(in micrograms per milliliter): Hg, 0.001; Cd, 0.004; Cu, 0.015; Pb, 0.004; Zn, 0.20; As, < 0.02. Two samples of ethyl alcohol, in which commercial 95 percent solutions were diluted to 70 percent with distilled water, had the following ranges of variation (in micrograms per milliliter): Hg, 0.001 in both; Cd, 0.004 to 0.005; Cu, 0.02 to 0.15; Pb, 0.043 to 0.1; Zn, 0.00 to 0.04; As, 0.02. These figures call attention to the considerable variability of metal concentrations in preservative solutions, even before any specimens, tags, or labels are placed in them.

Analyses of the specimens that had been preserved for 2 to more than 85 years (Table 2) suggest that concentrations of some metals continue to increase in specimens for years. If the two oldest samples (1914 and 1885) are omitted, concentrations of cadmium, arsenic, and lead do not appear to increase after 1 month in preservative, copper concentrations increase for 2 years (to 1969), and both zinc and mercury concentrations increase for at least 13 years (to 1958). Only the anomalously high levels of lead and zinc in the 1969 sample contradict these suggestions, but these levels may be attributable to contact of the specimens during pre-preservation handling with a source high in lead and zinc, such as the painted deck of a ship.

The possibility of sources other than preservatives contributing to increased metal concentrations in specimens is emphasized by the two oldest samples (Table 2, 1914 and 1885), in which all six metals analyzed have extremely high concentrations. In some cases (Cu, Pb, Zn) these concentrations could be interpreted as extending the time during which metal concentrations continue to increase in preserved specimens. It is also possible that, because the old specimens were fixed in alcohol, which does not harden the tissues to the extent that formalin does, they were more completely digested before being subjected to spectrometry. On the other hand, a tin tag bearing the catalog number was present in the container only with these two samples. Emission spectrography (*4*) of these two tin tags and of three others (old and new) that had never been in preservatives showed the presence in all five of mercury (0.003 to 0.005 percent), cadmium (0.0007 to 0.01 percent), copper (0.007 to 0.1 percent), lead (0.1 to 1.0 percent), and arsenic (0.007 to 0.25 percent), but zinc was not detected in any. Only lead constituted more than 1 percent of any tag, and this tag had never been used. There was no correlation of metal concentrations with either age of tag or use in or out of preservative. The tags, therefore, could provide a source of five of the metals (all except zinc) and contribute to the high concentrations of these metals in associated liquid-preserved specimens.

Zinc concentrations are apparently not related to the presence of either the tin catalog tags or the vulcanized paper catalog tags that are currently used. Although no zinc was detected in the metal tags, emission spectrography (*4*) showed that the vulcanized paper contained 0.5 percent zinc, along with copper (0.03 percent) and lead (0.03 percent), but no detectable mercury, cadmium, or arsenic. Yet the only sample associated with a vulcanized paper tag (1958) had a lower concentration of zinc than was found in samples with no catalog tag (1969) or with the metal ones (1914 and 1885).

In conclusion, we have demonstrated that increased concentrations of some metals and decreased concentrations of others occur in specimens within a month after initial preservation and that the concentration after this short time varies in different preservatives. We have presented evidence that both the properties of preservatives and species differences in fish tissues can influence metal concentrations in preserved specimens, as can the presence of metal tags or other materials in the preservatives. Whereas some metals reach their maximum concentrations in preserved specimens within a month, others appear to increase in concentration for years. Until the effects of preservation are properly understood, fluid-preserved museum specimens cannot be used for meaningful comparisons of metal concentrations, either with other museum specimens or with frozen specimens.

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2. The Ocean Acre program has been supported by contracts from the U.S. Navy Underwater Systems Center, New London, Conn.
3. Liquid preservatives were analyzed by flame atomic absorption spectrometry after first being evaporated to dryness in a water bath at 60°C and then being redissolved in "ultrapure" (U.S. National Bureau of Standards) nitric acid. Analyses of undiluted formaldehyde and one sample of alcohol were accomplished at the Federal Drug Administration Laboratory, Washington, D.C. The diluted formalin and one sample of alcohol were analyzed at the Skidaway Institute of Oceanography.
4. Emission spectrographic analysis of metal and vulcanized paper tags was accomplished by the Conservation Analytical Laboratory at the Smithsonian Institution.
5. Supported by National Science Foundation–International Decade of Oceanic Exploration grants GX-28745 to Smithsonian Institution and GX-33615 to Skidaway Institute of Oceanography.

4 January 1974

Mycoplasma Contamination of Cultured Amniotic Fluid Cells: Potential Hazard to Prenatal Chromosomal Diagnosis

Abstract. *Amniotic fluid cell cultures were screened for mycoplasma contamination. Mycoplasma RNA's were observed in more than half the cultures examined. Karyotypic analyses of these contaminated cell cultures revealed a significant increase in chromosomal aberrations. These studies emphasize the need for screening for mycoplasma in cultured amniotic cells.*

In utero diagnosis of chromosomal abnormalities in early gestation by amniocentesis has led to the establishment of prenatal diagnostic centers (*1*). Since the finding of an abnormal karyotype usually leads to therapeutic abortion, the chromosomal analysis of the cultured amniotic fluid cells must reflect the true endowment of the fetus, and false positive results must be avoided. Mycoplasma, a prokaryotic organism, is a frequent tissue culture contaminant (*2*) which can infect cells without obvious alterations in their growth or morphology (*3*) and can cause chromosomal aberrations in cultured human diploid fibroblasts (*4*). In view of the potential hazard to prenatal diagnosis that this microorganism represents, amniotic fluid cell cultures grown in our laboratory were screened for mycoplasma contamina-

tion and examined for chromosomal abnormalities.

Cell cultures of amniotic fluid were established and cultured as previously described (*1*). To ensure against loss of a cell culture by contamination, all amniotic fluid specimens were routinely divided into two parallel cultures which were kept in separate incubators, subcultured on alternate days, and grown in different batches of media.

These cultures were screened for mycoplasma shortly after chromosomal analysis. Each cell culture was examined by microbiological (*2*), histological (*5*), and biochemical (*6, 7*) techniques. The results of mycoplasma screening of the replicate cultures from ten diagnostic amniocenteses are summarized in Table 1. Slightly over half (11 of 20) the cultures examined revealed prominent $23S_E$ (Svedberg constant by electrophoresis) and $16S_E$ prokaryotic RNA peaks in addition to the human 28*S* and 18*S* ribosomal RNA peaks on polyacrylamide gel electrophoresis (PAGE). Coelectrophoresis of contaminated cellular RNA with both mycoplasma and bacterial RNA indicated that these prokaryotic $23S_E$ RNA peaks were mycoplasmal in origin (*8*). Results of differential incorporation of [^{3}H]uridine and [^{3}H]-uracil into RNA (Udr/U), a recently developed biochemical test for mycoplasma contamination (*7*), agreed with PAGE analysis in all cultures examined. Orcein staining was concordant with PAGE results in 17 of the 20 cultures. Aerobic and anaerobic cultures of amniotic fluid cells and their media for mycoplasma (*2*) were consistently negative as were microbiological cultures for bacteria and fungi. This disparity between biochemical and microbiological testing for mycoplasma has been observed frequently and indicates the difficulty of growing these fastidious organisms on cell-free media (*5, 9, 10*).

We are aware of the general consensus that a microorganism should not be classified as a mycoplasma unless growth on solid medium is accomplished (*10*). Although we agree with this statement to the extent that formal classification should include this property, it is our contention, supported by the findings of others (*11*), that cell cultures can be contaminated with mycoplasmas that cannot be grown on currently available solid media. Thus, when other noncultural methods indicate the presence of these organisms, we feel justified in describing them as mycoplasmas. Therefore, an amniotic fluid cell culture was considered to be contaminated if Udr/U values were below 350 and mycoplasma RNA species were observed by PAGE.

By phase microscopy the contaminated and uncontaminated cells were morphologically indistinguishable, and the interval between amniocentesis and growth of sufficient cells for karyotyping was not significantly different: 19.5 days (contaminated) as compared to 19.7 days (uncontaminated). Establishment and growth of the amniotic fluid cell cultures in the presence of the antibiotics, aureomycin (Lederle) and gentamycin (Schering), did not prevent mycoplasma contamination (Table 1).

Chromosomal analysis of cultured amniotic fluid cells was performed as described (*1*). Metaphases suitable for karyotyping were examined and scored without knowledge of the results of mycoplasma screening, and the frequency of chromosomal gaps and breaks, polyploidy, and aneuploidy was determined. In addition, routine chromosome analysis was performed in the clinical cytogenetics facility. There was a significant ($P < .02$) fourfold increase in chromosomal breaks and gaps observed in the metaphase preparations from the mycoplasma infected cultures [24/307 (7.8 percent)] when compared with

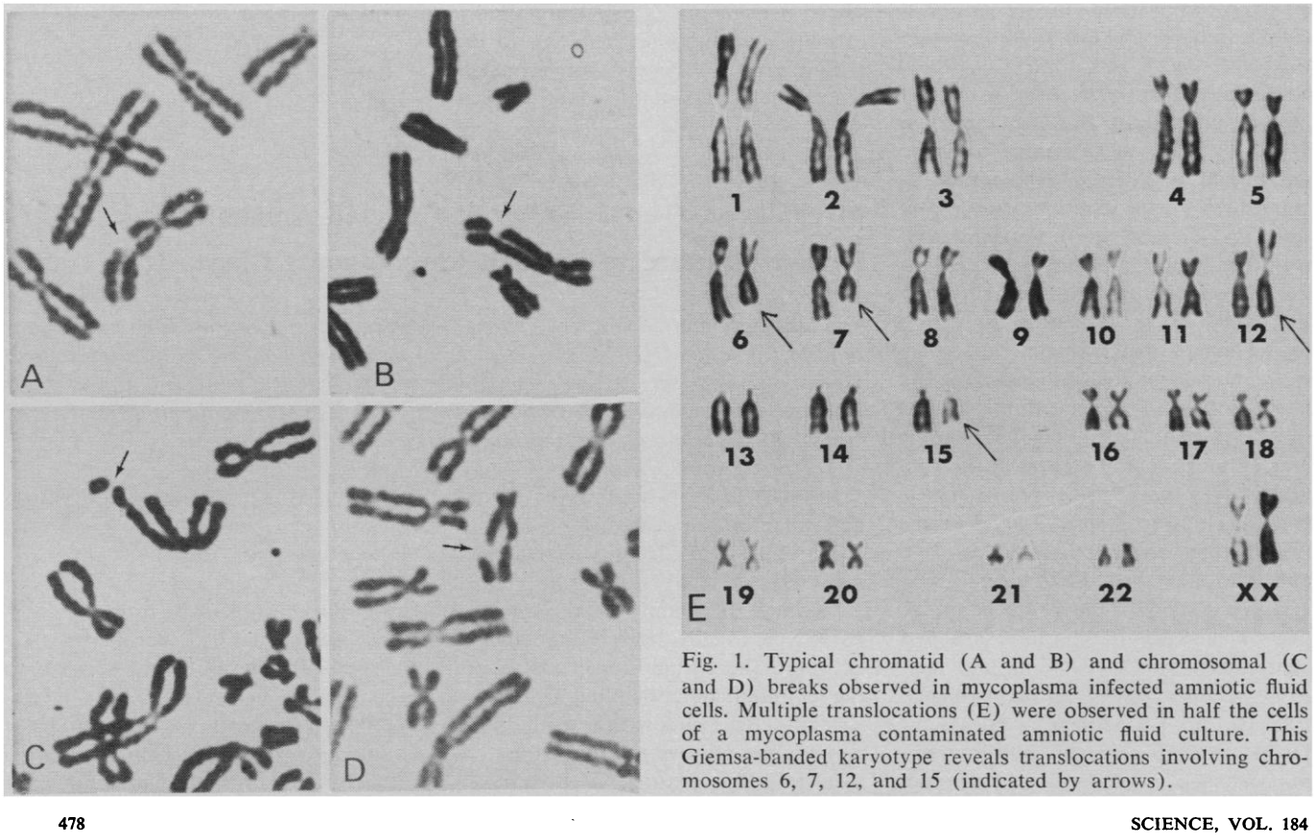

Fig. 1. Typical chromatid (A and B) and chromosomal (C and D) breaks observed in mycoplasma infected amniotic fluid cells. Multiple translocations (E) were observed in half the cells of a mycoplasma contaminated amniotic fluid culture. This Giemsa-banded karyotype reveals translocations involving chromosomes 6, 7, 12, and 15 (indicated by arrows).

uninfected cultures [3/166 (1.8 percent)]. Typical breaks and gaps are seen in Fig. 1, A to D. Aneuploidy, defined as alteration in chromosome number, was observed twice as frequently in mycoplasma contaminated cultures [39/307 (14.7 percent) as compared to 11/166 (7.1 percent), $.10 > P > .05$]. Polyploidy, a not infrequent finding in normal amniotic fluid cell cultures, was not significantly different in uncontaminated [11/307 (3.7 percent)] and mycoplasma contaminated cultures [5/166 (3.1 percent)].

Analysis of one mycoplasma infected culture by the clinical cytogenetics laboratory revealed a highly unusual mosaicism with half the cells examined possessing consistent multiple translocations. The chromosome rearrangements (Fig. 1E) were not those observed in known human chromosomal disorders, but were more likely the result of mycoplasma contamination. A parallel uncontaminated culture derived from the same amniocentesis revealed only normal karyotypes. Continuation of the pregnancy was advised and subsequent chromosome analysis of the newborn child was normal. However, without the availability of a parallel uncontaminated culture and evidence of mycoplasma contamination in the amniotic fluid cell culture with chromosomal abnormalities, prenatal diagnosis would have resulted in false positive results and possible therapeutic abortion.

Mycoplasma contamination was detected by biochemical testing in amniotic fluid cell cultures obtained from two other prenatal diagnostic centers (*12*). To ensure against contamination by the testing laboratory, replicate amniotic fluid cell cultures from an outside center were received, grown, and tested by separate tissue culture facilities. Mycoplasma were detected in the same two of four cultures examined separately, an indication that the contamination did not originate in the testing laboratory. Therefore, mycoplasma contamination would appear to be as widespread in cultured amniotic fluid cells as it is in other types of cultured human diploid cells (*2, 6, 7*).

Microbiological studies have indidicated that contamination of cultured cells can arise from various sources including faulty aseptic technique, aerosolization from other infected cultures, and from the use of contaminated cell culture medium (*13*). Since mycoplasma can be found in the female genital tract (*14*), amniotic fluid from ten amniocenteses were inoculated directly into specially prepared mycoplasma broth. After 3 weeks, all cultures were negative. Unfortunately, direct biochemical testing of the amniotic fluid could not be performed, since cultured cells are required. Therefore, we cannot exclude the possibility that mycoplasmas which do not grow in defined medium are present in utero. However, in our opinion, the most likely sources of the observed contamination of amniotic fluid cell cultures are growth media constituents, particularly fetal calf serum, and faulty sterile technique.

Our results indicate that mycoplasma infection of cultured amniotic fluid cells leads to a significantly increased incidence of chromosomal alterations. Breaks and gaps induced by mycoplasma should not confuse prenatal chromosomal diagnosis except, perhaps, in Bloom's syndrome or Fanconi's anemia. However, translocations and alterations of chromosome number could lead to false positive results. We therefore recommend that all amniotic fluid cell cultures which reveal chromosomal mosaicism, multiple or unusual translocations, or frequent breaks be examined for mycoplasma contamination. Parallel cultures of all amniotic fluid cells are also advised since, as in our case of chromosomal mosaicism, it may provide a source of uninfected cells. Biochemical as well as microbiological screening should be done since our study and previous studies indicate that mycoplasma contamination can be missed by microbiological testing alone (*6, 7*).

Table 1. Results of biochemical, histological, and microbiological screening of cultured amniotic fluid cells. Amniotic fluid cell cultures were supplemented with antibacterial drugs: penicillin (100 μg/ml) and streptomycin (100 μg/ml) (P), gentamycin (Schering) (50 μg/ml) (G), or aureomycin (Lederle) (50 μg/ml) (A). [^{3}H]Uridine-labeled cellular RNA's extracted from each culture were subjected to electrophoresis on 2.5 percent polyacrylamide gels (PAGE) and then examined for the presence (+) and absence (−) of prokaryotic $23S_E$ and $16S_E$ RNA's. Duplicate amniotic fluid cultures were incubated for 18 hours with either [^{3}H]uridine or [^{3}H]uracil (5 μc/ml, specific radioactivity >20 c/mole). The cells were then removed with 0.1 percent Pronase (Calbiochem) and washed with saline; the nucleic acids were precipitated with 5 percent trichloroacetic acid and then hydrolyzed at 90°C for 15 minutes with 5 percent perchloric acid (PCA). Portions of the PCA supernatant were taken for measurement of RNA content by the orcinol reaction and for radioactivity by scintillation counting. The ratio of the specific activities of [^{3}H]uridine-labeled RNA to [^{3}H]uracil-labeled RNA (Udr/U) was determined (*7*). Values of 350 or above were considered negative for mycoplasma contamination. Cell cultures were grown on slide-flaskettes (Lab-Teck) and then fixed and stained with orcein (*4*). Extranuclear staining was indicative of mycoplasma contamination. Microbiological cultures were made for mycoplasma, bacteria, and fungi.

Cell culture	Antibiotic	$23S_E$ and $16S_E$ RNA's on PAGE	Udr/U	Aceto-orcein staining	Microbiological culture
91	P	−	840.0	+	−
96	P	−	699.0	−	−
98	P	−	408.0	+	−
98	A	−	374.6	−	−
99	P	−	492.5	−	−
99	A	−	681.0	−	−
107	P	−	482.4	−	−
107	A	−	778.5	−	−
115	P	−	850.0	−	−
90	P	+	176.5	+	−
90	G	+	1.4	+	−
91	G	+	4.3	+	−
95	P	+	4.6	+	−
95	G	+	10.4	+	−
96	G	+	8.7	+	−
97	P	+	215.0	+	−
97	G	+	178.0	+	−
110	P	+	62.7	−	−
110	A	+	82.7	+	−
115	A	+	57.9	+	−

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8. [^{3}H]Uridine-labeled cellular RNA from contaminated amniotic fluid cultures were subjected to coelectrophoresis on parallel 2.5 percent polyacrylamide gels at 5 ma for 2 hours with [^{14}C]uridine-labeled *Escherichia coli* and *Mycoplasma hyorhinis* RNA; *M. hyorhinis* and *E. coli* had identical 16*S* RNA peaks. The larger mycoplasma ribosomal RNA peak migrated to 23S_E position coincident with the prokaryotic RNA peak observed in the contaminated cells, whereas the bacterial RNA peak migrated to a 22S_E position.
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15. We thank Drs. M. Kaback, L. Karp, L. Hayflick, T. Makinodan, F. Conte, and R. Schneider, and Ms. H. Lawce. Supported by NIH grants GM-19,527 and AI-00,082 and grants from the John A. Hartford Foundation, the Maternal and Child Health Service, and the National Foundation–March of Dimes.

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14 November 1973; revised 16 January 1974

Human Acid Phosphatase in Somatic Cell Hybrids

Abstract. *The human enzyme, lysosomal acid phosphatase ACP_2, is expressed in man-rodent somatic cell hybrids as a dimeric molecule. The human-rodent heteropolymer, as well as the human and rodent homopolymer, is associated with lysosomes in these cells. The genes specifying lysosomal acid phosphatase ACP_2 and LDH A are syntenic.*

Somatic cell hybrids formed between aneuploid rodent cells and human fibroblasts or white blood cells selectively lose human chromosomes (*1, 2*). Human genes can be assigned to particular chromosomes by correlating the presence or absence of the gene product in a series of hybrid clones with the presence or absence of specific chromosomes (*3*). These hybrids provide a unique opportunity to study the organization and regulation of genes coding for enzymes and proteins associated with particular subcellular structures. We describe the expression of human lysosomal acid phosphatase ACP_2 in man-mouse and man-hamster somatic cell hybrids and the syntenic relationship of the gene specifying this enzyme with the gene specifying LDH A.

Rodent parental cells used for the preparation of hybrids included RAG, a mouse cell deficient in hypoxanthine–guanine phosphoribosyl transferase; 3T3 4E, a mouse cell deficient in thymidine kinase; and E 36, a hamster cell deficient in hypoxanthine–guanine phosphoribosyl transferase (*4*). Human parental cells included white blood cells from two normal males, white blood cells from two unrelated females carrying different translocations involving chromosomes X and 19, or diploid fibroblasts from two normal males. Parental cells were treated with Sendai virus inactivated by β-propiolactone (*2, 5*), and hybrid colonies were selected in Dulbecco's modification of Eagle's medium supplemented with 5 or 10 percent fetal calf serum, $1 \times 10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine (*6*) (HAT medium). For electrophoretic analysis, cells from primary hybrid clones grown in HAT medium were harvested by trypsinization 9 to 12 weeks after hybridization.

Cell extracts were analyzed for acid phosphatase (E.C. 3.1.3.2.) by means of the starch gel systems of Lundin and Allison (*7*) and Swallow and Harris (*8*). Enzyme activity was detected with an α-naphthyl phosphate–fast garnet GBC salt or a β-glycerol phosphate–lead nitrate stain (*7*). The hybrid clones were analyzed by starch gel, agarose, acrylamide, or Cellogel electrophoresis for the presence of human and rodent forms of a number of enzymes which included: adenosine deaminase (E.C. 3.5.4.2), adenylate kinase 2 and 3 (E.C. 2.7.4.3), aspartate aminotransferase (E.C. 2.6.1.1), α-galactosidase (E.C. 3.2.1.22), β-glucuronidase (E.C. 3.2.1.31), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), glucose phosphate isomerase (E.C. 5.3.1.9), hexosaminidase A and B, indophenol oxidase dimer and tetramer, isocitrate dehydrogenase (E.C. 1.1.1.42), lactate dehydrogenase A and B (E.C. 1.1.1.27), malate oxidoreductase (E.C. 1.1.1.37), malate oxidoreductase (decarboxylating) (E.C. 1.1.1.40), purine nucleoside phosphorylase (E.C. 2.4.2.1), peptidases A, B, and C, phosphoglucomutase 1 (E.C. 2.7.5.1), phosphogluconate dehydrogenase (E.C. 1.1.1.43), phosphoglycerate kinase (E.C. 2.7.2.3), and phosphopyruvate hydratase (E.C. 4.2.1.11) (*9*).

Starch gels of extracts of certain hybrid clones showed two new bands of acid phosphatase activity in addition to the rodent cathodal band (Fig. 1). The electrophoretic mobility of the anodal band is comparable to the major component of extracts of human diploid fibroblasts (see below). The second new component occupies a position intermediate to those of the rodent and human isozymes and presumably is a heteropolymer composed of rodent and human subunits. The formation of a heteropolymer is consistent with the

Table 1. Expression of human lysosomal acid phosphatase ACP_2 and human LDH A in man-mouse and man-hamster hybrid clones. The column designations are +/+, ACP_2 present and LDH A present; +/−, ACP_2 present and LDH A absent; −/+, ACP_2 absent and LDH A present; and −/−, ACP_2 and LDH A absent.

Source	Number of clones			
	+/+	+/−	−/+	−/−
RAG/WBC (X/19 translocation W)	3	0	0	5
RAG/WBC (X/19 translocation B)	7	0	0	7
RAG/WBC (D, normal male)	2	0	0	0
E 36/WBC (X/19 translocation W)	13	2	0	8
E 36/WBC (G, normal male)	2	0	0	1
3T3 4E/fibroblasts (normal male A)	3	0	0	1
3T3 4E/fibroblasts (normal male B)	4	0	0	1
Total	34	2	0	23