and in WI-38 human diploid fibroblasts (*6*) was studied by incubating the cells for 48 hours in medium 9 (Table 1), to which was added 0.5 mc of C^{14}-glucose (specific activity, 15 mc/mmole). The cells were then harvested by scraping and centrifugation and subjected to conditions employed for the total hydrolysis of protein (6*N* HCl in evacuated sealed tubes at 110°C for 21 hours). Samples of the concentrated hydrolyzates were analyzed on the long column of an automatic amino acid analyzer (*7*). The serine peak was collected and its radioactivity determined by liquid scintillation counting.

Table 1 shows mean percentages for incorporation of radioactive precursors of DNA, RNA, and protein during 24- and 48-hour labeling periods. These data are derived from nine different experiments with six CGL patients. All but one of the patients had received treatment; this factor was ignored in our studies since it did not seem to affect the amino acid requirements of the cells. Partial inhibition of incorporation occurred because of the lack of asparagine (medium 4); however, the lack of serine (medium 8) was more inhibitory to incorporation and was similar to the effect produced by minimal medium (medium 9). The results with media 2 through 7, except 4, do not differ significantly from 100 percent; results with media 8 and 9 are significantly different from results with the other media. Experiments with cell concentrations of 1×10^5 to 15×10^5 cells per milliliter showed that the serine requirement was not dependent on cell density. We have also examined cells from three patients with acute granulocytic leukemia; the results were essentially the same as with CGL cells.

Table 2 shows the results of similar experiments with four different samples of normal bone marrow cells. In the absence of either asparagine or serine the cells exhibited a degree of metabolic inhibition. Again, the effect due to the lack of serine was more pronounced than that due to the lack of asparagine.

Experiments similar to those reported in Table 2 were performed with WI-38 (*6*) human diploid fibroblast cells. These cells were not inhibited by the lack of any of the nonessential amino acids.

In a glucose-containing culture medium, human cells that are capable of synthesizing serine use glucose almost exclusively to form the carbon skeleton of serine (*8*). We therefore examined the synthesis of C^{14}-serine from C^{14}-glucose in CGL cells, in normal marrow, and in WI-38 cells under similar conditions. Results in terms of radioactivity in total cellular serine for the three cell types were: CGL cells, 2100 count min^{-1} $\mu mole^{-1}$; bone marrow, 1395 count min^{-1} $\mu mole^{-1}$; and WI-38 cells, 317,700 count min^{-1} $\mu mole^{-1}$. Thus, while WI-38 cells could readily synthesize C^{14}-serine from C^{14}-glucose, CGL cells and normal bone marrow cells were only minimally capable of this synthesis.

Both the incorporation data reported in Tables 1 and 2 and the minimal C^{14}-serine synthesis from C^{14}-glucose seem to suggest that, although bone marrow and CGL cells may have a partial asparagine requirement, they also have a serine requirement that appears to be more severe.

Our results suggest that normal human bone marrow cells and cells derived therefrom, whether leukemic or not, may require preformed serine. If true, it is conceivable that therapeutic measures involving the enzyme serine dehydratase or serine antimetabolites could be beneficial in leukemia, providing the in vivo growth rate of the leukemic cells is sufficiently elevated above that of normal bone marrow cells.

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References and Notes

1. H. Eagle, *Science* **130**, 432 (1959).
2. Annotations, *Lancet* **1967-I**, 431 (1967).
3. J. D. Regan and E. H. Y. Chu, *J. Cell Biol.* **28**, 139 (1966).
4. F. J. Bollum, *J. Biol. Chem.* **239**, 2733 (1959).
5. R. Mans and G. D. Novelli, *Arch. Biochem. Biophys.* **94**, 48 (1961).
6. L. Hayflick and P. S. Moorhead, *Exp. Cell Res.* **25**, 585 (1961).
7. D. Spackman, W. H. Stein, S. Moore, *Anal. Chem.* **30**, 1190 (1958).
8. H. Eagle and K. Piez, in *Amino Acid Pools: Distribution, Formation and Function of Free Amino Acids*, J. T. Holden, Ed. (Elsevier, Amsterdam, 1962), pp. 694–705.
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Lymphocytoid Lines from Persons with Sex Chromosome Anomalies

Abstract. *Permanent lymphocytoid cell lines have been established from patients with the XYY and XXY chromosome constitutions. Large amounts of these and other cell lines with genetic defects can be provided for various studies.*

A series of technical innovations have made possible the discovery and study of many genetic abnormalities in temporary cultures of human cells. For example, Jacobs and Strong (*1*) described cells with an extra chromosome in Klinefelter's syndrome, and Hauschka *et al.* (*2*) reported the XYY anomaly. Subsequently a large number of anomalies of the sex chromosomes have been described (*3*).

Seemingly permanent human lymphocytoid cell lines have been established from patients with various malignant and nonmalignant diseases (*4*) and from normal persons (*5*). Over 500 such cell lines have been initiated in our laboratory. The cell lines have been valuable for viral, biochemical, immunological, and clinical studies. An effort is now being made to establish a library of lymphocytoid cell lines from patients with chromosome anomalies and other genetic abnormalities.

A mentally retarded 28-year-old man with antisocial behavior and a history of suspected arson had an XYY phenotype (*6*). Leukapheresis of 500 ml of peripheral blood was performed on 24 July 1968, and six cultures were initiated by techniques reported previously (*7*). On 16 September 1968 two cultures began to grow rapidly and were considered established; they were labeled RPMI cell lines No. 1608 and No.1618. These cell lines had a 46 XYY chromosome constitution (plus an extra Y) and have continued to grow vigorously.

Leukapheresis was performed on blood from a 31-year-old man with Klinefelter's syndrome. Eight cultures were started on 2 July 1968; after several cultures were combined, one culture began growing rapidly on 21 October

Table 1. Distribution of chromosome counts.

Cell line (RMPI No.)	Chromosome counts			Total cells	Tetraploidy* (%)
	45	46	47		
1608 (XYY)		9	92	101	0.7
1608 (XYY)	1	1	48	50	0.6
1638 (XXY)	2	18	80	100	1.0

* Determined from a sample of 600 to 800 metaphases under low-power optics.

1968. This culture, designated RPMI cell line No. 1638, has subsequently been divided every few days; portions of cells are being preserved in our cell bank. The cultured cells revealed a predominant karyotype 47 XXY chromosome constitution (Table 1).

The theoretical importance of maintaining permanent human cell lines with various chromosomal defects is self-evident. It remains to be seen whether or not critical biochemical expressions of such defects are retained by the cultured cells. Evidence in support of this hope has been provided by the continuing production of a Bence Jones protein by a lymphocytoid cell line derived from a patient with multiple myeloma (*8*). We are interested in establishing permanent cell lines from persons with genetic defects. Our laboratory is equipped to grow gram and kilogram amounts of these cell lines (*9*).

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References

1. A. Jacobs and J. A. Strong, *Nature* **183**, 302 (1959).
2. T. S. Hauschka, J. E. Hasson, M. N. Goldstein, G. F. Koepf, A. A. Sandberg, *Amer. J. Hum. Genet.* **14**, 22 (1962).
3. M. Bartalos and T. A. Baramki, *Medical Cytogenetics* (Williams and Wilkins, Baltimore, 1967).
4. G. E. Moore, H. Kitamura, S. Toshima, *Cancer* **22**, 245 (1968).
5. G. E. Moore and W. F. McLimans, *J. Theor. Biol.* **20**, 217 (1968).
6. S. Kelly, R. Almy, M. Barnard, *Nature* **215**, 405 (1967).
7. G. E. Moore, J. T. Grace, Jr., P. Citron, R. E. Gerner, A. Burns, *N.Y. State J. Med.* **21**, 2757 (1966).
8. Y. Matsuoka, G. E. Moore, Y. Yagi, D. Pressman, *Proc. Soc. Exp. Biol. Med.* **125**, 1246 (1967).
9. G. E. Moore, P. Hasenpusch, R. E. Gerner, A. Burns, *Biotechnol. Bioeng.* **10**, 625 (1968).

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Protein Digestion in Isolated Lysosomes Inhibited by Intralysosomal Trypan Blue

Abstract. *Control rats and rats treated with subcutaneous trypan blue were injected intravenously with denatured albumin-I^{125}. Lysosome-rich fractions of their livers, when incubated at 22°C in osmotically protected medium (pH 7.4), retained their capacity to digest albumin-I^{125}. The rate of digestion was lower in suspensions prepared from rats treated with trypan blue than in control suspensions, but rates of lysosome breakage were not different. These results and other experimental evidence suggest that trypan blue concentrated within lysosomes can inhibit intralysosomal digestion, probably by inhibition of lysosomal proteinases.*

There is now evidence that the principal function of lysosomes is the intracellular digestion of macromolecules (*1*). In endocytic cells this process should be uniquely susceptible to modification by foreign compounds, and De Duve (*2*) has recently summarized the different forms such modification might take. One possibility is a reduced digestive capacity caused by uptake of enzyme inhibitors, and such a mechanism of action has been proposed for trypan blue (*3*) on the basis of its uptake into lysosomes and its ability to inhibit lysosomal enzymes in vitro (*4*). The experiments described have been undertaken to test this hypothesis.

Mego *et al.* (*5*) have demonstrated that after intravenous injection into mice formaldehyde-treated albumin-I^{131} is taken up into the liver heterolysosomes. They showed further that when a suspension of the resulting albumin-laden lysosomes was incubated at 37°C, in osmotically protected medium, degradation of their contained protein continued, with release of labeled iodotyrosine. In the work reported here, similar methods have been used to study the effect of trypan blue on intralysosomal digestion of protein. In our experiments male Wistar rats (270 to 310 g) were given an intravenous injection of denatured albumin-I^{125} (2.5 mg/kg in 0.1*M* saline). The labeled albumin was prepared by the method of McConaghey and Dixon (*6*) and denatured as described by Mego *et al.* (*5*).

Some rats received a subcutaneous injection of purified trypan blue (75 mg/kg) 24 hours before the albumin. Rats were killed 0.5 hours after injection with albumin, and their livers were removed immediately and homogenized in ice-cold 0.25*M* sucrose (10 ml per gram of liver) with a Teflon-on-glass homogenizer. After removal of cell debris and nuclei by low-speed centrifugation (1100*g* for 10 minutes) a large granule fraction containing lysosomes was sedimented by centrifugation at 16,500*g* for 20 minutes. This fraction was carefuly resuspended in 0.01*M* tris buffer (*p*H 7.4), containing 0.25*M* sucrose (5 ml per gram of liver), and immediately incubated at 22°C, the temperature being selected in order to reduce injury to the lysosomes during incubation. Samples were removed at intervals of up to 2 hours, placed in trichloroacetic acid (TCA) (final concentration 10 percent), and centrifuged to remove precipitated protein. This procedure served to precipitate the labeled albumin while allowing the products of digestion (iodotyrosine-I^{125}) to remain in solution. The supernatants were therefore decanted and counted in an automatic gamma well counter (Nuclear-Chicago Gammatic).

Seven experiments were performed in which the rate of digestion in a granule suspension prepared from a rat treated with both trypan blue and albumin was compared with that in a suspension prepared at the same time from a rat that had received albumin alone. In every case the rate was lower in granules from rats treated with the dye, a result that is consistent with the role of trypan blue as an intralysosomal inhibitor. Figure 1 shows the results of a typical experiment.

Conditions known to disrupt rat liver lysosomes, such as the absence of an adequate protective medium (that is, incubation in buffer only), the addition of 0.1 percent Triton X-100, or freezing and thawing the suspension prior to incubation, each abolished the breakdown of albumin. Furthermore, the addition of trypan blue in high concentration (20 mg/ml) to a control suspension did not decrease the rate of the breakdown of albumin, compared with that of controls. These experiments establish the site of the proteolysis illustrated by Fig. 1 to be intralysosomal and show that the dye must be concentrated within lysosomes to exert an effect on intralysosomal digestion. There is no reason to suppose that these wholly intralysosomal events would be modified in any way by the