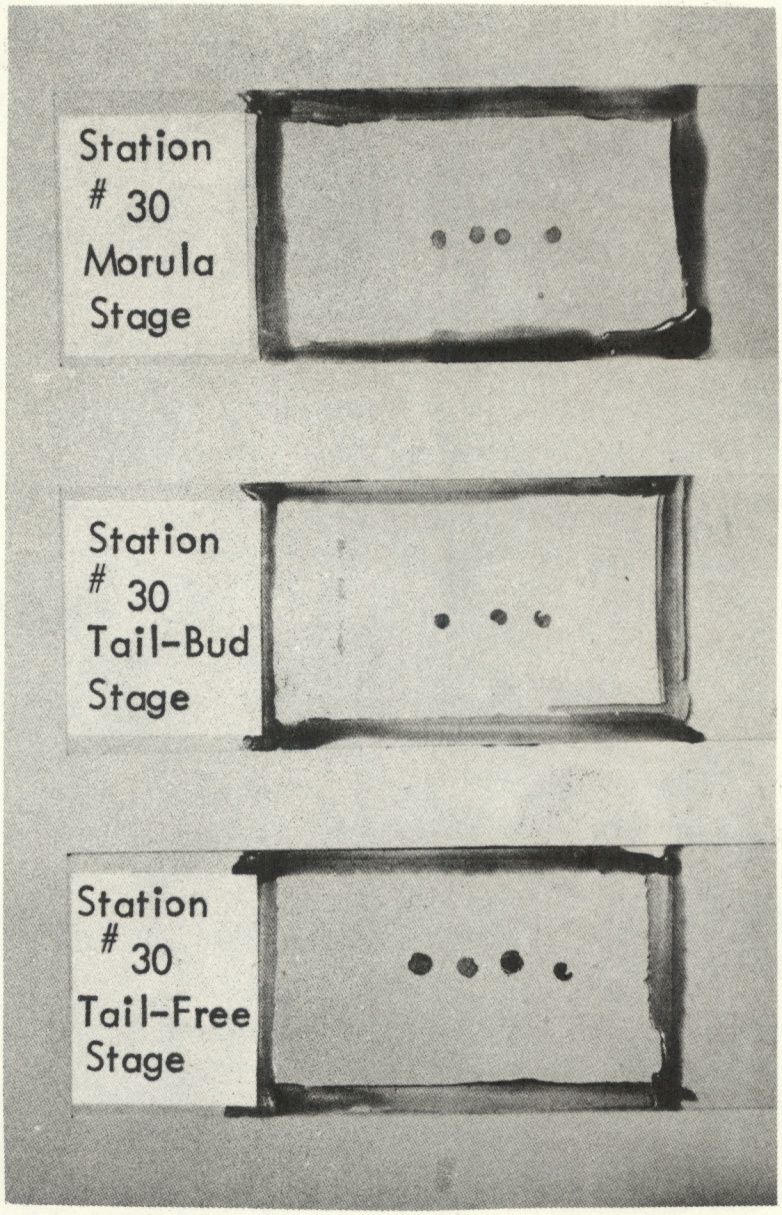

Station
30
Morula
Stage
Station
30
Tail-Bud
Stage
Station
30
Tail-Free
Stage

Plate 3

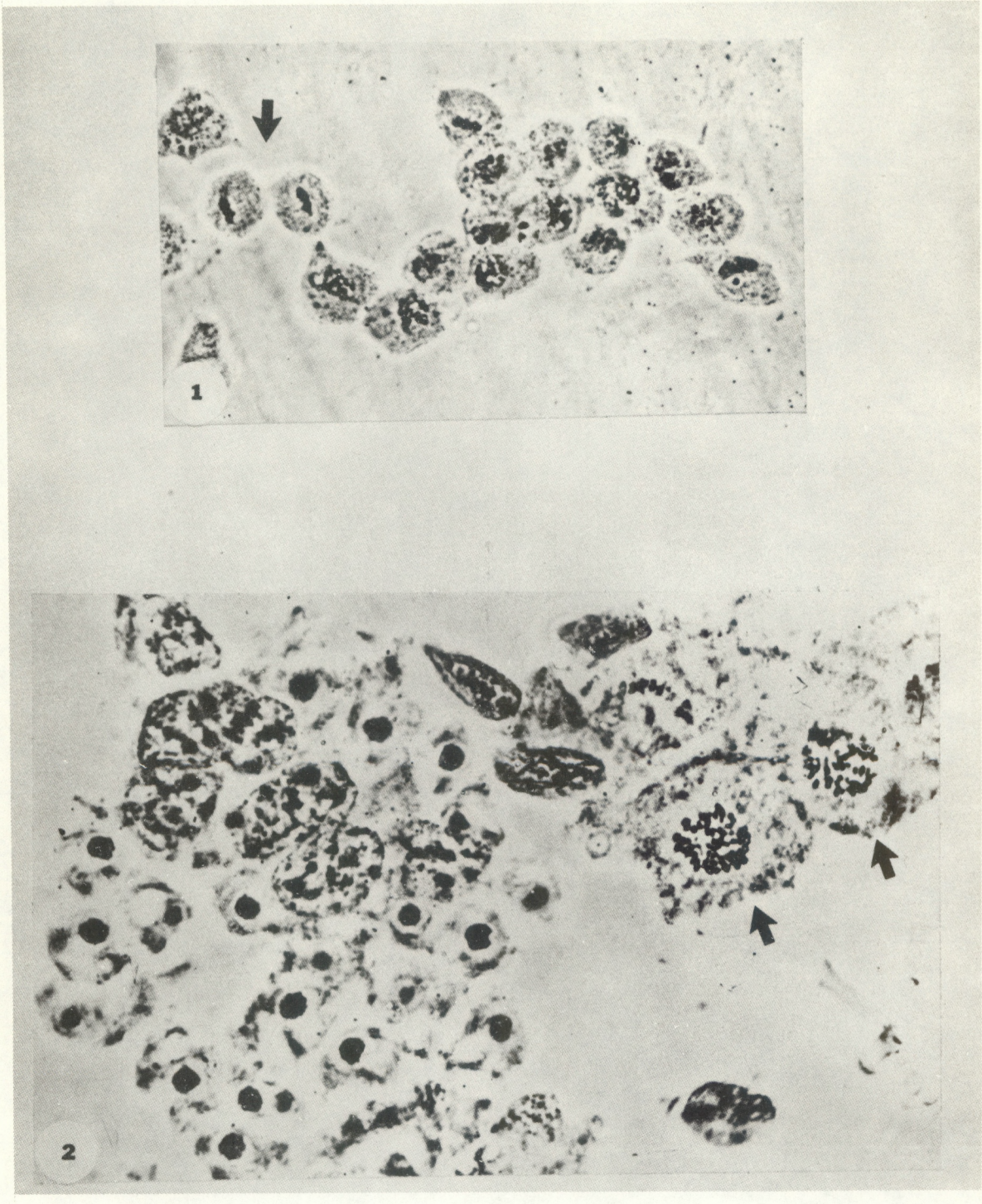


Plate 4

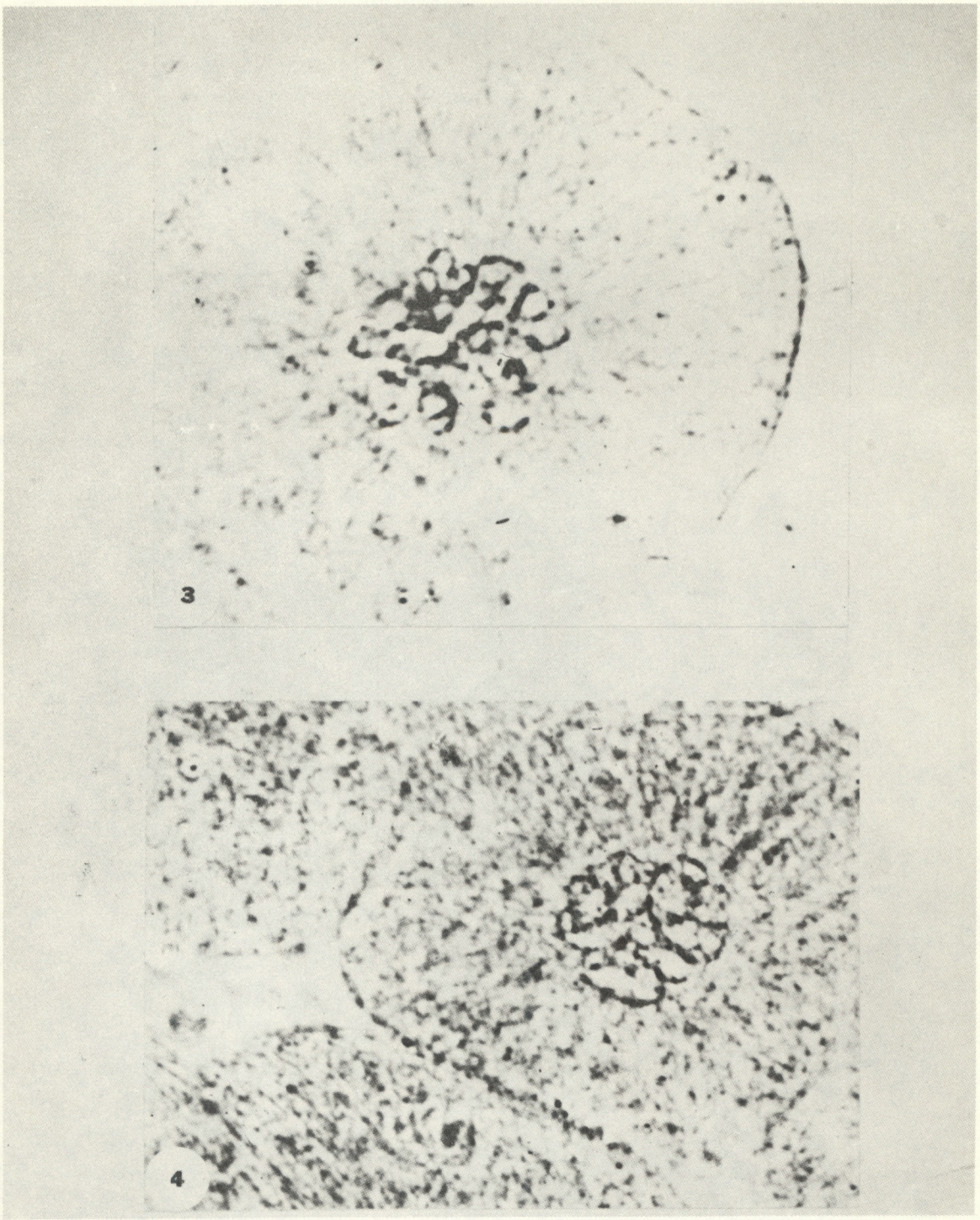


Plate 5

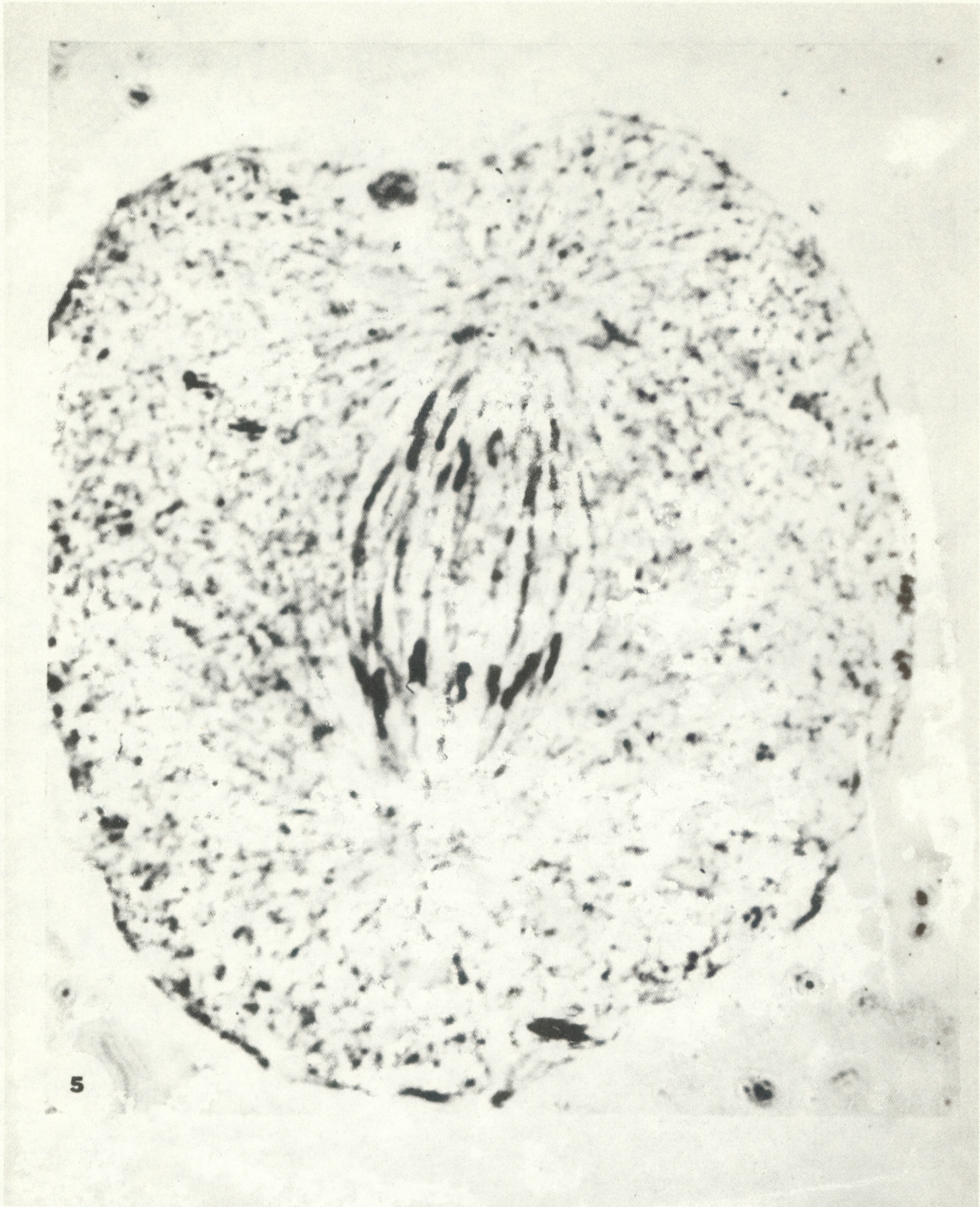


Plate 6

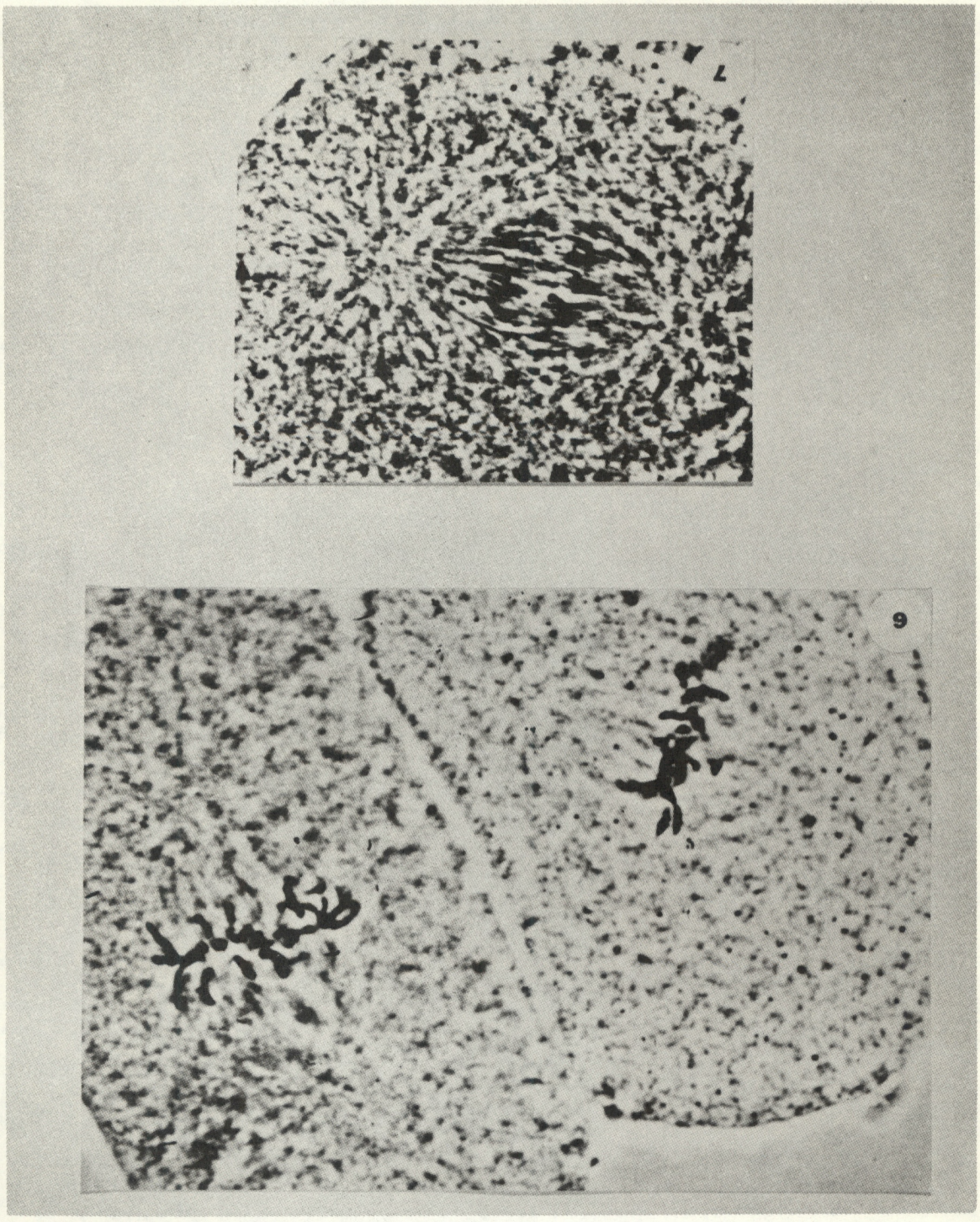


Plate 7

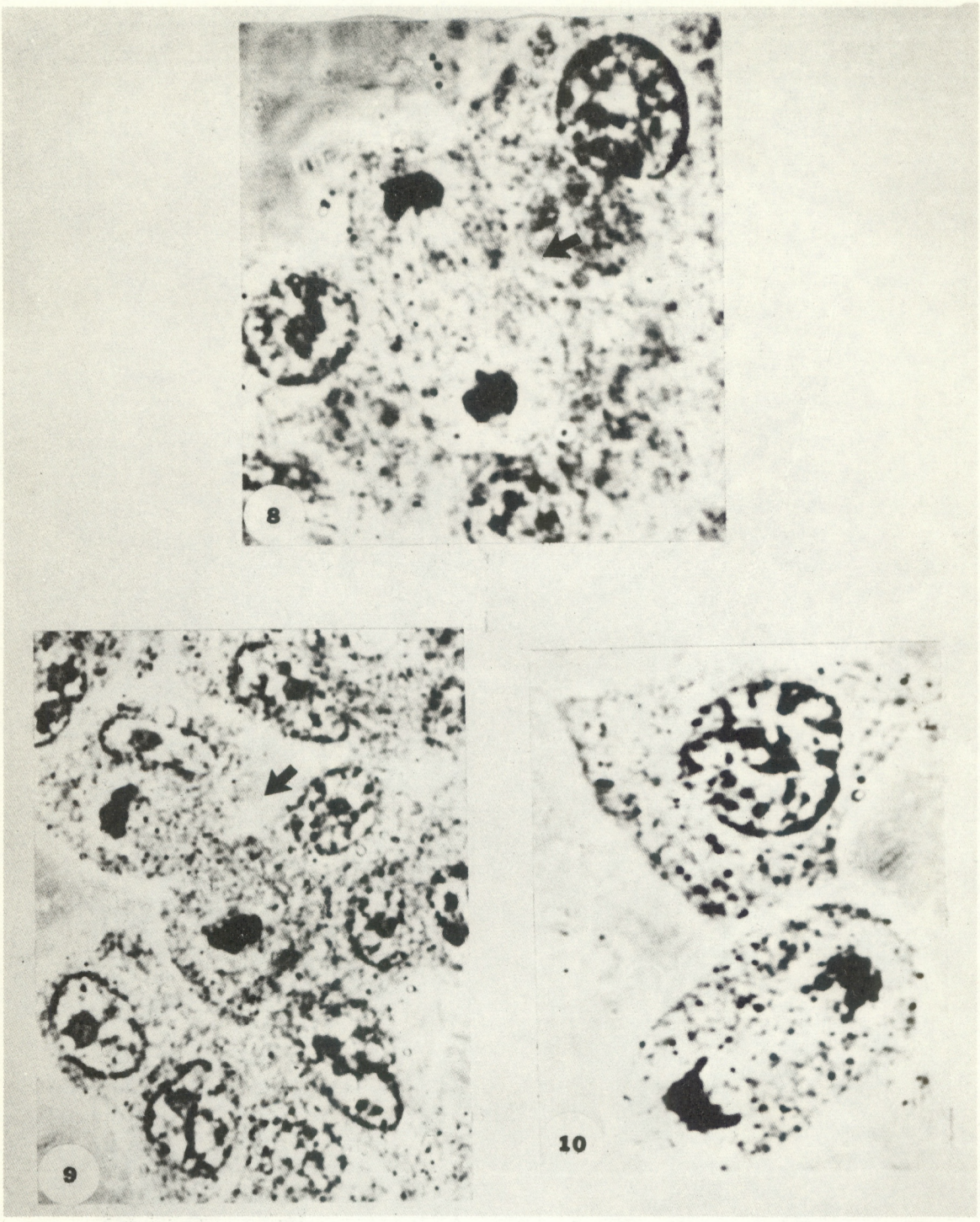
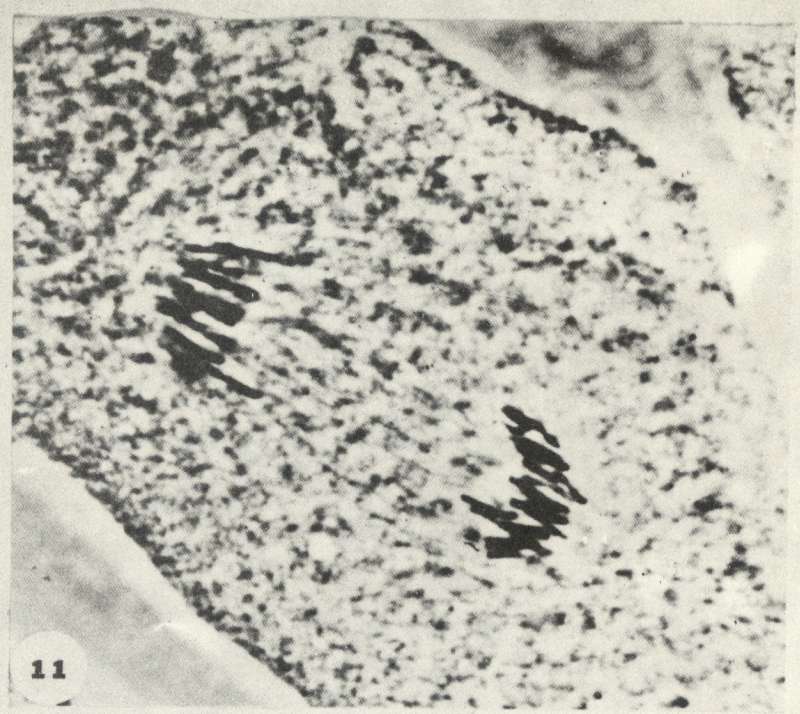
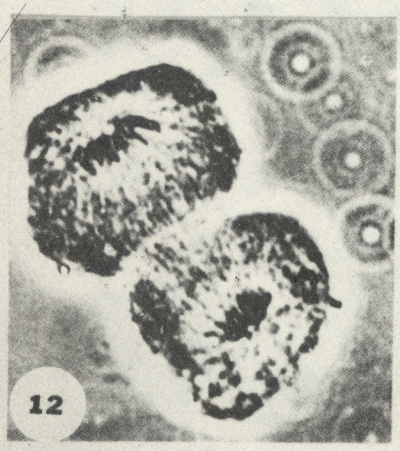
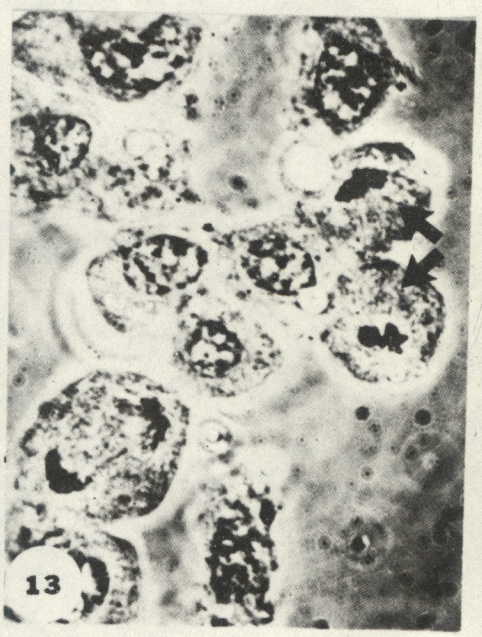


Plate 8







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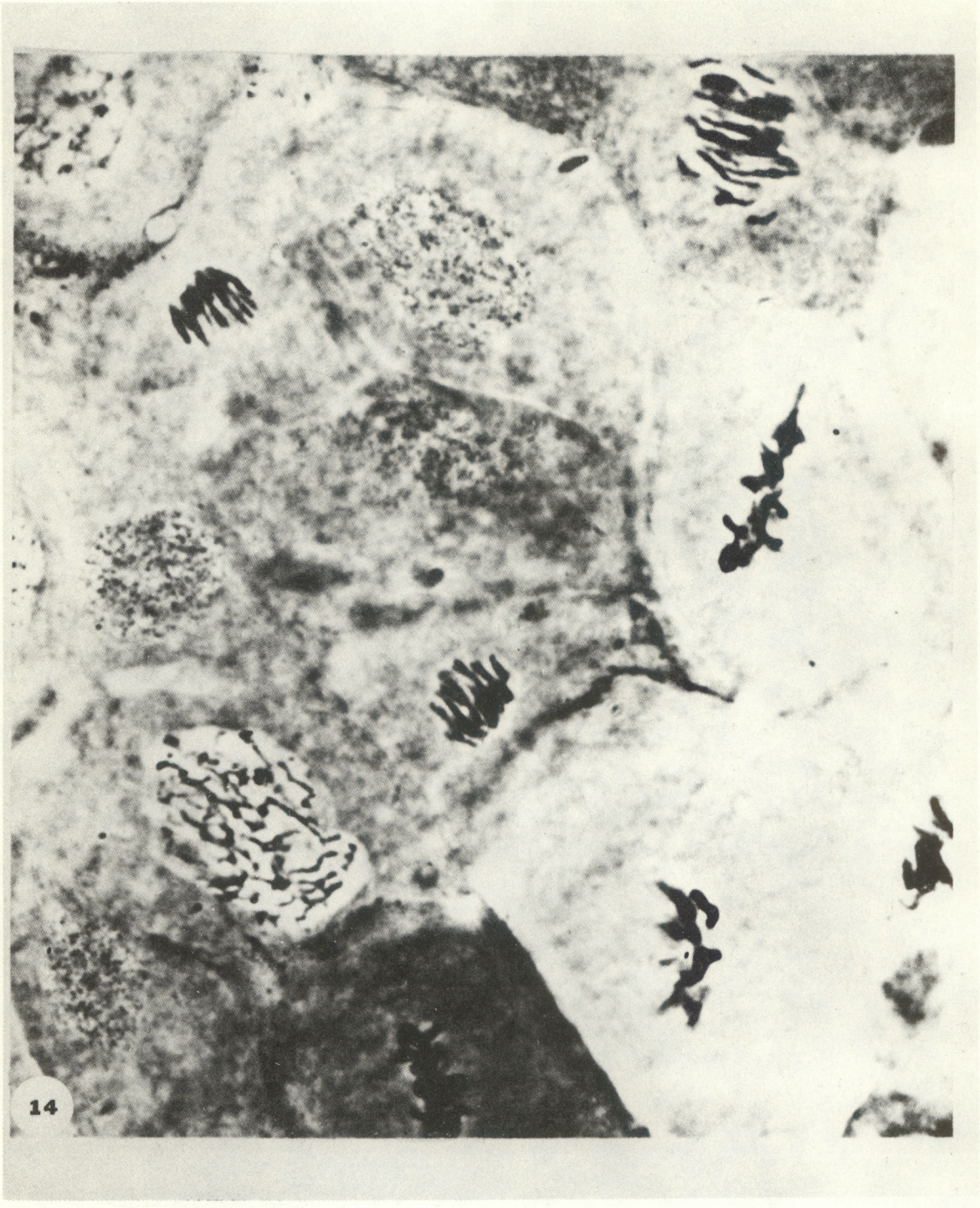

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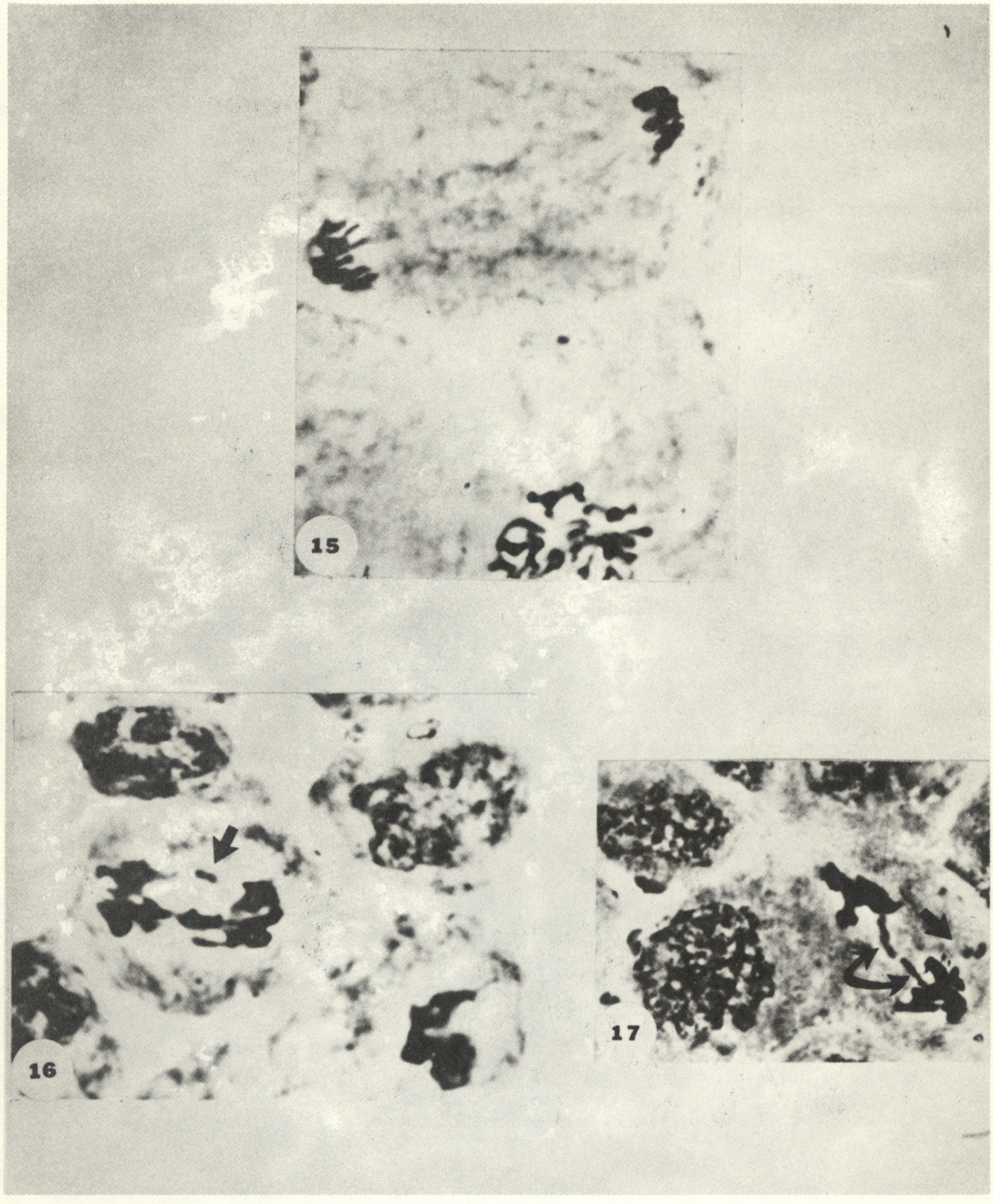
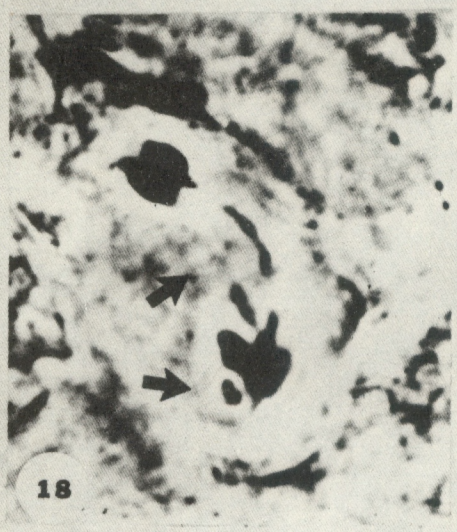


Plate 11



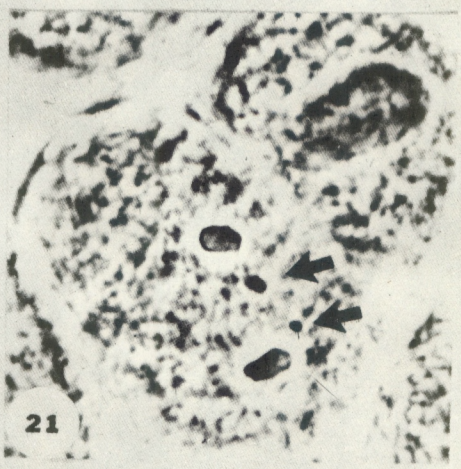
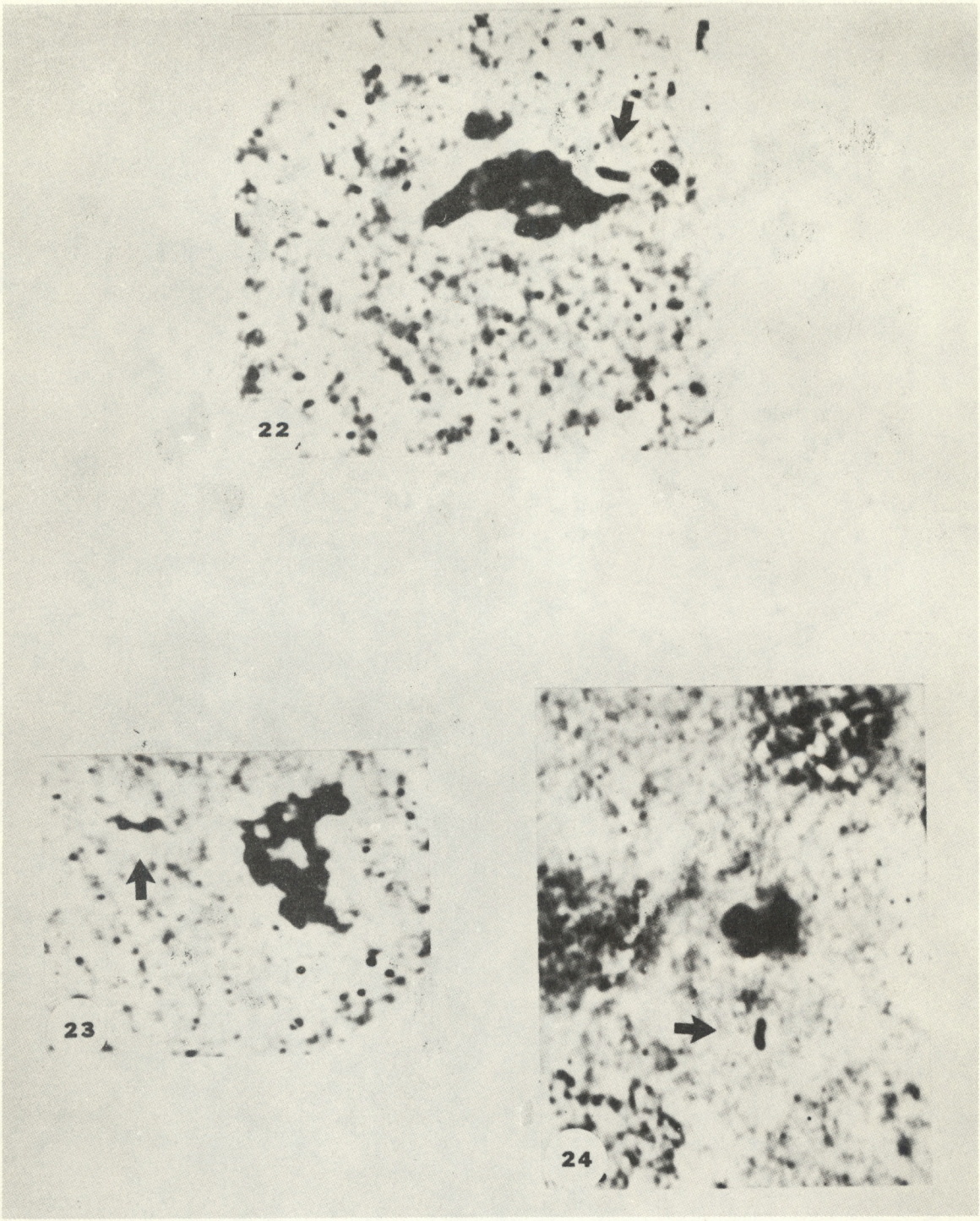


Plate 12



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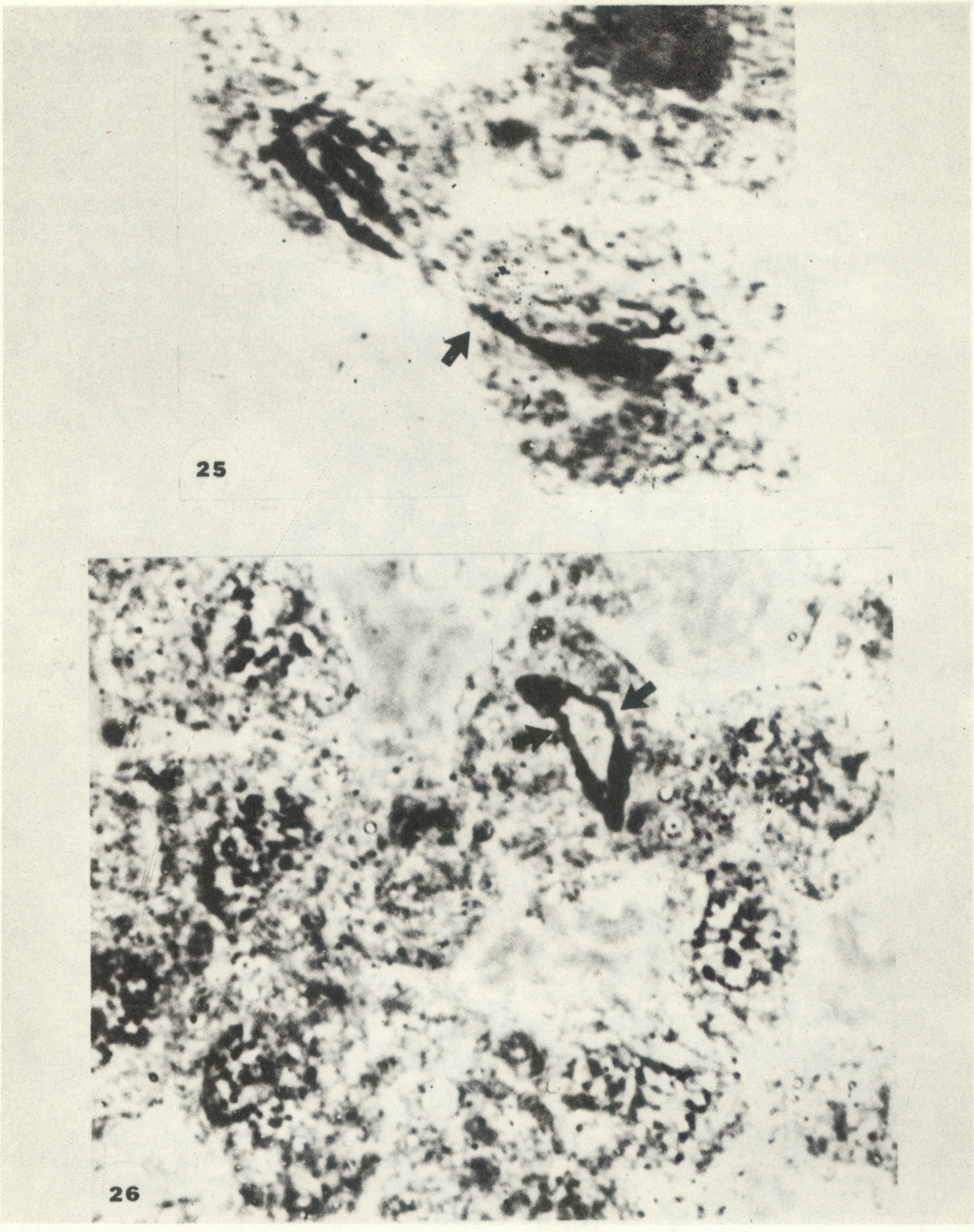

Plate 14

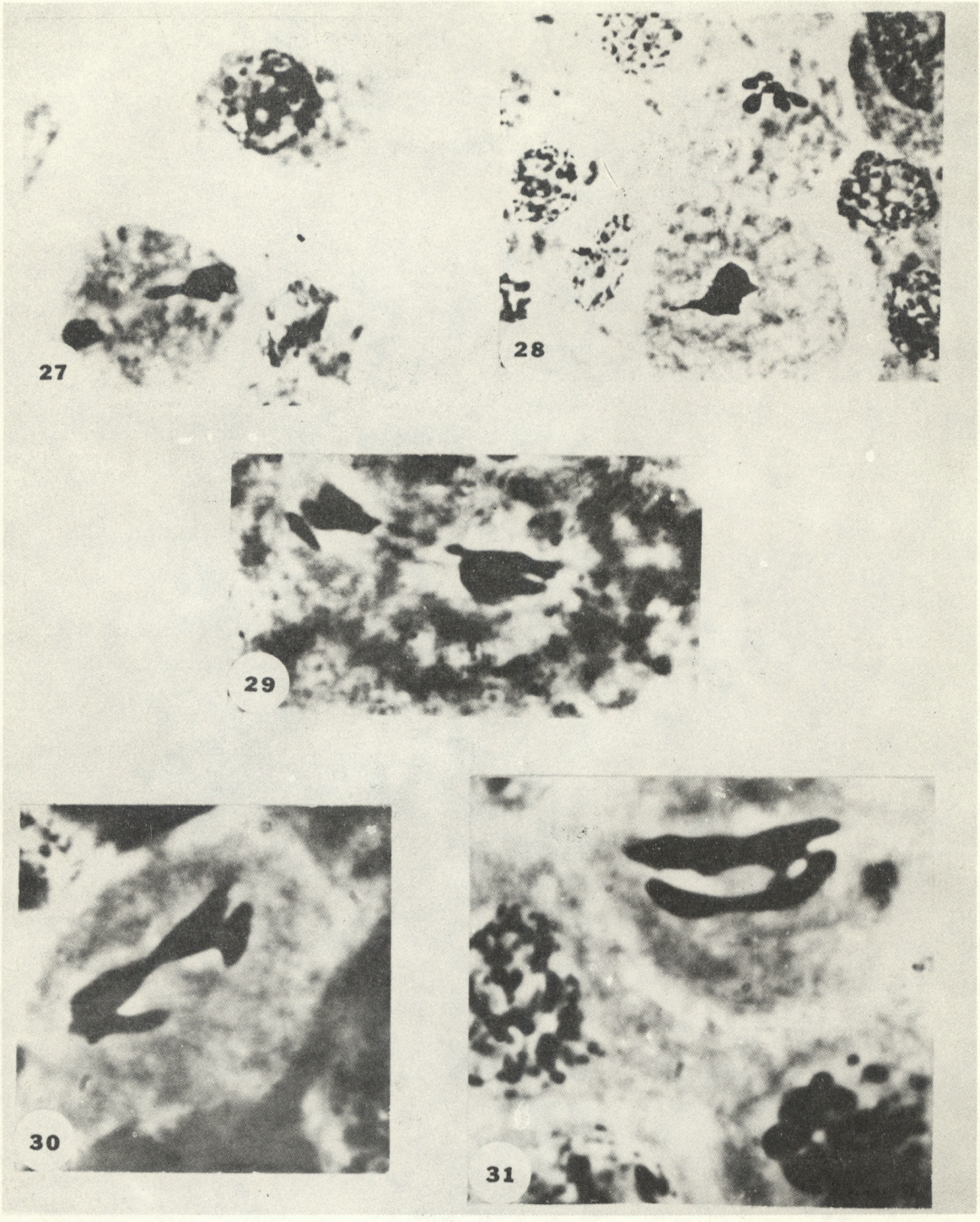

Plate 15

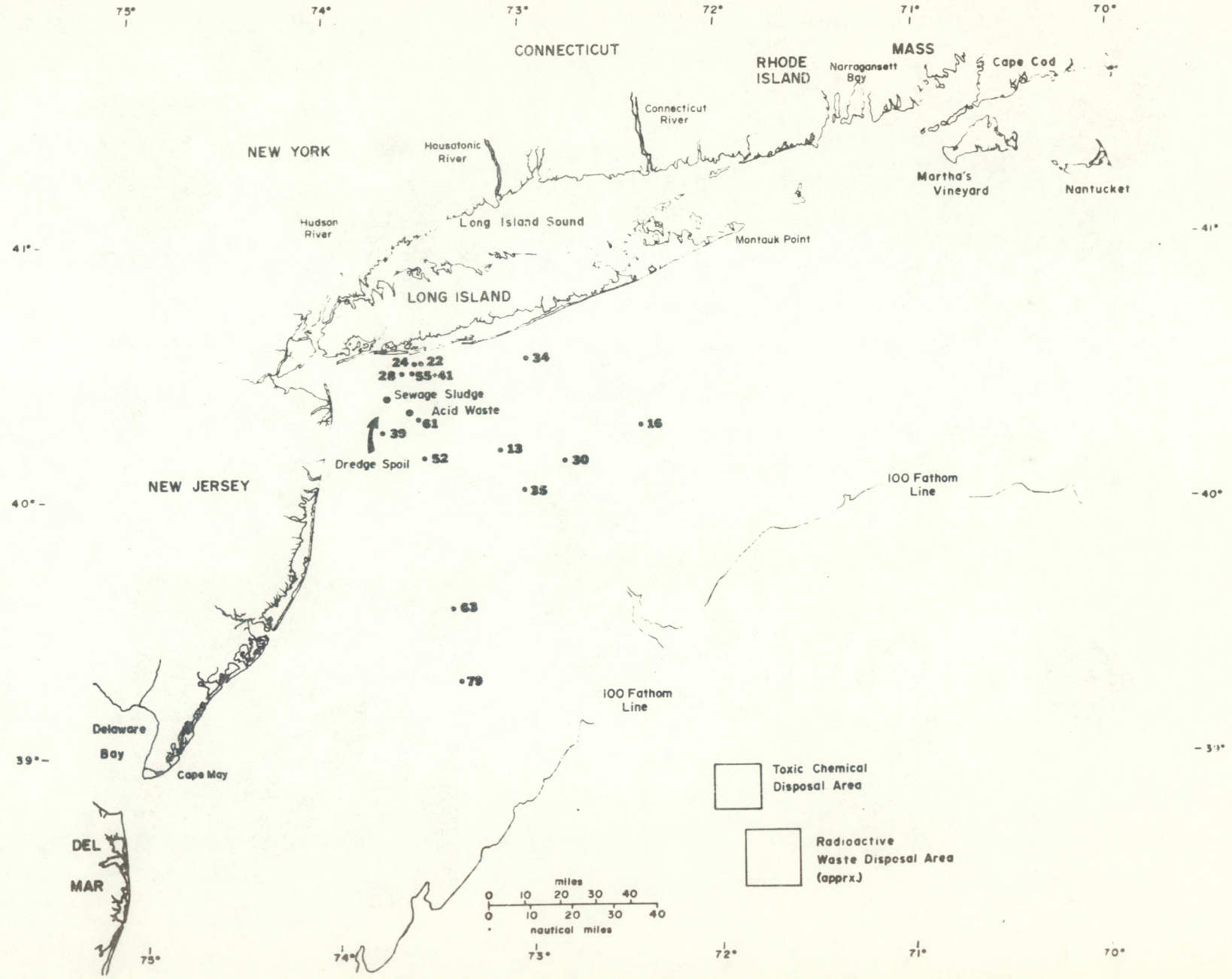
75°
74°
73°
72°
71°
70°
CONNECTICUT
RHODE ISLAND
MASS
Narragansett Bay
Cape Cod
Connecticut River
NEW YORK
Housatonic River
Martha's Vineyard
Nantucket
Hudson River
Long Island Sound
Montauk Point
41°
LONG ISLAND
24
22
34
28
55
41
Sewage Sludge
Acid Waste
61
16
39
13
Dredge Spoil
52
30
NEW JERSEY
35
100 Fathom Line
40°
63
79
100 Fathom Line
Delaware Bay
39°
Cape May
DEL
MAR
Toxic Chemical Disposal Area
Radioactive Waste Disposal Area (approx.)
miles
0 10 20 30 40
0 10 20 30 40
nautical miles

Plate 16

Environmental Research LABORATORIES

The mission of the Environmental Research Laboratories is to study the oceans, inland waters, the lower and upper atmosphere, the space environment, and the Earth, in search of the understanding needed to provide more useful services in improving man's prospects for survival as influenced by the physical environment. The following laboratories contribute to this mission.

MESA *Marine EcoSystems Analysis Program Office.* Plans and coordinates regional programs of basic and applied research directed toward the solution of environmental problems which involve the functioning, health and restoration of marine ecosystems.

OCSEA *Outer Continental Shelf Environmental Assessment Program Office.* Plans and directs assessments of the primary environmental impact of energy development along broad areas of the outer continental shelf of the United States; coordinates related research activities of federal, state and private institutions.

W/M *Weather Modification Program Office.* Plans and directs ERL weather modification research for precipitation enhancement and severe storms mitigation; operates ERL's research aircraft.

NHEML *National Hurricane and Experimental Meteorology Laboratory.* Develops techniques for more effective understanding and forecasting of tropical weather. Research areas include: hurricanes and tropical cumulus systems; experimental methods for their beneficial modification.

RFC *Research Facilities Center.* Provides aircraft and related instrumentation for environmental research programs. Maintains liaison with user and provides required operations or measurement tools, logged data, and related information for airborne or selected surface research programs.

(CIRES) *Theoretical Studies Group.* Provides NOAA participation in the Cooperative Institute for Research in Environmental Sciences (CIRES), a joint activity with the University of Colorado. Conducts cooperative research studies of a theoretical nature on environmental problems.

AOML *Atlantic Oceanographic and Meteorological Laboratories.* Research areas include: geology and geophysics of ocean basins and borders, oceanic processes, sea-air interactions and remote sensing of ocean processes and characteristics (Miami, Florida).

PMEL *Pacific Marine Environmental Laboratory.* Research areas include: environmental processes with emphasis on monitoring and predicting the effects of man's activities on estuarine, coastal, and near-shore marine processes (Seattle, Washington).

GLERL *Great Lakes Environmental Research Laboratory.* Research areas include: physical, chemical, and biological limnology; lake-air interactions, lake hydrology, lake level forecasting, and lake ice studies (Ann Arbor, Michigan).

GFDL *Geophysical Fluid Dynamics Laboratory.* Research areas include: dynamics and physics of geophysical fluid systems; development of a theoretical basis, through mathematical modeling and computer simulation, for the behavior and properties of the atmosphere and the oceans (Princeton, New Jersey).

APCL *Atmospheric Physics and Chemistry Laboratory.* Research areas include: processes of cloud and precipitation physics; chemical composition and nucleating substances in the lower atmosphere; laboratory and field experiments toward developing feasible methods of weather modification.

NSSL *National Severe Storms Laboratory.* Research is directed toward improved methods of predicting and detecting tornadoes, squall lines, thunderstorms, and other severe local convective phenomena (Norman, Oklahoma).

WPL *Wave Propagation Laboratory.* Research areas include: theoretical research on radio waves, optical waves, and acoustic gravity waves; experimental research and development on new forms of remote sensing.

ARL *Air Resources Laboratories.* Research areas include: diffusion, transport, and dissipation of atmospheric contaminants; development of methods for prediction and control of atmospheric pollution; geophysical monitoring for climatic change (Silver Spring, Maryland).

AL *Aeronomy Laboratory.* Research areas include: theoretical, laboratory, rocket, and satellite studies of the physical and chemical processes controlling the ionosphere and exosphere of the Earth and other planets, and of the dynamics of their interactions with high-altitude meteorology.

SEL *Space Environment Laboratory.* Research areas include: solar-terrestial physics, service and technique development in the areas of environmental monitoring and forecasting.

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