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DEVELOPMENT OF A DIPLOID CELL LINE FROM FETAL CALF LUNG FOR VIRUS VACCINE PRODUCTION(*)

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ABSTRACT

Details of isolation and replication of a fetal calf diploid cell (FCDC) is given. From the Karyological point of view, the fairly large number of chromosomes existing in metaphase spreads made counting rather tedious . Lack of practical classification was another problem which made reference to individual chromosomes difficult . By increasing the population doubling of this cell , a tendency of telocentric chromosomes to undergo centric fusion was observed . Susceptibility of FCDC to different viruses is described .

It is generally Known that strains of fibroblast cells fron mammalian embryo tissues can be serially cultured *in vitro* for a limited period without evidence of changes in cultural behaviour or morphological pattern . The human diploid cells (HDC) such as Wi - 38 of Hayflick and Moorhead (1961) or MRC - 5 of Jacobs et al. (1970) are now widely used in the pharmaceutical industry for the production of human viral vaccines. Several diploid cells form fetal tissues of subhuman primates were also isolated and characterized by Petricciani et al. (1971). These cells are also susceptible to a number of human viruses and are under investigation for use in human viral vaccine production. This report suggests the feasibility of establishing a diploid cell system from tissues of other mammals. We tried to isoslate diploid cells from fetal calf lung. The following is our preliminary report.

PROPAGATION OF CELL CULTURES

Embryos of pregnant cows were isolated in the Tehran slaughterhouse and brought to the laboratory. Cows imported from a western country, having health

(*) Joint WHO/IABS Symposium on the standardization of cell substrates for the production of virus vaccines Geneva, Dec. 1976. Develop. biol. Standard., Vol, 37, pp. 53-57 (S. Karger, Basel 1977)

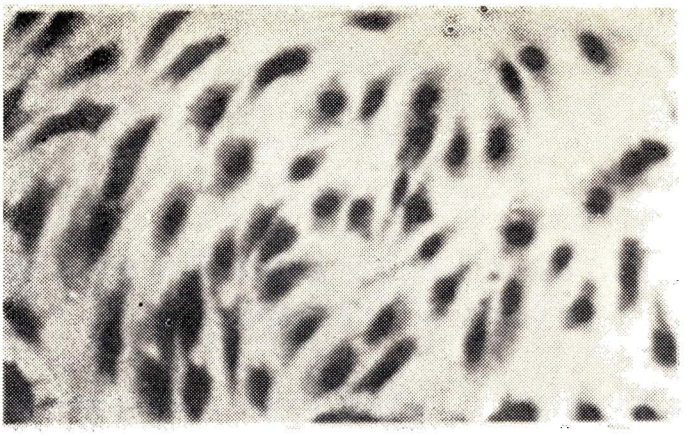

Fig. 1. Fetal calf diploid cells FCDC-2 at passage 30.

certificates, were inspected by veterinarians before and after killing. The carcass of cows giving fetus were carefully examined and a portion of lung of the fetus was also subjected to meticulous histological survey. No signs of any disease were recorded.

Serial propagation of trypsinized lung cells was effected according to the method of Hayflick and Moorhead (1961). Growth medium was Eagle Basal Medium (BME) (Grand Island Biological Company, N.Y.), supplemented with X 1 amino acids and vitamins of Eagle medium and 5% fetal calf serum. 4-oz bottles were seeded each with 6 ml of cells at a concentration of 10^5 cells per ml. After two days incubation at 36° C a confluent sheet of fibroblast cells (Fig. 1) was formed. Cells were subcultured every 2-3 days. Split ratio was 1:2. Up to the 12th subculture the confluency was obtained in 2-3 days; this time was increased gradually to 4-5 days when the number of subcultures reached 40. A slight sign of decline was exhibited when subcultures reached 50. At this stage a medium change was necessary and the confluency of cells was completed in 6 days. When subcultures reached 6, most cells were harvested and frozen in the presence of 10% dimethyl sulphoxide in BME.

All conventional *in vitro* and *in vivo* tests were used to control the presence of adventitious agents. So far no sign of contamination has been observed.

The absence of tumorigenicity was shown in suckling mice treated with anti-lymphocyte serum (ALS). KB cell used as control in ALS-treated baby mice induced tumors in control animals.

KARYOLOGICAL STUDY

The cows under experiment had a diploid number of $2n = 60$, with chromosome structures outlined by Hsu and Benirschke (1967) on *Bos torus*.

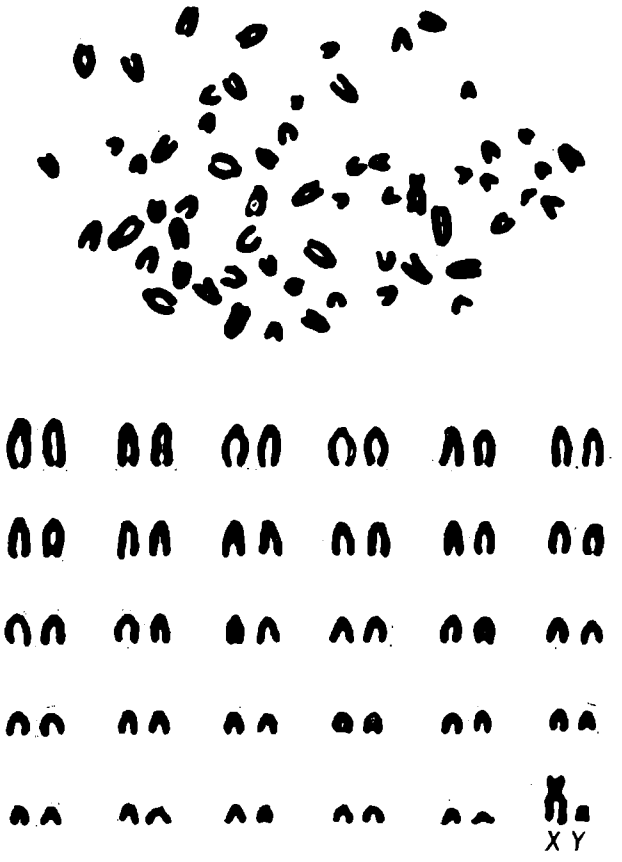

Fig. 2. Arbitrary arrangement of fetal calf diploid chromosomes.

Table I. Karyological data on fetal calf diploid cells FCDC-2 (2n = 60)

Culture Passage	Hypodiploidy	Hyperdiploidy	Polyploidy	Break & Gap	Structural abnormalities
5	5/500(1%)	0%	3/500(0.6%)	15/500(3%)	0
10	3/300(1%)	0%	2/300(0.66%)	10/300(3.3%)	0
20	9/300(3%)	0%	6/300(2%)	6/300(2%)	9/300(3%)
38	18/300(6%)	0%	12/300(4%)	9/300(3%)	9/300(3%)
44	30/300(10%)	0%	15/300(5%)	9/300(3%)	10/300(3.3%)
51	45/300(15%)	0%	16/300(5%)	15/300(5%)	15/300(5%)
60	54/300(18%)	2%	24/300(8%)	24/300(8%)	15/300(5%)

As demonstrated in Fig. 2 the authosomes included 58 acro (telo) centric chromosomes with even and gradually decreasing long arms. The readily distinguishable sex chromosomes consisted of an X which was a large submetacentric and a Y that appeared as a very small submetacentric chromosome.

The dgeree of gaps, breaks and polyploidy did not significantly alter, as the passage level of the cell was elevated up to 50. However at the neighbourhood of population doubling 40 onwards, structural abnormalities, that is, the appearance of di-and tricentric and elongated chromosomes start to emerge, reaching a level of 5% at doubling 50. These changes were still within the acceptability limit at population doubling 60 considering the fact that endoreduplications have also been included (Table I).

VIRUS SUSCEPTIBLILITY

Viruses used were: vesicular stomatitis (New Jersey serotype), vaccinia virus, foot-and-mouth disease virus type O, bovine viral diarrhea (NADL strain), infectious bovine rhinotrachitis virus, para-influenza type 3, rinderpest virus, vaccine strain KAO of Dr Plowright and measles virus (Sugiyama vaccine strain).

Three fetal calf diploid cells (FCDC) were isolated in our laboratory. FCDC-2 was selected for tests of viral susceptibility. Subcultivations 10 and 40 were selected for these studies. Different stock viruses were first adapted by one or two passages in FCDC-2 and were then seeded in 4-oz bottles containing a confluent sheet of FCDC-2 culture. Fluids were harvested when cytopathic effects (CPE) were sufficiently progressed and titrations were made in primary calf kidney or in BHK or vero cells depending on the previous adaptation of viruses to these cells. Results are reflected in Table II. The data showed that there was no significant difference in susceptibility of FCDC-2 cells at subcultures 10 and 40 for different viruses.

Several pilot lots of live attenuated measles vaccine (Sugiyama strain) and rinderpest vaccine were produced in FCDC-2.

Table II. Virus susceptibility of fetal calf diploid cells FCDC-2

Cell Passage	Measles	Vaccinia	V.S	R.P.	F.M.D	E.R.V	P.I.3	V.D.	I.B.R
10	4.0*	7.0	5.0	5.5	5.0	5.0	>6.5	5.0	>6.5
40	4.5 (4.5-5.5)	7.0 (7.5-8.0)	5.5 (5.0-6.0)	6.0-6.5 (5.0-7.0)	5.0 (6.0-7.0)	6.5 (6.5-7.5)	7.5 (6.0-7.5)	7.0 (5.0-6.0)	7.5 (6.0-7.5)

* Reciprocal Log 10 of the TCID 50 Per ml

V.S=Vesicular Stomatitis Virus. R.P=Rinderpest Virus. F.M.D=Foot and Mouth Disease Virus. E.R.V=Equine Rhinopneumonitis Virus. P.I.3=Parainfluenza 3 Virus. V.D=Vrial diarrhea Virus. I.B.R=Infectious Bovine Rhinotracheitis Virus

+ Figures in Parentheses are titers obtained in Control Culture systems

DISCUSSION

By applying the methodology of Hayflick and Moorhead (1961) 3 diploid cell lines derived from lung tissues of fetal calves were developed. One of these cells, FCDC-2 was investigated as a candidate for veterinary vaccine production. By karyological studies it was possible to follow the cytogenetic characteristics and the number of chromosomes was determined; but because of the great number of chromosomes, their unique acrocentric pattern, and the unavailability of a practical classification so that reference could be made to individual chromosomes or at least to individual groups of chromosomes in reporting abnormal findings, the karyotype was not established. The chromosome aberrations and polyploidy were rare, even when the number of doublings reached 50. The only noticeable change during the subcultivation of these cell lines was the formation of metacentric chromosomes by fusion of two acrocentrics. The number of metacentrics increased gradually by repeated subcultivations (Figs 3 and 4). This phenomenon observed first by Robertson in 1916 (5) and called "robertsonian translocation" or centric fusion seems to be the result of fusion of two acrocentric chromosomes. Referring to the wide range of susceptibility of this diploid cell to animal viruses, its use in production of animal virus vaccines should be economic and practical.

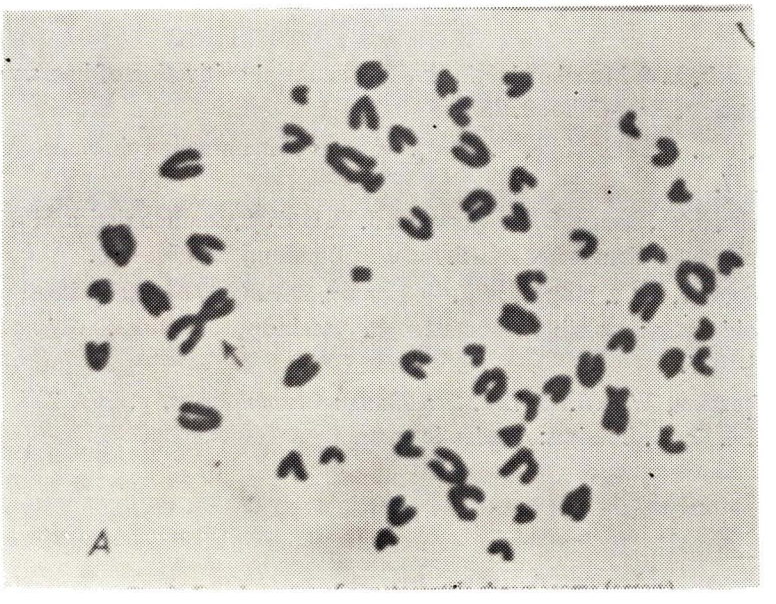

Fig. 3. Development of a metacentric chromosome (arrow).

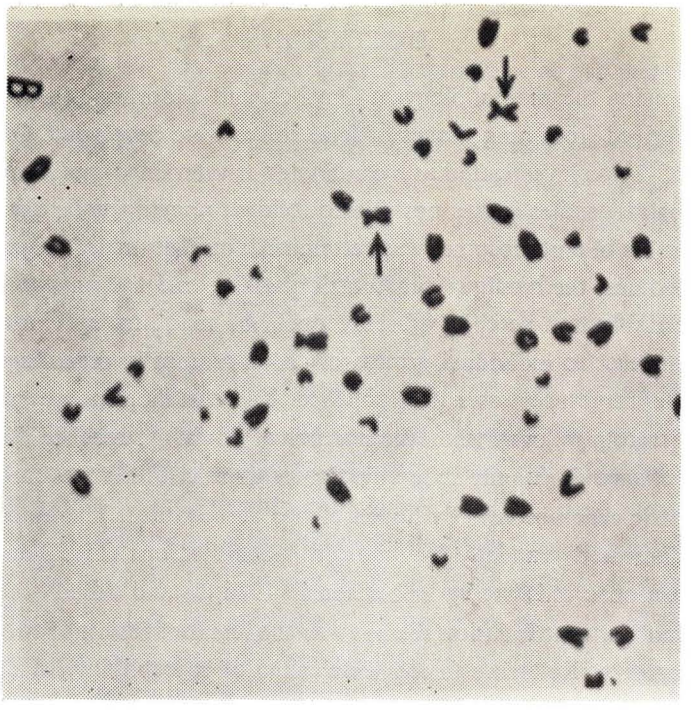

Fig. 4 - Development of 2 (Sub) Metacentric Chromosomes (arrow)

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