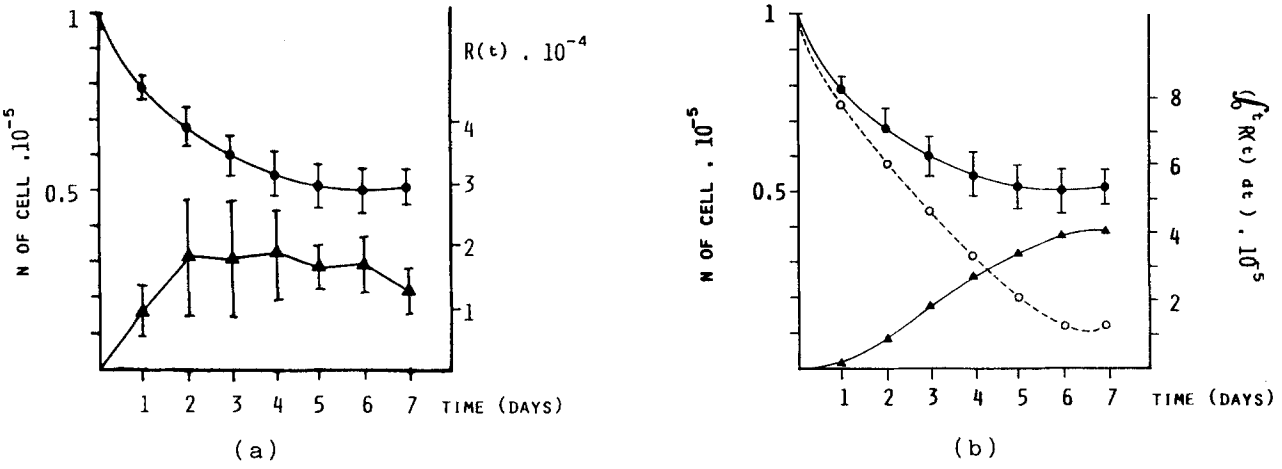

Figure 2. *a* Time-course of the cell number (circles) and of R (t) (triangles) related to Sp 2 leukemic cells, cultured in liquid phase for 7 days. *b* Time-course of the cell number decrease (closed circles) and of $\int_0^t R(t)\,dt$ (triangles), related to leukemic cells from Sp 2, cultured in liquid phase for 7 days. Each point represents the mean of six experiments; the vertical bars represent SEM. The graphic representation of $\int_0^t R(t)\,dt$ could represent also the numerical increase in Sp 2 disguised by a loss greater than that which was experimentally determined. The open circles represent the evaluation of the real loss, performed by subtracting, point by point, the actual increase from the experimental loss.

terized by a cell loss during incubation, shows a substantial [3H]-Tdr uptake up to day 7, indicating the presence of a not negligible number of cycling cells in this fraction. Applying to the Sp 2 the same mathematical procedure used for the data in figure 1, it is possible to calculate the actual growth kinetics of this fraction and, using the same k obtained for Sp 1, it is now possible to resolve the kinetics of the total cell number in Sp 2 into the kinetics of the real cell increment and cell loss as shown in figure 2b.

Based on this data we conclude that Sp 2, far from being devoid of cells endowed of proliferative capacity, includes a cohort of cells which re-enter the cycle and undergo an expansion not dissimilar to that of the Sp 1. Thus the main difference between Sp 1 and Sp 2 is not the presence of proliferating cells, but the amount of cell loss, which is much larger in Sp 2. This conclusion, which stresses the importance of evaluating leukemic subpopulation kinetics by means of a method which takes into account both cell proliferation and cell loss, should be borne in mind in attempts to set up cytokinetic systems capable of analyzing the effects of drugs or biological factors involved in the control of human leukemic growth.

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Aneuploidy of glandular epithelial cells in histologically normal prostate glands

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Summary. The percentage of aneuploidy in normal prostate glands from subjets 13–38 years old and 45–66 years old ranged from 0–78% and 0–63%, respectively. In contrast to adults, aneuploidy was absent in fetal and postnatal prostates. It is concluded that aneuploidy is a fundamental attribute of histologically normal adult prostate glands.
Key words. Prostate; aneuploidy; flow cytometry.

The age-related propensity of human prostate glands to undergo hypertrophic or neoplastic transformation is firmly established [1-3]. The extent of pathologic alterations of prostatic epithelium is usually assessed histologically in accordance with the relatively well-defined classification schemes of hypertrophic and neoplastic states [4-6]. In their turn, these classification categories are closely linked to the incidence of aneuploidy in hyperplastic or neoplastic prostatic epithelium [7-11]. There seems to be a consensus that the amount of aneuploidy can be correlated with the magnitude of cellular anaplasia, and that the onset of aneuploidy occurs either during the initial tumor formation or during progression of the tumor's growth [7, 11-14]. A possibility that normal prostatic epithelium may likewise contain aneuploid cells is thereby implicitly excluded. Literature is lacking in detailed analysis of DNA content of epithelial cells from normal prostate glands.

We now report that, contrary to the accepted views, aneuploid cells represent a numerically significant fraction of histologically normal prostate glands. Flow cytometric analysis of prostate glands from 5 fetuses, one 2-month-old infant, and 18 adults was performed as described elsewhere [15]. All prostates were obtained under sterile conditions [16] either from 'heart beating' cadavers or from cadavers less than 12 h post mortem. Samples of the prostate glands were excised from the mid-portions of the postero-lateral lobes away from the urethra. In the prostate glands particular care was exercised not to include tissues from the hyperplastic nodules. Nuclei were isolated from the tissue samples and their DNA concomitantly stained. Flow cytometric analysis allowed us to quantify the amount of DNA per nucleus in the isolated prostate cells as described elsewhere [15].

As is indicated in the table, no aneuploidy was detected in fetal prostates whereas 13 out of 18 adult glands contained a large number of aneuploid cells in addition to diploid and tetraploid cells, containing 8.1 and 16.2 pg DNA per nucleus, respectively. These hyperploid cells contained from 9 to 12 pg DNA per nucleus. Within this hyperdiploid range there existed at least two distinct subpopulations of aneuploid cells. Their characteristic DNA quantities were 10.2 and 11.3 pg DNA per nucleus. Some samples contained both of these subpopulations of cells while others were characterized by one aneuploid population. It is particularly noteworthy that high incidence of aneuploidy was repeatedly demonstrated in young adults and that, irrespective of age, the number of proliferating cells was quite small in all prostates (fig.). In fact, the number of cells in G_2 + M phase of the cell cycle was essentially identical in fetal and adult prostates. Prostate glands from adults (table) failed to reveal increased aneuploidy as a consequence of aging. As in the case of young adults, a few aged prostates were characterized by the absence of aneuploid cells detected by flow cytometry. The highest incidence of aneuploidy was again registered in young adults as well as in the aged specimens (table).

In light of the data summarized here, we conclude that in most healthy adults cellular aneuploidy is a fundamental attribute of histologically normal prostates. Since no aneuploidy was detected in fetal prostate tissues, but it was determined unambiguously in 72% of adults, we surmise that its onset was probably co-incidental with puberty and may be genetically determined. We also suggest further that it is singularly unlikely that these findings are confined solely to

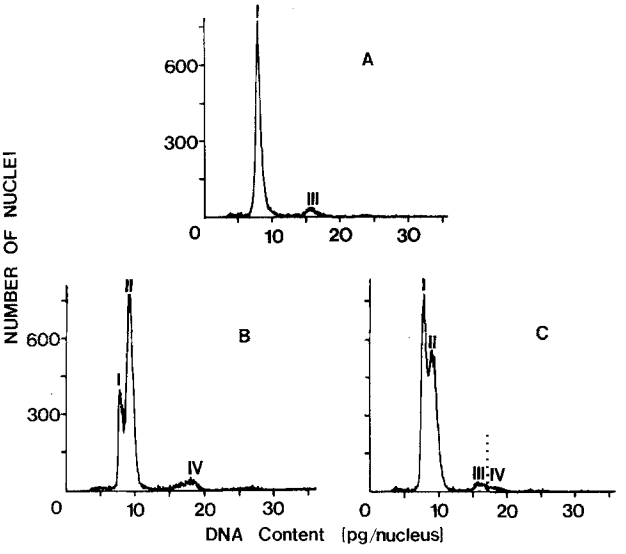
Flow cytometric quantification of aneuploid cells in histologically normal prostate glands

Group	Number per group	Age	% Aneuploidy X_i	Median	Mean ± SD	Range
I	(6)	fetal and postnatal	0	0	0	0
II	(13)	18 yrs	64.3			
		19	65.5			
		20	78.3			
		22	32.9			
		22	0			
		23	70.7			
		27	16.0	32.9	37.5 ± 30.8	0–78.3
		28	71.7			
		28	55.0			
		36	18.6			
		37	13.9			
		37	0			
		38	0			
III	(5)	45	0			
		56	0			
		60	46.7	46.7	31.5 ± 29.5	0–63.3
		62	47.5			
		66	63.3			

The percent aneuploidy (X_i) was determined as follows:

$$\frac{\text{number of nuclei with greater than diploid DNA content}}{\text{total number of nuclei}} \times 100$$

Three groups were defined as shown above. The median, mean, and range of the percentage of aneuploid cells is given for each group.



Predominantely epithelial nuclei from histologically normal prostate glands were isolated in nuclear isolation medium (NIM) [15], filtered and stained concomitantly with 4,6-diamidino-2-phenylindole (DAPI) [15]. DAPI-stained nuclei were analyzed with a modified high-resolution PHYWE flow cytometer [15]. A minimum of 20,000 nuclei were analyzed for each DNA histogram. For the reasons fully considered elsewhere [15] consistently unambiguous resolution of aneuploid cells was obtained only with the PHYWE instrument. This included the occasional presence of more than one aneuploid peaks thus suggesting the existence of distinct aneuploid cell populations in some prostate glands.

Histogram *A* of fetal prostate cells. Peak I represents diploid $G_{0/1}$ cells (84.5%) whereas peak III and the areas between peaks I and III depict proliferating cells [(S–8.0%), (G_2 + M–7.6%)]. Histogram *B* reflects a representative cellular distribution pattern contained within 19-year-old prostate. Peak I, diploid $G_{0/1}$ cells (22.5%); peak II, aneuploid $G_{0/1}$ cells (65.5%); peak IV, proliferating aneuploid G_2 + M cells (5.6%); area between peaks II and IV, S-phase diploid and aneuploid plus G_2 + M diploid cells (6.3%). Histogram *C* of histologically normal prostate cells from a 60-year-old subject. Peak I, diploid $G_{0/1}$ cells (45.4%); peak II, aneuploid $G_{0/1}$ cells (46.7%)*; peak III, diploid G_2 + M cells (3.5%)**; peak IV, aneuploid G_2 + M cells (2.2%); area between peaks II and III, diploid and aneuploid S-phase cells (2.2%).

*A negligible number of diploid S-phase cells is present; ** a negligible number of aneuploid S-phase cells is present.

prostatic tissues, and that analogous determinations should be made on the epithelial cells of other normal glandular structures.

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Effects of anti-EGF serum on newborn mice

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Summary. Administration of anti-EGF serum to newborn mice led to delay of eyelid opening and incisor tooth eruption, acceleration of hair growth and delay of weight gain. These results indicate that in the first week after birth EGF still has a physiological function, which can be abrogated by anti-EGF serum.
Key words. EGF; anti-EGF serum; newborn mice.

Epidermal growth factor (EGF) has been generally suggested to play a role in growth and differentiation of epithelial tissues of the epidermis, the cornea, the respiratory and intestinal tracts and the mammary gland[1–3], in spermatocyte maturation[4], and also in skin wound repair processes[5]. It has been found, moreover, to act as a powerful cocarcinogen[6]. The biological significance of a number of further data, largely obtained in vitro, has however remained questionable and needs clarification. No clinical symptoms associated with or attributable to failure or dysfunction of EGF have been discovered to occur spontaneously in man or animals. Surgical removal of the submandibular glands, the major site of EGF biosynthesis in mice, is capable of influencing proliferative processes like, for example, the development of the lactating mammary gland[3] or tumor incidence and growth[6], but contradictory data on its effect on serum EGF levels in adult animals (mice, hamsters) have been reported[4,7,8]. Therefore the existence of further sites of EGF biosynthesis is highly probable[9,10].

In order to stimulate a syndrome of systemic loss of EGF function, newborn mice were treated with high-titre anti-EGF serum and observed for the emergence of biological effects.

Material and methods. Mouse EGF was prepared from submandibular glands as described by Savage and Cohen[11]. Purity was assessed by SDS gel electrophoresis in 15% polyacrylamide gels[12] following reduction and denaturation. A single band of M_r = 6000 could be demonstrated. The EGF preparation was subjected to partial amino acid sequence analysis and the specificity, in addition, confirmed by competition in a quantitative ^{125}-I-EGF binding assay to cell membranes[13].

Antisera to mouse EGF were prepared in rabbits according to Rizzino et al.[14] by immunization with the EGF prepared and purified as referred to above. The specificity of the antisera was assessed by neutralization of EGF-dependent enhancement of colony formation of NRK cells in soft agar (unpublished results) and by an enzyme-linked immunoassay according to Engvall and Perlmann[15] with EGF and NGF (Wellcome, Beckenham) as antigens.

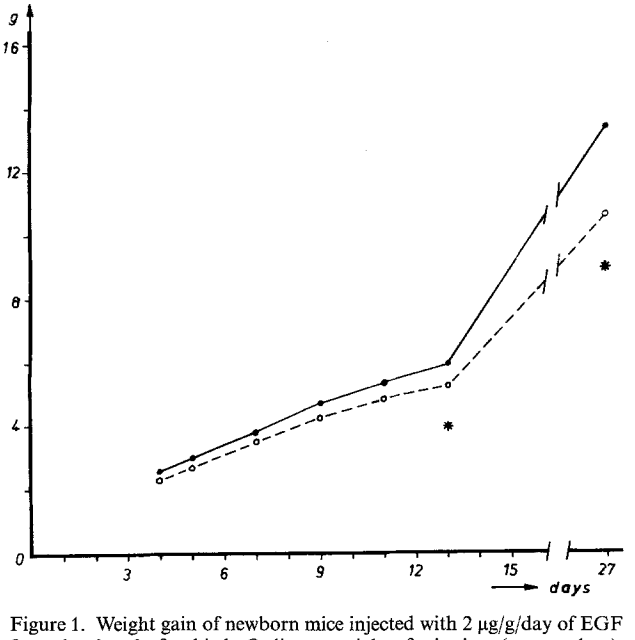

Figure 1. Weight gain of newborn mice injected with 2 µg/g/day of EGF from day 0 to 9 after birth. Ordinate: weight of mice in g (mean values), ●—● PBS (n = 9), o—o EGF (n = 8). Asterisks indicate statistically significant differences (Student's t-test, $p < 0.01$).