liquid in which it is dissolved together determine transmittance spectra.

The adsorption process of the carbonyl compounds on the surface of the active adsorbent is a phenomenon which is apparently well suited for kinetic studies. Available data, although of a more qualitative nature, indicate that the process of disintegrating the solid particles of the adsorbates and spreading and adsorbing them uniformly over the surface of the adsorbent covers a period of several days and may be easily followed by visual, microscopic, and spectral inspection. The spectral and microscopic characteristics of the products after reaching equilibrium can in no way be distinguished from samples prepared by adsorbing the carbonyl compounds on the same solids out of solution in a solvent of low dipole moment. This was confirmed by measuring by reflectance the absorbance maxima of the three carbonyl compounds adsorbed on the same types of adsorbents from solution after removal of all traces of solvent. They were identical with those obtained from the solid-solid mixtures when the adsorption processes were complete. Reflectance spectroscopy has provided two further convenient methods for following the progress of adsorption quantitatively: (i) measurement of the change of wavelength maximum with time until equilibrium is reached, and (ii) the establishment of the position of the final absorption maximum and the measurement of the increase in absorbance with time at this fixed final wavelength.

Of considerable theoretical interest was the observation, with each of the combinations of adsorbents and adsorbates, that during the slow adsorption process a hypsochromic shift of the absorption maximum occurs, which ranges in magnitude from 2 to 13 mμ and covers numerous other values between these two extremes, depending on the system under investigation. The data were precise enough to be reproducible and to verify, unequivocally, the hypsochromic trend observed.

The shift toward the shorter wavelength is attributed to the fact that in the original crystal lattice of the organic compound the molecules, immediately after mixing, are in a lower energy state than they are after being adsorbed on the active surface. The energy required for this process is presumably supplied by energy of adsorption. This interpretation is in harmony with the observation (*4, 5*) that stable mercuric iodide could be converted into a metastable yellow modification by adsorption on active alumina and magnesium oxide respectively. In the mercuric iodide-alumina system the energy required is possibly furnished by (*4*) heat of adsorption.

HARRY ZEITLIN
PHILIP ANTHONY
WERNER JORDAN
Department of Chemistry, University of Hawaii, Honolulu 14

References and Notes

1. E. H. McRae, *J. Phys. Chem.* **61**, 562 (1957).
2. P. Anthony and H. Zeitlin, *Nature* **187**, 936 (1960).
3. Supported by a grant from the Research Corporation of New York.
4. H. Zeitlin and H. Goya, *Nature* **183**, 1041 (1959).
5. G. Kortüm, *Trans. Faraday Soc.* **58**, 1624 (1962).

20 May 1963

Polyploidy and Endoreduplication in Human Leukocyte Cultures Treated with β-Mercaptopyruvate

Abstract. *Treatment of cultured human peripheral blood leukocytes with β-mercaptopyruvate resulted in marked increases in polyploidy and endoreduplication in squash preparations of mitoses in the metaphase stage. Since β-mercaptopyruvate occurs in man as an intermediary metabolite in cysteine degradation, it might contribute to the development of polyploidy in vivo, especially in tumors lacking desulfurase enzymes.*

β-Mercaptoethanol ($SH\text{-}CH_2\text{-}CH_2\text{-}OH$) induces polyploidy, endoreduplication, and chromosome aberrations in cultured normal human leukocytes (*1*). A similar sulfhydryl compound, β-mercaptopyruvate ($SH\text{-}CH_2\text{-}CO\text{-}COOH$), occurs physiologically in man, and occupies a central position in the degradative pathways for cysteine. Therefore, the effect of β-mercaptopyruvate on cultured human leukocytes was investigated, since it could be of importance in the development of polyploidy in vivo.

Peripheral blood leukocytes from healthy human donors were cultured by a modification (*1*) of the method of Moorhead *et al.* (*2*). One milliliter of leukocyte-laden plasma separated from heparinized venous blood by dextran sedimentation was added to 4 ml of Parker's medium (TC 199) with antibiotics and crude phytohemagglutinin. β-Mercaptopyruvate (*3*) was immediately added to a final concentration ranging from $6.4 \times 10^{-4} M$ to $1.9 \times 10^{-3} M$, and the cultures were incubated at 37°C in tightly-capped bottles. After the addition of 0.02 μg of colchicine per ml for the final 4 hours of culture, the cells were treated in hypotonic sodium citrate, acid-fixed and squashed in aceto-orcein. Metaphase mitoses were examined microscopically for determination of ploidy. At least 200 consecutive mitoses were scored from each culture, except when the total number of mitoses was less than that because of toxic effects from the treatment.

In treated cultures, the percentage of cells containing multiples of the diploid chromosome complement was greatly increased in comparison to controls. The optimal concentration for producing polyploidy was close to the toxic dose, since general cell number as well as the total number of mitoses was considerably reduced when polyploidy exceeded 10 percent. There were no mitoses and there were only very few surviving interphase cells at the highest concentration. Preliminary experiments with cultures from six different donors indicated that the effective and the toxic concentrations varied depending on the donor, so that no single concentration was optimal. Therefore, a range of concentrations and culture periods was used, the results of which are summarized in Table 1.

Almost all of the polyploid mitoses were tetraploid, but there were occasional octoploid mitoses. Over 60 percent of the polyploids were also endoreduplicated, with typically paired diplochromosomes (Figs. 1 and 2). The control values never exceeded 0.5 percent polyploidy and when combined there was an overall incidence of 0.25 percent polyploidy. This compares favorably with other studies of similar material where the range was 0.1 to 2.0 percent (*4*). Chromatid breaks and rearrangements were rarely noted in the mercaptopyruvate-treated mitoses. The severe chromosome erosion in certain metaphases treated with mercaptoethanol (*1*) was not observed after treatment with mercaptopyruvate.

One interpretation of these results might be that under the unfavorable culture conditions produced by the addition of β-mercaptopyruvate, already existing polyploid cells were relatively increased by selection because somehow they were more resistant. A more likely explanation is that diploid cells became polyploid by a mechanism similar to

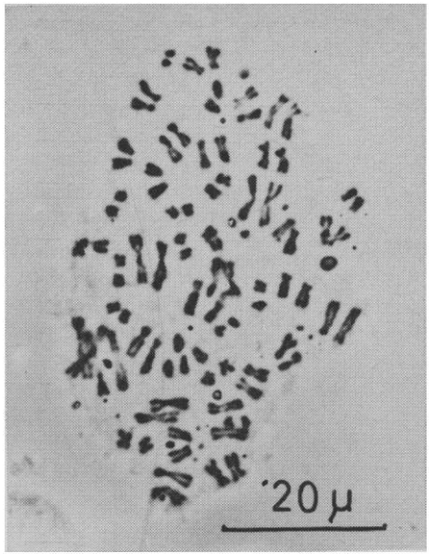

Fig. 1. Tetraploid endoreduplication, metaphase, with typically paired chromosomes, aceto-orcein stain, phase contrast.

that resulting from mercaptoethanol treatment, wherein the spindle mechanism is disturbed and centriole duplication is inhibited, without inhibition of DNA synthesis (*5*). Although the overall mechanism of action is obscure, inactivation of enzymes participating in the production of a functional spindle could be responsible for the development of polyploidy in cells treated with β-mercaptoethanol or β-mercaptopyruvate. Sulfhydryl compounds can inactivate certain enzymes by binding sulfhydryl groups necessary for combination with substrate (*6*). Another possibility is that disulfide bonds necessary for the configuration of the enzyme molecule may be broken.

That treatment with β-mercaptopyruvate causes polyploidy is important, since this compound occurs in man in the pathways of cysteine degradation. In addition to desulfuration directly to pyruvic acid, ammonia, and hydrogen sulfide, cysteine can undergo transamination with an α-ketoacid to form β-mercaptopyruvate (*7*). A desulfuration system distinct from cysteine desulfurase, yielding pyruvic acid and hydrogen sulfide, is probably the most important pathway for degrading β-mercaptopyruvate (*7*). Its reduction by diphosphopyridine nucleotide ($NADH_2$) and lactic dehydrogenase to mercaptolactic acid, however, is sufficiently rapid to be of physiologic importance (*8*). Transsulfuration may also yield pyruvate from β-mercaptopyruvate along with thiosulfate (*9*) or thiocyanate (*10*) depending on the sulfur acceptor. Finally, β-mercaptolactate can be decarboxylated to β-mercaptoethanol in cells such as yeast, which are deficient in sulfur-removing enzyme and pyridine nucleotide-linked lactic dehydrogenase (*11*).

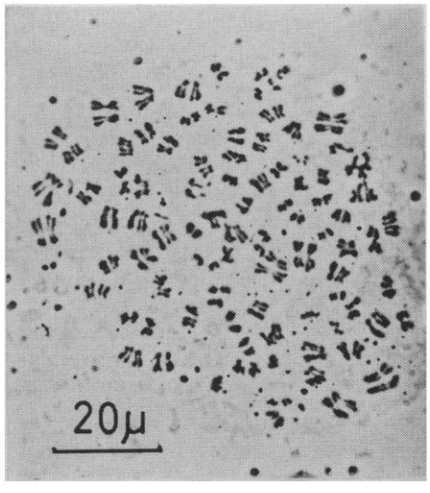

Fig. 2. Endoreduplicated octoploid mitosis, metaphase, aceto-orcein stain, phase contrast.

β-Mercaptoethanol produces polyploidy, endoreduplication, and chromosome aberrations such as breaks, rearrangements, and severe chromosome erosion or fragmentation at a concentration of $1.28 \times 10^{-3} M$ (*1*). But so many alternate pathways are available for cysteine degradation that the production of significant amounts of mercaptoethanol in vivo would hardly be expected. The concentration of mercaptopyruvate in the current experiments would also seem to be unlikely to occur in vivo. However, the actual intracellular concentration is not known, and may be considerably less. In addition, once mercaptopyruvate has gained entrance to the cell, there must be a sufficient amount to overcome a presumably normally functioning degradative enzyme system within the cell. Concentrations sufficient to produce similar effects might conceivably occur in such tissues as skin and hair follicles, where the formation of keratin may require up to 15 percent cysteine (*12*). Increased concentrations would be even more likely to occur if the degradative enzyme activity was reduced.

Mechanisms for the occurrence of increased β-mercaptopyruvate by loss of enzyme activity have already been demonstrated. Cysteine desulfurase in Bagg lymphosarcoma and Walker carcinoma 256 was only half that of normal liver and kidney (*13*). In Novikoff hepatoma, mercaptopyruvate desulfurase was reduced to about one-third that of normal liver (*7*). Thus in tumors, progressive loss of enzymatic activity in the pathways of cysteine catabolism might allow sufficient accumulation of intermediary sulfhydryl compounds to produce polyploidy.

Table 1. Incidence of tetraploid (T.) mitoses with random chromosome locations, and endoreduplicated tetraploid (T.E.) mitoses compared to normal diploid mitoses in human leukocyte cultures treated with β-mercaptopyruvate. The first series of 6-day cultures were from one blood sample, and all the remaining cultures were from a single sample from another donor.

Dosage ($10^{-3}M$)	T.	T.E.	Total mitoses	Polyploidy (%)
6-Day cultures				
0	1	0	200	0.5
0.64	2	0	200	1.0
1.28	9	17	300	9.0*
4-Day cultures				
0	0	0	200	<0.5
0.64	1	2	200	1.5
1.28	0	0	10	
5-Day cultures				
0	1	0	200	0.5
0.64	2	0	200	1.0
1.28	1	3	30	13.3
6-Day cultures				
0	0	0	200	<0.5
0.64	0	0	200	<0.5
1.28	3	10	117	11.1

* Includes one octoploid endoreduplication.

Heteroploidy with a near-triploid modal chromosome number has been reported in cytologic studies of the Walker carcinoma 256 (*14*). The Novikoff hepatoma in vivo consists of roughly 40 per cent tetraploid cells (*15*). When transplanted in vitro, there occurs first an increase in tetraploidy, then a widening numerical variation with the establishment of a narrow range of heteroploid cells between diploid and tetraploid. During the period of increasing tetraploidy, the cells cultured in vitro induced tumors when reintroduced into hosts. But coincident with the highly aneuploid transformation, the cell strains of the Novikoff hepatoma finally failed to produce tumors when reintroduced into hosts (*15*).

Particularly pertinent is the very high incidence of endoreduplication found by chromosome analysis in a case of acute leukemia during treatment with the sulfhydryl compound 6-mercaptopurine (*16*). Polyploidy and chromosomal abnormalities play important roles in the evolution of malignant cell lines from normal tissues cultured in vitro (*17*) and in the progression of tumors (*18; 19*).

JOHN F. JACKSON*
KERSTIN LINDAHL-KIESSLING
Institute for Medical Genetics, University of Uppsala, Sweden

References and Notes

1. J. F. Jackson, *Exptl. Cell Res.*, in press.
2. P. S. Moorhead *et al.*, *ibid.* **20**, 613 (1960).
3. Generously supplied as the sodium salt by B. H. Sörbo, Karolinska Inst., Stockholm.
4. Y. Ohnuki, A. Awa, C. M. Pomerat, *Ann. N.Y. Acad. Sci.* **95**, 882 (1961).
5. N. L. R. Bucher and D. Mazia, *J. Biophys. Biochem. Cytol.* **7**, 651 (1960).
6. R. Cecil and J. R. McPhee, in *Advances in Protein Chemistry*, C. B. Anfinson, Jr., M. L. Anson, K. Bailey, J. T. Edsall, Eds. (Academic Press, New York, 1959), vol. 14, p. 255.
7. A. Meister, P. E. Fraser, S. V. Tice, *J. Biol. Chem.* **206**, 561 (1954).
8. E. Kun, *Biochim. Biophys. Acta* **25**, 135 (1957).
9. B. H. Sörbo, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1962), vol. 5, p. 990.
10. J. W. Hylin, *ibid.*, p. 987.
11. E. Kun and H. G. Williams-Ashman, *Experientia* **18**, 261 (1962).
12. A. White, P. Handler, E. L. Smith, D. Stetten, *Principles of Biochemistry*, (McGraw-Hill, New York, ed. 2, 1959), p. 850.
13. A. M. Rutenberg, R. Goffstein, A. M. Seligman, *Cancer Res.* **10**, 113 (1950).
14. M. Cerny, *Casopis Lekaru Ceskych* **100**, 709 (1961).
15. T. C. Hsu and O. Klatt, *J. Natl. Cancer Inst.* **22**, 313 (1959).
16. C. Bottura and I. Ferrari, *Blood* **21**, 207 (1963).
17. A. Levan and J. J. Biesele, *Ann. N.Y. Acad. Sci.* **71**, 1022 (1958).
18. T. C. Hsu, in *International Review of Cytology*, G. H. Bourne and J. F. Danielli, Eds. (Academic Press, New York, 1961), vol. 12, p. 69.

19. Supported by a grant (to K.L.-K.) from the Swedish Cancer Society. Part of a research program on human cytogenetics under the direction of Professor J. A. Böök, aided by grants from the Swedish Medical Research Council and the National Institutes of Health (grant B 4320). On leave from Tulane University, J.F.J. received a U.S. Public Health Service training stipend in human genetics at the Institute for Medical Genetics, Uppsala, Sweden, for 1962–63.

* Present address: Department of Medicine. 1430 Tulane Ave., New Orleans.

3 June 1963

Congenital Malformations in Hamster Embryos after Treatment with Vinblastine and Vincristine

Abstract. *Intravenous injection of vinblastine or vincristine, two antitumor chemotherapeutic agents used in humans, into pregnant golden hamsters on the 8th day of gestation, causes an increase in the fetal mortality rate and the appearance of a significant number of congenital malformations in the surviving fetuses.*

The teratogenic effect of colchicine in the pregnant hamster has been reported (*1*). In a survey of the permeability of the hamster placenta to colchicine and other possible mitotic inhibitors, two more compounds, vinblastine (vincaleukoblastine, VLB) and vincristine (VCR), showed a profound embryocidal and teratogenic effect (*2*). Since these compounds, which are obtained from the common periwinkle plant (*Vinca rosea* Linn.) (*3*), have been used very effectively as chemotherapeutic agents in human tumors (*4*), it seems advisable to mention the possibility of their teratogenic effect in man. Both compounds have similar chemical structures (*5*), yet they appear to produce definite differences in their effects on tumors (*3*). The antimitotic activity of VLB in the hamster has been demonstrated by Cardinali *et al.* (*6*) and VCR has exhibited an antimitotic effect in the bone marrow of the mouse (*7*). Sokal and Lessmann (*8*) have reviewed the literature on the effects of cancer chemotherapeutic agents during human pregnancy and have concluded that aminopterin and the combined therapy of busulfan-6-mercaptopurine have been the only agents thus far studied in this class of compounds that have any known relationship to human congenital malformations.

The hamsters were anesthetized with Nembutal, and a small 1-cm incision was made over the femoral vein into which various concentrations of each compound (Table 1) were injected directly on the 8th day of gestation. Control animals received equal volumes of saline intravenously in a similar manner. The fetuses were recovered on the 14th day of gestation and examined for gross congenital malformations. The mortality rate was calculated by determining the number of resorption sites when the animals were killed. All maternal animals survived the treatment without any evidence of anorexia, weight loss, or diarrhea during the course of the experiment. Ten pregnant hamsters, used as controls, were injected intravenously with equal volumes of normal saline.

Table 1 shows that both compounds have a distinct embryocidal effect in that an increase in dosage causes a progressive rise in the embryonic mortality rate, an effect similar to that of colchicine (*1*). Evidence of gross malformations in the recovered fetuses was recorded and suspicious areas were prepared for microscopic examination. In addition, all fetuses which appeared to have skeletal defects at autopsy were cleared in 1 percent KOH and the skeletons were stained with alizarin red (*9*).

Malformations noted in the group treated with VLB included microphthalmia, anophthalmia, spina bifida, and skeletal defects consisting mainly of rib fusions and vertebral arch deformities. When administered on the 8th day of gestation, 0.25 mg/kg appears to be the most effective teratogenic dose of VLB. The fetuses from those animals receiving VCR showed malformations consisting of microphthalmia, anophthalmia, mild exencephaly, and rib defects. Its most effective teratogenic dose for hamsters is 0.1 mg/kg. It is probable that the incidence of malformations with these two compounds would be greater if a more detailed search by serial histologic sectioning were made.

Table 1. Effect of vinblastine and vincristine on fetal survival and gross congenital malformations when injected intravenously into golden hamsters on the 8th day of gestation.

Dosage (mg/kg)	Litters (No.)	Fetuses			
		Treated (No.)	Surviving (No.)	Mortality (%)	Grossly abnormal (No.)
Vinblastine					
0.1	4	51	42	18	3
0.25	10	134	56	58	16
0.5-2.6	3	38	11	71	2
Vincristine					
0.1	4	51	39	23	6
0.25	3	43	15	65	2
0.5	4	53	7	85	1
0.6-2.3	6	70	9	87	1
Controls					
0 (saline only)	10	119	111	7	0

Colchicine-induced malformations under identical experimental conditions (*1*) showed a great similarity to the malformations described here for VLB and VCR. It would thus appear that one explanation for the common teratogenic action of these compounds might by their mitosis-arresting activity on the developing embryonic tissue. However, in view of the marked difference in the tumor-spectrum activity of these two drugs, other unknown mechanisms may be responsible for their teratogenic action (*10*).

VERGIL H. FERM

Department of Pathology, Dartmouth Medical School, Hanover, New Hampshire

References and Notes

1. V. H. Ferm, *Proc. Soc. Exptl. Biol. Med.* **112**, 775 (1963).

2. Supported by U.S. Public Health Service grant GM 10210-01.

3. I. V. Johnson, G. H. Svoboda, H. F. Wright, *Proc. Am. Assoc. Cancer Res.* **3**, 331 (abstr.) 1962.
4. O. S. Selawry and J. Hananian, *J. Am. Med. Assoc.* **183**, 741 (1963).
5. N. Neuss, M. Gorman, H. E. Boaz, N. J. Cone, *J. Am. Chem. Soc.* **84**, 1509 (1962).
6. G. Cardinali *et al.*, *Proc. Soc. Exptl. Biol. Med.* **107**, 891 (1961).
7. G. Cardinali, G. Cardinali, M. A. Enein, *Blood* **21**, 102 (1963).
8. J. E. Sokal and E. M. Lessmann, *J. Am. Med. Assoc.* **172**, 1765 (1960).
9. A. B. Dawson, *Stain Technol.* **1**, 123 (1926).
10. The VLB and VCR were supplied to Dr. F. G. Ebaugh, Jr., Dartmouth Medical School, by Dr. J. G. Armstrong, Eli Lilly Co., Indianapolis, Ind.

29 April 1963