extensive periods of time with retention of the diploid configuration. Other workers [41, 59] have reported similar results with adult human diploid cells.

Morphology of diploid human fibroblast cell strains.—Figs. 1 and 2 represent fibroblasts of the WI-1 strain of diploid human fetal lung cells after 35 subcultivations and 9 months in *vitro*. Characteristically these cells are extremely elongated fibroblasts averaging about 185 $\mu \times 15$ μ. The single nucleus contains from 1 to 4 nucleoli which vary from oval to branching bodies. Individual cells are markedly transparent with characteristically

TABLE I. History of human diploid cell strains.

Strain designation	Fetus no.	Tissue of origin	Months in serial cultivation[a]	No. of subcultivations
WI-1	1	Lung	11	51
WI-2		Skin and muscle	6[b]	20[b]
WI-3	2	Lung	5	35
WI-4	3	Kidney	6	29
WI-5		Muscle	7	33
WI-6	4	Heart	2.5	10
WI-7		Thymus and thyroid	5	25
WI-8	5	Skin	8.5	32
WI-9		Kidney	8.5	29
WI-10	6	Kidney	5[c]	32[c]
WI-11	7	Lung	5[c]	30[c]
WI-12	8	Skin and muscle	8	41
WI-13		Kidney	8	40
WI-14	9	Skin	8	43
WI-15	10	Kidney	7.5	28
WI-16	11	Lung	8	44
WI-17		Liver	5[b]	24[b]
WI-18	12	Lung	8	53
WI-19	13	Lung	8	50
WI-20	14	Skin and muscle	5[b]	25[b]
WI-21	15	Heart	5	26
WI-22	16	Heart	1	5
WI-23	17	Lung	8	55
WI-24	18	Lung	7	39
WI-25	19	Lung	6[c]	38[c]

[a] Continuously passaged cells, never reconstituted from frozen stock (Series A).
[b] Serial cultivation of strain lost through bacterial contamination but cells from previous passages stored at −70°C.
[c] Still in culture as of February 28, 1961.

19 ABORTIONS

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Cytological Virological and Chromosomal Studies of Cell Strains From Aborted Human Fetuses.* (31037)

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1961 HAYFLICK
WI-1 – 25
= 19
+ 13
in this paper
= 32
elective abortions
43 miscarriages

Spontaneous abortion, usually without obvious cause, is a frequent occurrence in human pregnancies. To test the hypothesis that viral infections may play a part in the development of spontaneous abortion, a technique was sought to obtain dividing cells from human embryos that might be carrying latent viruses. We used a method developed by Jensen *et al*, for studying mouse tissues, in which cells could be obtained readily from organ explants. In the course of this work we collected cytological and chromosomal data on human fibroblast cell strains.

Materials and methods. Collection and preparation of specimens. Embryos were obtained from 2 sources: (A) surgical abortions performed in Scandinavia for social and psychiatric reasons, and (B) spontaneous abortions that occurred at the Philadelphia General Hospital and the Hospital of the University of Pennsylvania. The surgically removed embryos were placed in antibiotics containing Hanks' solution and shipped to us by air at a temperature of approximately 0°C. The spontaneous abortions were refrigerated in plastic bags without solution or antibiotics until collected, usually within 12 hours. Only those embryos which were expected to have viable tissues were studied. Aside from the decomposed external appearance, one of the best indicators of the embryo's condition appeared to be the physical aspect of the liver. All assays performed on embryos with friable and discolored livers were discarded, because the cells failed to grow.

Organ culture technique. The organ culture technique described by Jensen *et al*(1) was used: a grid of stainless steel mesh‖ was enclosed in a small Petri dish containing 10 ml of double strength Eagle's Basal Medium in isotonic Earle's solution with 10% calf serum; a small disc of open mesh paper (tea bag paper)** was moistened in the medium and applied to the top of the grid. Fragments of organs were cut into pieces about one cubic mm with a surgical blade and placed directly on the tea bag paper without being washed. Two explants were placed on top of each paper; the volume of the individual explants did not exceed 2 cu mm. The

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RA273 = 27 ELECTIVE ABORTIONS "ATTEN OF RA273 IN WI-38 69 PLOTKIN"
+ 40 THCHANG (IN MY DOCS RUBELLA)
67

TABLE I. Cell Growth Under Organ Cultures.

Organ	Embryos studied from Nov. '63 to May '64 — No. of embryos studied	Confluent cultures	Cell colonies	No growth	Successful*	Embryos studied Mar. to July '62 Successful	Total
Pituitary	21	15	4	2	19/21	25/27	44/48
Lung	30	27		3	27/30	12/15	39/45
Skin	30	25		4	26/30	8/8	34/38
Kidney	14	10	4		14/14	6/7	20/22
Spleen	12	3	8	1	11/12	4/7	15/19
Thymus	15	11	2	2	13/15	2/2	15/17
Heart	8		1	7	1/8	1/5	2/13
Intestine	8	1	4	3	5/8	0/4	5/12
Liver	7		6	1	6/7	0/4	6/11
Thyroid	5	5			5/5	2/3	7/8
Salivary glands						5/5	5/5
Adrenals						2/5	2/5
Pharyngeal mucosa	2	2			2/2		2/2
Whole embryo	1	1				1/1	2/2
Cornea						1/1	1/1
Meningea						1/1	1/1
Tongue						1/1	1/1

+19 sweat orig = 32

* Denominator: No. of embryo studied; numerator: No. of cultures with successful growth.

November, 1963, and May, 1964, 13 were sent from Scandinavia, 20 came from PGH, and 7 from HUP. Successful cultures were obtained from 12, 16 and 7 embryos, respectively. Table I presents the results of organ cultures initiated with tissues from 60 embryos (31 from Scandinavia, 16 from PGH, and 13 from HUP). At least one organ culture from this group was successful.

There is a distinction between confluent culture and cell colonies: in the former case, the cultures came to confluence and could then be used to establish a cell strain, while in the latter case, only discrete colonies formed.

From these results it appears that, with the exception of heart organ cultures, most preparations resulted in cell growth on the glass. It was usually possible to obtain confluent cultures from such tissues as skin, lung, pituitary, kidney, thymus, thyroid, and pharyngeal mucosa.

The extremely low proportion of bacterial and fungal contaminations (2 of 76) in these organ cultures was noteworthy.

Establishment of cell strains. Table II summarizes the results of attempts to establish cell strains from the confluent cultures developed under the grids. While cell strains were easily established from skin, lung, pharyngeal mucosa and pituitary, it was difficult to establish strains from intestine, thymus and thyroid.

All the cell strains were composed of fibroblast-like cells. With skin, lung and pharyngeal mucosa organ cultures, the cells under the grid were already predominantly fibroblastic; in the case of other organ cultures such as pituitary, thymus and thyroid the cultures at first appeared to be epithelial, but after the first trypsinizations became fibroblastic.

All of the cell strains had the previously described characteristics (2) for human diploid cell strains.

Virological studies. Two types of speci-

TABLE II. Establishment of Cell Strains.

Organ	No. of embryos studied	Culture sucessful for: More than 4 splits 1:2	Fewer than 4 splits 1:2	Culture unsuccessful at 1st split
Skin	16	15	1	
Lung	12	10	2	
Kidney	5		4	1
Pituitary	5	3	1	1
Pharyngeal mucosa	4	4		
Intestine	4	1		3
Liver	3			3
Thymus	3	1	2	
Thyroid	3	1	2	
Whole embryo	1	1		