very young fetuses when they are most susceptible to many virus infections. The normal gestational age of the fetal lamb is 150 days. We have shown that fetuses as young as 117 days can produce interferon. Mendelson (*5*) has shown that older fetal rats can produce interferon after virus challenge whereas younger fetal rats do not.

One must not conclude that, because the fetal lamb can produce great amounts of interferon after inoculation with CV, this will obtain for all viruses. It is still possible that diminished production of interferon after infection with a particular virus in the fetus near term is one of the factors underlying enhanced susceptibility to that virus.

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Sexual Reproduction in Candida lipolytica

Abstract. Candida lipolytica *is a rather common yeast isolated more frequently from substrates containing lipids or proteins, such as dairy products, than from substrates rich in sugars. This species assimilates hydrocarbons and is currently being studied for its potential to convert petroleum into yeast cells for use in feeds and foods. We have found* C. lipolytica *to exist in nature primarily in the heterothallic haploid state. When appropriate strains of opposite sex are mixed on a suitable sporulation medium, conjugation occurs followed by the production of ascospores. Since heterothallism permits laboratory control of hybridization, this characteristic of* C. lipolytica *enhances the possibility of improving its strains for technological uses.*

So far as we are aware, strains of *Candida lipolytica* (Harrison) Diddens et Lodder are isolated from nature primarily in the haploid state. Because haploid strains apparently are strongly heterothallic, they do not sporulate when grown individually on sporulation media. When cells of opposite sex are mixed on yeast extract–malt extract medium (*1*) and incubated at 25°C, mating occurs followed by the production of ascospores. Although diploid cultures may be isolated from nature, the occurrence of ascosporogenous diploids is rare. Such a culture of *C. lipolytica*, NRRL YB-423, was isolated from material obtained from a corn processing plant in Illinois. The culture produces one to four ascospores per ascus with an unusually wide range of spore shapes, including spheroidal, angular, hemispheroidal, hat-shaped, and shallow bowl–shaped. The asci are produced either laterally or at the tips of hyphal cells, rarely from conjugated blastospores. The asci commonly rupture when mature.

Asexually reproducing clones were obtained from ascospores isolated from intact asci of strain YB-423 by micromanipulation. When these ascosporic isolates were mated, the same variety of ascospore shapes was noted. However, when ascosporic strain YB-423-12 was mated with a haploid (YB-421) isolated from the same material that supplied strain YB-423, the pair produced an abundance of ascospores all, or nearly all, shaped like a shallow bowl. Their germinability was approximately 12 percent as compared with approximately 0.1 percent for ascospores of strain YB-423.

Candida lipolytica is outstanding among yeasts for its ability to produce lipase (*2*) and extracellular protease. It has potential for utilization of industrial wastes containing sugars, lipids, proteins, and hydrocarbons; currently, its greatest potential is the conversion of hydrocarbons to foods and animal feeds (*3*). Hybridization of selected lines and production of large-celled diploids, characterized by more rapid growth and greater ease of harvesting the larger cells, may improve the economics of the process. Examples of similar improvements are the production of soy sauce and of the Japanese fermented food, miso, through diploid hybridization of selected strains of the heterothallic haploid yeast *Saccharomyces rouxii* Boutroux (*4*).

A Latin description validating the perfect form of the species as *Endomycopsis lipolytica* will be published elsewhere.

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1 December 1969

Isolation and Zonal Fractionation of Metaphase Chromosomes from Human Diploid Cells

Abstract. *A simple method for isolating large quantities of human metaphase chromosomes has been developed. Fractionation of chromosomes from peripheral lymphocytes on a large scale is accomplished by velocity sedimentation in an A-XII zonal rotor.*

There have been several reports on the isolation and fractionation of mammalian metaphase chromosomes (*1*). For chemical examination of isolated chromosomes large quantities of cells in metaphase are needed; for this reason aneuploid cell lines such as mouse L cell, mouse lymphoblast tumor, Chinese hamster, and HeLa cells have been used. Fractionation of chromosomes into groups of distinct size has been achieved by sucrose gradient sedimentation. Dependence on this technique has severely restricted the quantity of

Table 1. Photomicrographs were taken at × 2000 of random fields of well-spread chromosomes from chromosomal fractions (CF) 1 to 6. The chromosomes were measured, and the centromeric indices were calculated by dividing the short chromosomal arm length by total length. Sucrose concentration was measured with a Bausch and Lomb refractometer. Standard error, S.E.

Fraction	Mean length (μ)	S.E. (μ)	Mean centromeric index	S.E.	Sucrose (%)	Chromosomes measured (No.)
1	1.5	.06	28	0.4	16.7	100
2	1.7	.06	34	.9	19.1	105
3	2.5	.12	35	.8	21.5	91
4	2.7	.09	33	1.2	23.6	81
5	3.0	.10	34	1.0	25.7	100
6	3.8	.16	36	0.8	27.7	88
Unfractionated chromosomes	3.3	.11	35	.7		210

Table 2. Unfractionated chromosomes assigned to Denver classification by length and centromeric index. The observed distribution of chromosomes compares favorably with the expected distribution in a normal XY karotype with chi square (χ^2) equal to 9.07, $P > .20$.

Chromosomal group	Number per group	Distribution of chromosomes (%)		Length (μ)	S.E.	Centromeric index	S.E.
		Observed	Expected				
A	25	11.9	13.0	6.4	0.2	43.9	0.8
B	9	4.3	8.7	4.9	.3	28.4	.9
C+X*	74	35.2	32.6	3.7	.1	39.2	.6
D	23	10.9	13.0	2.5	.1	16.4	.8
E	28	13.3	13.0	2.5	.1	39.1	1.1
F	20	9.5	8.7	2.0	.1	46.6	0.4
G+Y*	31	14.8	10.9	1.5	.1	22.4	.9
Total	210			3.3	.1	35.0	.7

* Over 90 percent of blood donors are male, XY.

material that can be fractionated.

To obtain a large number of euploid mammalian cells of a well-defined genetic source, we have performed chromosome fractionation studies with human peripheral lymphocytes. Human lymphocytes can be grown under simple conditions of culture and can be stimulated to divide by the addition of phytohemagglutinin. Up to 20 percent of the cells can be harvested in metaphase arrest after previous treatment with vinblastine. Since a unit of blood contains 1×10^9 lymphocytes there is a ready supply of a large number of lymphocytes. To permit separation of large numbers of chromosomes in a single velocity sedimentation run, fractionation was performed in the A-XII zonal rotor (*2*) with an 1100-ml sucrose gradient.

Fractions rich in white blood cells and their autologous plasma were obtained from a local commercial blood bank, combined with heparin (10 unit/ml), and allowed to sediment at 37°C for 1 hour. The supernatant was removed and diluted with RPMI media (*3*) supplemented with glutamine, penicillin, and streptomycin, the final lymphocyte concentration being 1×10^6 cells per milliliter. Phytohemagglutinin (Burroughs Wellcome), 0.4 ml of 100 ml of media, was added, and lymphocyte cultures were prepared containing 1×10^8 cells per 32-ounce prescription bottle and incubated at 37°C. After 3 days, vinblastine sulfate (0.1 μg/ml) was added. Sixteen hours later the lymphocytes were harvested by low-speed centrifugation, and the cells were washed, treated with 1 percent sodium citrate, centrifuged, resuspended in a *p*H 3 buffer (*4*), and broken mechanically by vigorous shaking for 1 hour. At this point, the resultant nuclei and free chromosomes were collected by centrifugation, resuspended in 20 ml of 0.1*M* tris(hydroxymethyl)aminomethane (tris) (*p*H 7.4) containing 0.014*M* $MgCl_2$ and 0.01*M* KCl (RSB) with 3 percent sucrose and used in the zonal velocity sedimentation. A Beckman high-capacity gradient pump was used to load the A-XII zonal rotor at 550 rev/min with the 1100 ml of 15 to 40 percent sucrose gradient in RSB, followed by 250 ml of a 55 percent sucrose cushion in RSB. The sample was introduced through the center, displacing the 55 percent sucrose cushion through the periphery. Finally a 100-ml overlay without sucrose in RSB was added through the center. Velocity sedimentation was then performed at 2000 rev/min for 20 minutes. For un-

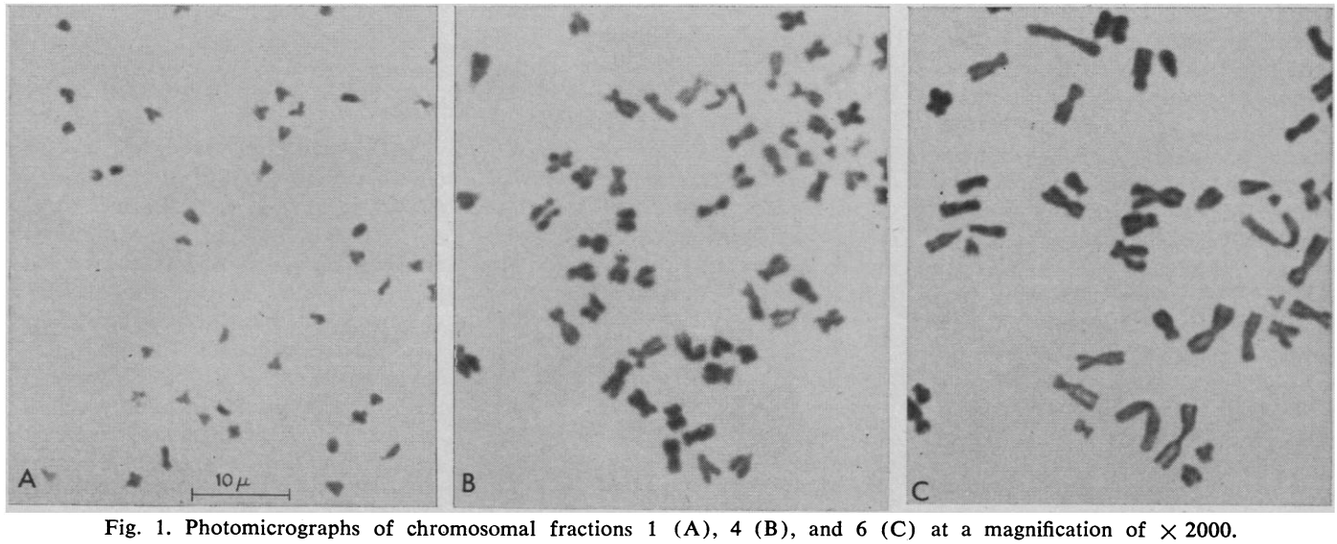

Fig. 1. Photomicrographs of chromosomal fractions 1 (A), 4 (B), and 6 (C) at a magnification of × 2000.

loading, the speed was decreased to 550 rev/min and the overlay, sample gradient, and cushion were unloaded through the center by displacement with 60 percent sucrose through the periphery. Eighteen 80-ml fractions were collected, and slides were made after centrifuging the chromosomes in each fraction at 3000 rev/min for 30 minutes. Photomicrographs were taken at × 2000, and approximately 100 chromosomes from each fraction were measured.

Of the 18 fractions collected, the initial two were from the overlay and sample; the following 13 represented the gradient chromosomal fractions (CF) 1 to 13, and the last three were the 55 percent sucrose cushion. The overlay fractions contained cellular debris, but only rare chromosomes or nuclei. The initial gradient fractions, CF 1 to 6, contained the fractionated chromosomes (Fig. 1) which showed minimum aggregation and no nuclear contamination. The next seven fractions, CF 7 to 13, were made up of the longest chromosomes and increasingly larger aggregates of chromosomes and nuclei. The bulk of the nuclei appeared in the 55 percent sucrose cushion fractions.

Table 1 is a tabulation of chromosomal lengths and their centromeric indices (CI) in the first six chromosomal fractions, CF 1 to 6. The CI's are calculated by dividing the length of the chromosomal short arm by the total chromosomal length and are a measure of the centromeric position. There is a clear increase in the mean chromosomal length with increasing sucrose concentration. Only in fraction 1 is the centromeric index significantly decreased over the other fractions and the control sample of unfractionated chromosomes. This is interpreted as an indication of enrichment for small acrocentric chromosomes. Five of these fractions have mean lengths below the unfractionated mean. Chromosomes of longer mean length are present in fractions CF 7 to 13. However, these fractions also contain aggregates of chromosomes of various sizes which interfere with valid measurements of mean length.

Identification of individual fractionated chromosomes presents certain difficulties. The Denver conference classified human chromosomes into seven groups based on their length and centromeric position in intact metaphases (*5*). In our isolation of chromosomes, the preparations reveal metaphase plates with various degrees of chromosomal contraction. Therefore, depending on the degree of contraction, there may be overlapping in length between adjacent chromosomal groups with similar centromeric indices, such as groups A and C. Realizing these difficulties, we have attempted to classify our free chromosomes into the seven Denver groups by measurement of size and centromeric index. When 210 chromosomes from the unfractionated chromosome preparation were measured and classified, the observed distribution into Denver groups compared favorably to the expected normal karyotype distribution with an χ^2 equal to 9.07, $P > .20$ (Table 2).

The first six chromosomal fractions were then classified into Denver groups to determine enrichment of these fractions for particular groups. To balance the numerical predominance of group C chromosomes in the normal karyotype, 33 percent of the total, we calculated an index of enrichment (IE) for each fraction. The IE is equal to the percentage of chromosomes of a particular Denver group in each fraction divided by the percentage of chromosomes in this Denver group in unfractionated chromosomes. In Fig. 2 histograms represent the index of enrichment for each chromosomal group in the first six chromosomal fractions. Fraction 1 shows the most striking enrichment for the small chromosome groups, with 88 percent of the chromosomes classified as groups F or G, and is almost completely free of larger chromosomes of groups A, B, and C. The enrichment of group G is greater than fourfold and reflects the finding that, in velocity gradient fractionation, the smallest chromosomes can be most easily separated. Fractions 2, 3, and 4 showed two- to threefold enrichments of the E and F groups; a lesser enrichment of B and C groups was seen in fractions 5 and 6. Fractions which might contain enrichment of group A, as mentioned earlier, contained too many aggregates of smaller chromosomes to be evaluated.

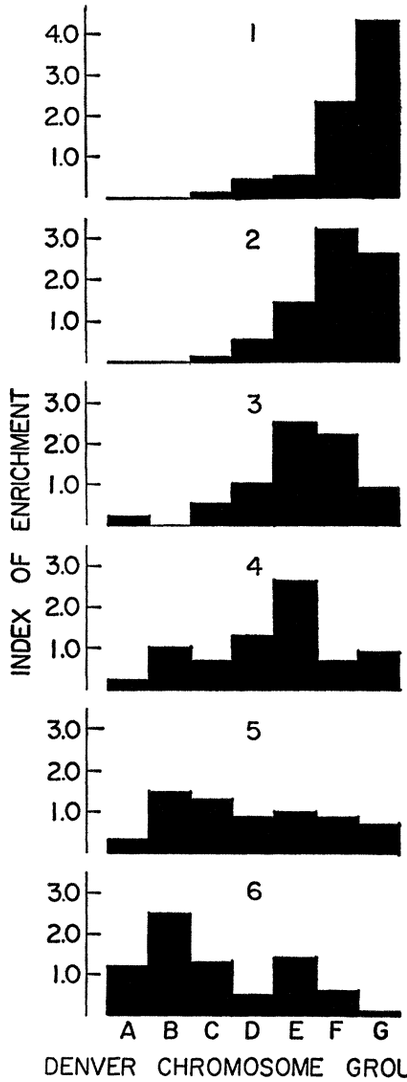

Fig. 2. Chromosomes in chromosomal fractions 1 to 6 are assigned by Denver classification into seven chromosomal groups (*A–G*) by length and centromeric index. An index of enrichments (IE) is then calculated for each fraction; IE greater than 1 indicates purification for a particular Denver group; IE less than 1 indicates a relative elimination.

Further purification of chromosomal fractions can be effected by subjecting them to a second cycle of differential zonal centrifugation. The development of methods to decrease aggregation will permit better fractionation of the large chromosomes.

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10 December 1969