not significant; however, this is still under investigation and all data reported herein are based solely on initial readings. Significant differences were observed between normal and buphthalmic animals as early as 35 days of age.

It should be noted that even though the AX strain was not used as a control it contained phenotypically normal animals with cornified cell counts analogous to the other normal animals. These were not used since it has not been determined at this point whether there is an effect in the heterozygote (*Bubu*) and we did not therefore wish to confound the data. The III strain control which is known not to contain the buphthalmic gene is a different subline from that in which the buphthalmics were observed.

There appears to be an increase in number of cornified cells with age in the early weeks of life followed by a plateau 5 to 14 weeks with a subsequent increase to a peak at about 130 days. The numbers then decline and stabilize. The peak at 134 days might be due to chance variation or possibly to a metabolic upset occurring concurrently or caused by reaching the age of puberty. Two months has now been taken as the standard age for observations because of the apparent stability at this early prepuberal age.

Summary. Pathological and preclinical stages in the buphthalmic rabbit's eye can be distinguished by the degree of corneal cornification. This is determined by enumeration of cells removed from the intact anesthetized eye. Five normal strains and 2 buphthalmic strains were investigated. The buphthalmics had cell counts significantly higher than those of genetically normal animals. No significant differences were observed either between any of the control strains or between buphthalmic groups.

1. Hanna, B. L., Sawin, P. B., Sheppard, L. B., Genetics, 1962, v47, 519.

2. Kolker, A. E., Moses, R. A., Constant, Marguerite, Becker, B., Invest. Ophthalmol., 1963, v2, 316.

3. Huber, W. G., Smith, G. S., J. Vet. Med., 1963, v58, 875.

Received February 1, 1965. P.S.E.B.M., 1965, v119.

Chromosome Changes in PPLO-Infected FL Human Amnion Cells.* (30145)

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Several reported effects of mycoplasma (pleuropneumonia-like organisms, PPLO) are very similar to virus effects. These include transmissable cytopathic effects(1,2,3) and ultrastructural changes(1) in cultured mammalian cells, hemagglutination(4), and change of host cell surfaces which makes them capable of hemadsorption(5). This report extends the observations on the similar capacities of PPLO and viruses. A study of FL human amnion cells(6), following PPLO infection, has shown the mycoplasma able to affect the genetic composition of the mammalian cells.

Major changes in the chromosomes of FL cells, cultured continuously for 9 years, have not occurred since the first analysis in 1957 (10). Chromosome numbers were determined again in 1961(11), more extensively in 1962(7), and, in addition, from control preparations for the present study. Other characteristics which distinguish these transformed human cells from the normal human karyotype have also been reported(7).

Materials and methods. A strain of PPLO (3) which we isolated from tissue cultures demonstrated the characteristics of mycoplasma by agar cultivation and electron microscopy and was sensitive to aureomycin.

* This investigation was supported in part by U.S.P.H.S. Research Grant CA-05883 and Research Career Award K6-CA-21607-01 to Jørgen Fogh from Nat. Cancer Inst.

For the present study FL cells cultured serially in LY medium(6) or Bodian's medium(7) with 20% human serum, penicillin, and streptomycin were inoculated with the PPLO. The mycoplasma was obtained either from infected cell culture filtrates or, in other experiments, from BYE broth(8) culture after many subpassages of the PPLO in this medium. For chromosome analysis the FL cells were prepared by a method based on the suspension technique developed by Moorhead *et al*(9) and with ignition of the fixative. Chromosome aberrations occurring in uninfected FL cells, as determined from control preparations for the present study, are shown in Table I.

Results. In the PPLO-infected FL cells the changes included reduction in chromosome number, increase in chromosome aberrations, and the appearance of new chromosome varieties.

A. *Reduction in chromosome number.* While the numbers for uninfected FL cells were predominantly between 70 and 76, the PPLO-carrying cultures showed a gradual decrease and, after a number of months, the chromosome numbers were in the range of 63 to 68 (Fig. 1). Within the first days after PPLO inoculation some cells with lower numbers could be observed (in one experiment 14% of the cells had fewer than 68 chromosomes at the third day). However, further experiments made it apparent that the early reduction in numbers was temporary; the chromosome numbers 10 or 30 days after the initial PPLO inoculation were essentially similar to the numbers in uninfected cells, indicating that the early-produced cells with low numbers might have lost their proliferative capacity. Further reduction was seen in the following months: The proportion of cells with numbers less than 68 was, at 47 days, 20%; at 67 days, 60%; at 246 days, 74%; and at 318 days, 78%. Cells with very low numbers were observed (one cell with 51 chromosomes at 67 days, and one cell with only 48 chromosomes after 246 days of infection). The majority of these cells with reduced chromosome numbers was obviously proliferative, as indicated by the growth of the cultures.

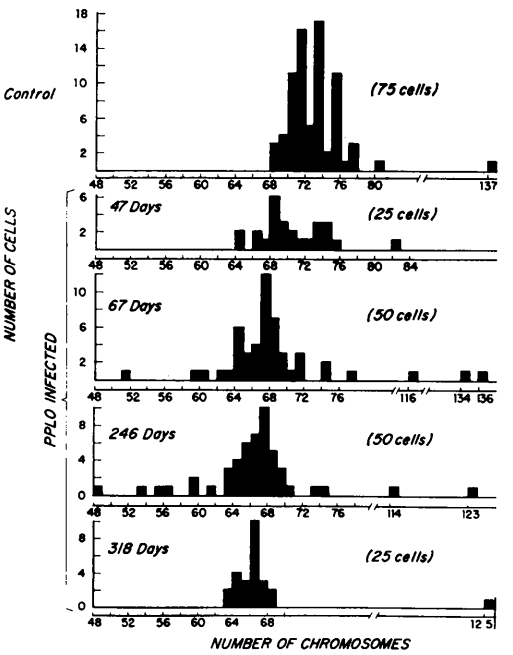

FIG. 1. Comparison of distribution of chromosome numbers in uninfected FL cells and FL cells from cultures carrying PPLO infection for various periods (Exp F 138).

B. *Increase in chromosome aberrations.* The types of changes observed are depicted in Table I, showing the data of one experiment (F 138), in which PPLO-infected cells presently have been carried for more than 10 months. The incidence of cells in endoreduplication increased with time of cultivation in the presence of PPLO; more than 10% of the colchicine-blocked metaphase cells showed endoreduplication after 318 days. Dicentric chromosomes and acentric fragments were also more frequently observed in the metaphases of the PPLO-carrying cells. Translocational exchanges, observed in only one uninfected FL cell of approximately 2000 metaphases examined, were found rather frequently in the infected cultures, and ring chromosomes (never before observed in FL cells) were seen after long periods of infection in this experiment. In other experiments they occurred as early as 4 and 10 days after infection. There was a possible increase in the appearance of secondary constrictions, and destruction of chromosomes was definitely more frequent. In addition,

TABLE I. Percentage of Metaphase Plates with Chromosome Abnormalities in Uninfected FL Cells (Control) and in FL Cells Carrying PPLO Infection for Various Periods (Experiment F 138).

Type of cell	Days after PPLO inoculation	No. metaphase plates	Metaphase plates with abnormalities (%)								
			Endoreduplication	Dicentric chromosomes	Acentric fragments	Minute chromosomes	Translocational exchanges	Ring chromosomes	Secondary constrictions	Chromatid breaks	Chromosome destruction
Control	—	387	.5	1.0	.8	1.8	.3	—	2.3	.8	.3
PPLO-infected	47	108	2.8	—	—	—	.9	—	.9	—	1.9
	67	99	7.1	3.0	1.0	—	1.0	—	2.0	4.0	2.0
	246	250	4.4	1.6	2.0	4.4	—	3.2	3.2	.8	2.4
	318	158	10.8	4.4	2.5	1.3	1.9	1.3	5.7	1.9	1.9

cells showing fragmentation of the entire chromosome complement have been observed. Chromatid breaks were, in several experiments, observed consistently within the first week of infection; their frequency occasionally appeared increased after longer periods of infection, although in some late preparations they were absent or observed only rarely.

Other similar experiments, at this time carried on for shorter periods, have confirmed the impression that although chromosome aberrations occurred during the first days after infection, most changes developed very slowly in the PPLO-infected cell cultures.

C. *New chromosome varieties.* Three new chromosome varieties, not observed in any of the approximately 2000 analyzed metaphase plates of uninfected FL cells, were found in repeated experiments in the PPLO-carrying cell populations. The data in Table II show the appearance of a very large teleocentric chromosome, which is shown in Fig. 3. In a recent experiment this chromosome was found in a single cell 28 days after the initial PPLO infection. In other experiments it appeared later. Our data show increasing frequency of this chromosome, with time, up to 28.5% following 10 months of serial cultivation of PPLO-infected cells.

A large metacentric chromosome (Fig. 4), occurring rather infrequently, was observed in one experiment as early as 4 and 10 days after infection. In another experiment it was seen after 10 months.

A large subteleocentric chromosome (Fig. 5) appeared early after infection. It was found in 20% of the metaphases after 2 months. Later, its frequency decreased, although after 10 months it was still seen in 4.4% of the analyzed cells.

The 3 chromosome varieties have now been observed in 4 independent experimental series.

Discussion. Chromosome changes, observed as a consequence of virus infection of mammalian cells, resemble in many respects the PPLO effects on FL cells reported here. Although all presently available data on virus-induced changes were obtained from studies with diploid or near-diploid cells, these

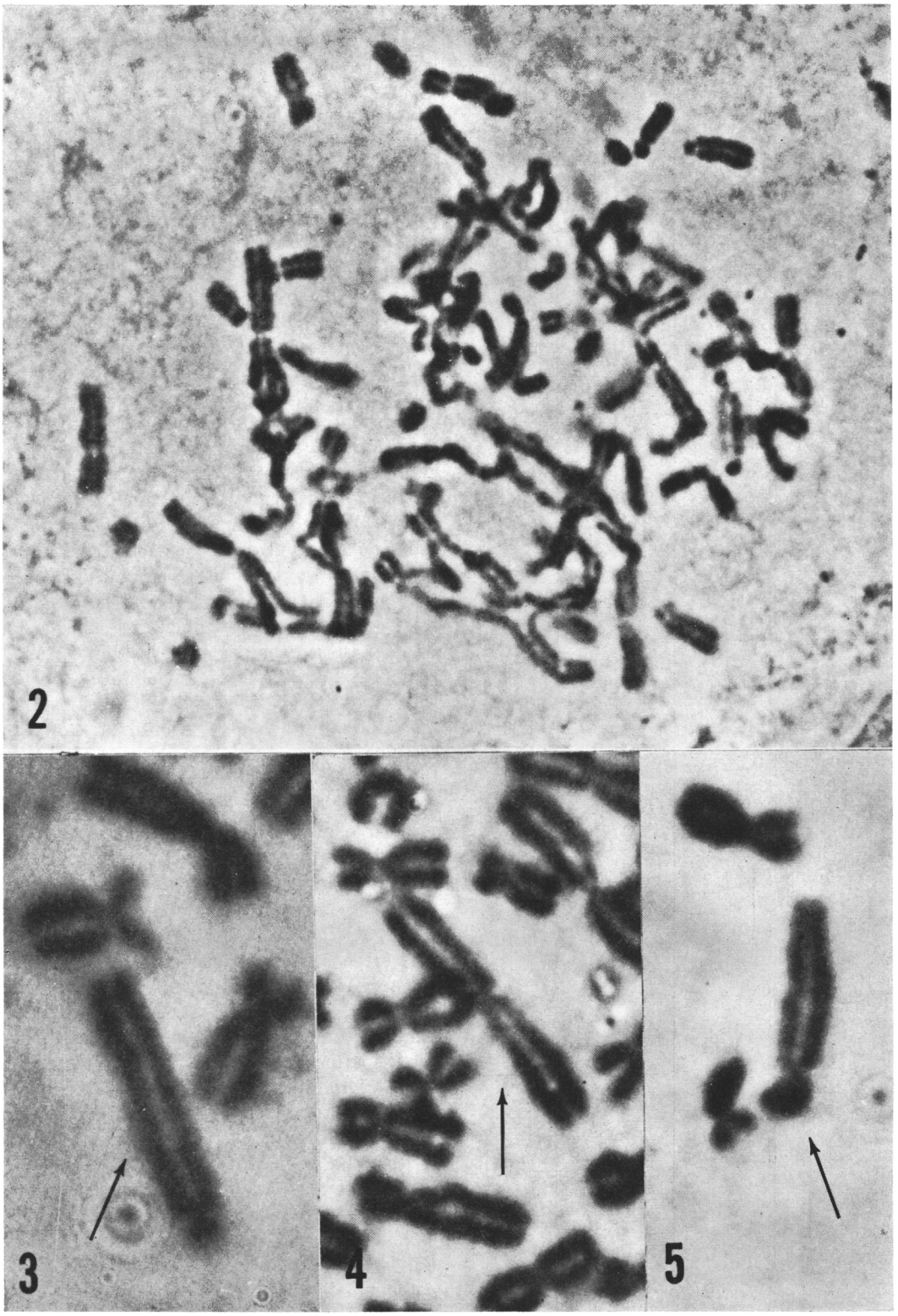

FIG. 2. Extensive complex chromosome aberrations indicating reunion of breaks and structural rearrangements in metaphase plate of PPLO-infected FL cell after 47 days infection of the cell line. Magn. 2640×.

TABLE II. Incidence of 3 New Chromosome Varieties Observed in PPLO-Infected FL Cells at Various Times After the Initial Infection (Data from 3 Experimental Series).

Exp No.	Days after PPLO inoc	No. of metaphase plates	New chromosome varieties: Large teleocentric	Large metacentric	Large subteleocentric
F 159	4	225	—	.4	1.3
	10	225	—	.9	—
	28	125	.8	—	—
F 138	47	108	—	—	.9
	67	99	3.0	—	20.2
HT 8	97	150	1.3	—	8.0
F 138	246	250	18.4	—	—
	318	158	28.5	1.3	4.4
Uninoculated controls		ca. 2000	—	—	—

changes and those observed in the PPLO-affected hypertriploid FL cells show striking similarities. Thus extensive changes in chromosome numbers, with many of the cells showing low counts, were also seen after SV40 infection of diploid human fibroblasts (12). Increase in dicentrics and acentric fragments followed infection with both herpes simplex virus(13) and SV40(12). A few dicentrics were also found in patients with chicken pox(14). Minute chromosomes were observed in SV40 transformed cells(12). Rearrangements of chromosomes with exchanges and translocations were effects of all the above viruses, of human adenovirus type 12 (15) and, to a small degree, of measles virus(16).

Chromosome breaks, observed as an early effect of most of the viruses studied(13,16,17, 14, 15), were not encountered in our material, even early after infection, at frequencies as high as those observed with measles and chicken pox infection, for example. In SV40 transformed cells yielding infectious virus, chromosome breaks did not appear to be a characteristic change(19,12). Fragmented chromosomes, observed in our PPLO-infected cells, were also a characteristic of the adenovirus type 12-infected Chinese hamster cells (15) and diploid human embryonic cells infected with herpes zoster virus(18).

New chromosome varieties have been reported in Chinese hamster cells after infection with herpes simplex virus(17) or adenovirus type 12(15). However, they differed from cell to cell. In the SV40 transformed human cells, several abnormal chromosomes were observed; including, in one line, an abnormal, long subteleocentric chromosome, such as seen in our PPLO-carrying cells(12). The consistency of the appearance of new chromosome varieties in 4 independent experiments following PPLO infection of FL cells and their increasing frequency, especially of the long teleocentric, with time of cultivation of the infected cells, indicates that PPLO-induced genetic changes is a more likely mechanism than an adaptation or selection process. This assumption is supported by the absence of such varieties from the extensive number of uninfected FL cells examined. In some cells both the large teleocentric and subteleocentric chromosomes were observed in the same metaphase plate, which excludes the possibility that these 2 chromosomes are identical. The large submetacentric marker chromosome characteristic of FL cells(7) was also seen in those cells containing the new varieties.

The appearance of the new varieties on separate occasions may indicate that specific loci on FL cell chromosomes are affected by

FIG. 3. New chromosome variety (arrow), a very long teleocentric observed only in PPLO-infected FL cells. Magn. 5040×.

FIG. 4. Another new variety (arrow), an unusual, large metacentric chromosome seen in PPLO-infected FL cultures. Magn. 5040×.

FIG. 5. A long subteleocentric chromosome (arrow) in PPLO-infected FL cell never observed in the uninfected cells. Magn. 5040×.

infection with this PPLO strain. Whether the effects here described are due to an intracellular action of the mycoplasma(1) or to mycoplasma-associated products, is yet to be determined. Other effects on FL cells after infection with this PPLO include not only early cytolytic effects, but also the subsequent appearance of a "PPLO-resistant" cell population showing changes in morphology and division rate of the cells(3). It seems reasonable to assume that these changes, and at least some of the chromosome aberrations, are related. The heteroploid FL cells were particularly susceptible to this mycoplasma and were therefore chosen for these initial studies. Further investigations now in progress will include other cell types, also diploid cells, in order to evaluate how generally the effects here described occur as a consequence of infection with this and other strains of mycoplasma.

Summary. Characteristic chromosome changes were observed in PPLO-infected FL human amnion cells. These changes included a gradual reduction in chromosome numbers, increase in chromosome aberrations, and the appearance of 3 new varieties. Although some of the aberrations appeared early after infection, most changes developed slowly, over a period of several months.

1. Edwards, G. A., Fogh, J., J. Bact., 1960, v79, 267.

2. Kraemer, P. M., Defendi, V., Hayflick, L., Manson, L. A., Proc. Soc. Exp. Biol. and Med., 1963, v112, 381.

3. Fogh, J., Hahn, E., Fogh, H., Exp. Cell Res., in press.

4. Tully, J. G., Proc. Soc. Exp. Biol. and Med., 1963, v114, 704.

5. Berg, R. B., Frothingham, T. E., ibid., 1961, v108, 616.

6. Fogh, J., Lund, R. O., ibid., 1957, v94, 532.

7. Petursson, G., Fogh, J., Cancer Res., 1963, v23, 1021.

8. Barile, M. F., Yaguchi, R., Eveland, W. C., Am. J. Clin. Path., 1958, v30, 171.

9. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battigs, D. M., Hungerford, D. A., Exp. Cell Res., 1960, v20, 613.

10. Levan, A., Genetics and Cancer, Univ. of Texas, Austin, 1959, p151.

11. Fogh, J., Biedler, J. L., Denues, A. R. T., Ann. N. Y. Acad. Sci., 1961, v95, 758.

12. Koprowski, H., Ponten, J. A., Jensen, F., Ravdin, R. G., Moorhead, P., Saksela, E., J. Cell. and Comp. Physiol., 1962, v59, 281.

13. Hampar, B., Ellison, S. A., Nature, 1961, v192, 145.

14. Aula, P., Hereditas, 1963, v49, 451.

15. Stitch, H. F., Van Hoosier, C. L., Trentin, J. J., Exp. Cell Res., 1964, v34, 400.

16. Nichols, W. W., Levan, A., Hall, B., Östergren, G., Hereditas, 1962, v48, 367.

17. Hampar, B., Ellison, S. A., Proc. Nat. Acad. Sci., 1961, v49, 474.

18. Benyesh-Melnick, M., Stich, H. F., Rapp, F., Hsu, T. C., Proc. Soc. Exp. Biol. Med., 1964, v117, 546.

19. Shein, H., Enders, J., Proc. Nat. Acad. Sci., 1962, v48, 1164.

Received February 3, 1965. P.S.E.B.M., 1965, v119.

Specificity of Enzyme Inhibition by DDD.* (30146)

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The chlorophenyl position isomers of 2,2-bis (chlorophenyl)-1,1-dichloroethane (DDD) produce adrenal cortical atrophy in the dog (1) and inhibition of steroidogenesis in the human and dog. The o,p′ isomer is more active in causing atrophy than the p,p′ and the m,p′ is the most active(2,3). In other species tested (rat, rabbit, guinea pig, cat, hamster) the atrophy does not occur under conditions of dosage and time similar to those producing it in the dog(1). In a non-responsive species Frenkel *et al*(4) have found lack

* This investigation was supported by USPHS Grants AM 08188-01 and AM 02767 from Arthritis and Metabolism Section.

† Markle Scholar in Academic Medicine.