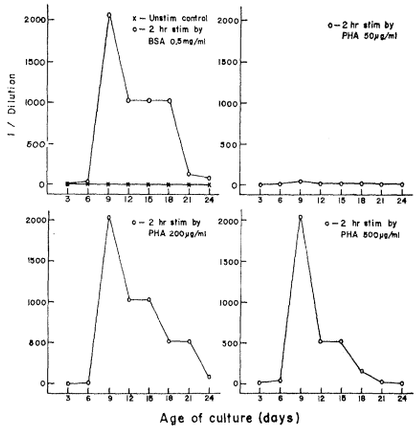

Fig. 1. Reciprocal titers of antibody to BSA in cultures of BSA-sensitized lymph node fragments following stimulation by BSA (0.5 mg/ml) and PHA in concentrations of 50, 200, and 500 μg/ml for 2 hours on day 0. Each point represents the mean value for duplicate cultures.

an example of the stimulation of antibody production and thymidine incorporation in cultures of HCG-sensitized node fragments following treatment with PHA. An increase in the titer of antibody to HCG appeared on the 2nd day, was maintained for 8 days, and then started to decline. The untreated control cultures and cultures stimulated with HCG were used for reference. The apparent shorter latent period and shorter duration of elevated antibody production in PHA-stimulated cultures, as compared to those stimulated with HCG, was not observed consistently when different concentrations of PHA and different rabbits were used. Figure 1 shows the effects of PHA in varying concentrations on the production of antibody to BSA in cultures of BSA-sensitized node fragments. PHA in concentrations of 500 and 200 μg/ml produced an anamnestic response almost exactly like that elicited by BSA, while a PHA concentration of 50 μg/ml showed only a questionable stimulatory effect. Preliminary experiments showed that the effective concentrations of PHA for mitotic stimulation were lower when serum-free medium (*10*) was used.

If the elicitation by PHA of an anamnestic response is truly a nonspecific one, it implies that sensitized cells can transmit to their progeny the necessary information for synthesizing a specific antibody in the absence of exogenous antigen. Thus the antibody titers in the cultures rise because of an increased number of antibody-producing cells resulting from the mitotic stimulation by PHA (Table 2). If this is the case, presence of the antigen is not necessary for the elicitation and maintenance of the anamnestic response.

On the other hand, the elicitation by PHA may be really a specific one in that PHA increases the sensitivity of previously primed cells to traces of antigen still present in the fragments. Normally, these concentrations must be insufficient to produce an anamnestic response, as shown by the negative response in the untreated cultures. If this is the case, PHA may resemble the inducer in the synthesis of induced enzymes (*11*) by increasing the permeability of the cell to the antigen; thus the intracellular antigen may reach a concentration sufficient to trigger synthesis of the antibody (perhaps by derepressing the directing gene). Phytohemagglutinin may also exert its effect by altering the membrane of the sensitized cell so that it is more responsive to the antigen or to whatever compound transfers the information, for example, RNA (*12*), or RNA-antigen complex. The effectiveness and persistence of the PHA stimulatory effect after such short contact raises the possibility that PHA may act on the level of the cell membrane; conceivably, for the same reason, so may the antigen. It is difficult at present to explain the three experiments in which the sensitized cells did not respond to PHA, but did respond to the specific antigen by producing an anamnestic response.

Elicitation by PHA of an anamnestic response in vitro thus provides a different approach for investigating the role of antigen in antibody formation.

TIEN-WEN TAO*
Department of Pathology, Harvard Medical School, Boston, Massachusetts

References and Notes

1. G. Dreyer and E. W. A. Walker, *J. Pathol. Bacteriol.* **14**, 28 (1909).
2. F. J. Dixon and P. H. Maurer, *J. Immunol.* **74**, 418 (1955).
3. Phytohemagglutinin was obtained from Difco; bovine plasma albumin, from Armour.
4. Follutein, E. R. Squibb & Sons.
5. T. W. Tao, thesis, Harvard Univ. (1962).
6. A. B. Stavitsky, *J. Immunol.* **72**, 360 (1954).
7. D. L. Joftes and S. Warren, *J. Biol. Phot. Assoc.* **23**, 145 (1955).
8. A. H. Coons, *J. Cellular Comp. Physiol.* **52** (Suppl. 1), 55 (1958).
9. T. F. O'Brien, M. C. Michaelides, A. H. Coons, *J. Exptl. Med.* **117**, 1053 (1963).
10. C. T. Ambrose, *ibid.* **119**, 1027 (1964).
11. A. Novick and M. Wiener, *Proc. Natl. Acad. Sci. U.S.* **43**, 553 (1957); J. Monod, in *Cellular and Humoral Aspects of the Hypersensitive States*, H. S. Lawrence, Ed. (Harper, New York, 1959), p. 628.
12. J. A. Mannick and R. H. Egdahl, *Science* **137**, 976 (1962); M. Fishman, R. A. Hammerstrom, V. P. Bond, *Nature* **198**, 549 (1963).
13. This study was supported by USPHS research grant HD-00137. I thank Prof. A. T. Hertig for his generous support and Prof. A. H. Coons for his valuable criticism and suggestions regarding the manuscript. To C. T. Ambrose, I owe gratitude for encouragement and assistance.
* Present address: Department of Medicine, New York University Medical Center, 500 First Ave., New York, N.Y.

20 July 1964

Chromosome Abnormalities in vitro in Human Leukocytes Associated with Schmidt-Ruppin Rous Sarcoma Virus

Abstract. *Chromosome breakage was observed in human leukocytes in vitro when Schmidt-Ruppin strain of Rous sarcoma virus was added to the cultures. Similar additions of Bryan strain of Rous sarcoma virus had no such effect.*

The Schmidt-Ruppin strain of the Rous sarcoma virus produces tumors in a wide variety of mammals as well as in fowl (*1*). Affected mammals include the mouse, rat, Syrian hamster, rabbit, and guinea pig. A similar strain of the Rous sarcoma virus (Zilber) produces tumors in primates (*2*). We have previously studied the effect of the Schmidt-Ruppin virus on chromosomes in the cells of rat tumors induced by the virus, in the cells of similar tumors in tissue culture, and in diploid rat embryo cells in tissue culture (*3*). In all three systems chromosome breaks, or rearrangements resulting from breaks, occurred. Because of the wide variety of mammals in which this virus produces tumors and the association of the virus with chromosome breakage, its effect was studied in a human cell system in vitro. The cells, human peripheral leukocytes, were chosen primarily because in culture they are induced to divide by addition of phytohemagglutinin (*4*); therefore the times of RNA and DNA synthesis and the state of mitosis when virus is added are known with relative accuracy (*5*).

Pools of cell-free virus suspension were prepared by harvesting rapidly growing tumors induced in the chicken. The tumors were minced and put in suspension (10 percent by weight) in tris-saline solution containing 10 per-

cent calf serum. The cellular debris was removed by multiple centrifugations, and the separated virus preparations were stored in flame-sealed glass ampules at −60°C until used. The human leukocytes were cultured, with slight modification (*6*), by the method of Moorhead *et al.* (*7*). The cells were exposed to virus suspension, either at the time of culture preparation or after DNA synthesis had begun, by centrifuging the cultured cells, decanting and saving the supernatant media, resuspending the cells in virus suspension, and allowing them to stand in the cold for 1 hour. The cells were centrifuged again and the virus suspension was decanted; the cells were then replaced in their former media. Controls were handled similarly, but without the addition of virus. On two occasions Bryan strain of the Rous sarcoma virus, a strain which does not produce tumors in mammals, was similarly added to additional controls. The cultures were then incubated until 72 hours, when chromosome preparations were made in the usual manner (*6*).

In the first four experiments, titrations of the Schmidt-Ruppin virus were not available. Later, we found that the Schmidt-Ruppin virus could be titrated in the same manner as the Bryan strain of Rous sarcoma virus on either chorioalantoic membranes of embryonated chicken eggs (*8*) or monolayers of chick fibroblasts (*9*). When virus suspension was added at the time the cultures were established, severe mitotic inhibition prevented chromosome analysis (Table 1). If the virus was added at 36 hours, after the onset of DNA synthesis, mitotic inhibition was less severe, and chromosome abnormalities were detected. Abnormalities detected after adding virus at 36 hours were in the form of chromatid breaks (Fig. 1) in five experiments in which colchicine was added and the cells were studied during c-metaphase. They were in the form of acentric fragments and bridges (Fig. 2) in one experiment from which colchicine was omitted to permit study of anaphases. Breaks seen in the metaphase preparations fitted the description by Östergren and Wakonig (*10*) of the delayed isolocus chromatid type. These authors postulated that a labile locus is formed when the chromosome is functionally a single unit. Then, after the chromosome has been functionally split into two chromatids, any range of abnormality from a secondary constriction in only one chromatid—or, as in the typical example, a secondary constriction in one chromatid, with a corresponding break in the other—to a complete break in both chromatids may be observed. (A complete break in both chromatids may also derive from chromosome breakage before the split.) This variation presumably depends on the stresses to which the chromatids are subjected during chromosomal contraction at mitosis (*11*). Chromosome rearrangements and reunions are rare.

The question arose whether chromosome abnormalities might be due to some component of the chicken tumor extract rather than to the virus: deoxyadenosine, a normal deoxyriboside found in all tissues, has been known to produce chromosome abnormalities at high concentration (*6*). However, when the Bryan strain of virus suspension, prepared from chicken tumors in the same way as the Schmidt-Ruppin virus suspensions, was added to similarly prepared cells, there was no significant chromosome breakage; the Bryan strain did seem to produce some degree of mitotic inhibition (Table 1).

In several cultures to which Schmidt-Ruppin virus had been added there seemed to be a defect in coiling or contraction, or both, of the chromosomes in a high percentage of the cells in mitosis. This is suggested by the smaller chromosome size and dif-

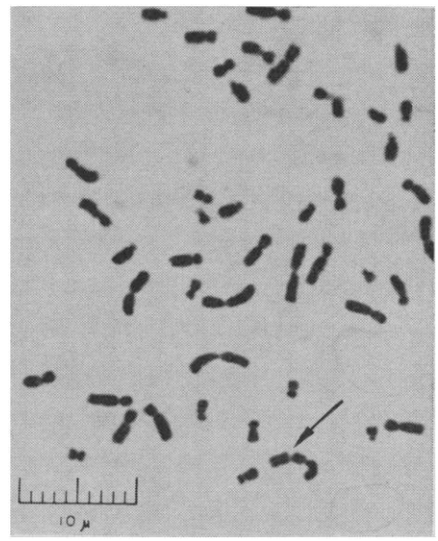

Fig. 1. Chromosome break in human leukocyte at metaphase treated with Schmidt-Ruppin Rous sarcoma virus. Break is at the arrow.

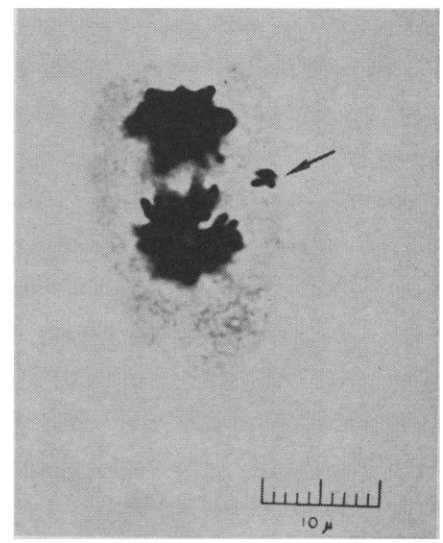

Fig. 2. Anaphase preparation showing acentric fragment remaining at equator of cell after treatment with Schmidt-Ruppin strain of Rous sarcoma virus.

Table 1. Schmidt-Ruppin Rous sarcoma virus in human leukocytes.

Stage examined	Time of virus addition (hour)	Schmidt-Ruppin Rous			Bryan strain of Rous			Control	
		Virus concn (FFU per 0.1 ml)	Cells with breaks* (%)	Mitotic index†	Virus concn (FFU per 0.1 ml)	Cells with breaks* (%)	Mitotic index†	Cells with breaks* (%)	Mitotic index†
Metaphase	Zero			0.3					3.1
	36		24	1.9				2	
Metaphase	Zero			0.2					2.3
	36		16	2.5				8	
Metaphase	Zero			0.3					1.4
	36		26	1.2				6	
Anaphase	36		20					2	
Metaphase	36	3×10^4	Severe inhibition		7×10^5	6	1.35	4	2.75
Metaphase	36	2×10^4	40	0.7	7×10^5	8	0.9	4	2.8
	36	2×10^5	Severe inhibition	0.3					

* 50 cells analyzed at each examination. † 2000 cells counted at each examination.

ference in staining quality, as compared with control preparations. This type of change is sometimes associated with decreased robustness of cells under unfavorable culture conditions.

When the Schmidt-Ruppin virus was concentrated by ultracentrifugation so that 2×10^6 focus-forming units (FFU) of virus were added to the culture containing approximately 2.25×10^6 cells, there was mitotic inhibition to a degree that prevented chromosome analysis. On two occasions serial titrations of the leukocyte culture fluids after virus addition revealed no evidence of virus multiplication.

W. W. NICHOLS, A. LEVAN*
L. L. CORIELL, H. GOLDNER
C. G. AHLSTRÖM*
South Jersey Medical Research Foundation, Camden, New Jersey, and **Institutes of Genetics and Pathology, University of Lund, Lund, Sweden*

References and Notes

1. C. G. Ahlström, N. Jonsson, N. Forsby, *Trans. Scand. Congr. Pathol. Microbiol. 13th, Turku*, 127 (1962).
2. J. S. Munroe and W. F. Windle, *Science* **140**, 1415 (1963).
3. W. W. Nichols, *Hereditas* **50**, 53 (1963).
4. P. C. Nowell, *Cancer Res.* **20**, 462 (1960).
5. M. A. Bender and D. M. Prescott, *Exptl. Cell Res.* **27**, 221 (1962).
6. B. A. Kihlman, W. W. Nichols, A. Levan, *Hereditas* **50**, 139 (1963).
7. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exptl. Cell Res.* **20**, 613 (1960).
8. V. Groupe, V. Dunkel, R. A. Manaker, *J. Bacteriol.* **74**, 409 (1957).
9. H. M. Temin and H. Rubin, *Virology* **6**, 669 (1959); H. Rubin, *ibid.* **10**, 29 (1960).
10. G. Östergren and T. Wakonig, *Botan. Notiser*, p. 357 (1954).
11. This idea that the weak points are broken during the spiralization movements on chromosome contraction is an unpublished interpretation by Östergren.
12. We thank M. Peluse for technical assistance, and Mary Federico for help with the photography. Supported by grants CA-03845, 06415, and 4953 and research career development award RC-17-64 from NIH, and by the Swedish Cancer Society and the Swedish Medical and Natural Sciences Research Council.

9 July 1964

Radiation-Chemical Oxidation of Peptides in the Solid State

Abstract. *Gamma-ray irradiation of polypeptides as highly dispersed fluffs under oxygen leads to chemical degradation of the peptide bond with the remarkably high oxygen consumption of about one molecule per 2 ev of absorbed energy. A radical chain mechanism appears to be involved, and there is evidence that excited states of the polypeptide aggregate undergo chemical quenching by molecular oxygen.*

The radiation-induced destruction of amino acids in solid proteins has been described by Alexander and Hamilton (*1*) and by Bowes and Moss (*2*) as not being significantly greater under oxygen than in a vacuum. However, neither of these studies was undertaken to determine optimum conditions for radiation-chemical reaction in a heterogeneous peptide-oxygen system; in the one study the dose rate was confined to the relatively high value of about 5×10^{21} ev g^{-1} min^{-1}, and in the other the solids were irradiated in a nondispersed form. We have, therefore, re-examined this question by irradiating —as highly dispersed fluffs (*3*)—a polypeptide, gelatin, and a polyamino acid, poly-D,L-alanine, in a vacuum and in an oxygen atmosphere under γ-rays at the relatively low dose rate of 1×10^{16} ev g^{-1} min^{-1}.

The very striking results are the remarkably high G values (see Table 1) for oxygen uptake and for oxidative degradation of both gelatin and poly-D,L-alanine. Chemical analysis of the irradiated peptides after a 3-hour period of hydrolysis in 2N hydrochloric acid established that ammonia and α-ketoacid or acids were principal degradation products. The quantitative procedures were those developed in our previous studies of the radiolytic oxidation of polypeptides in aqueous solution (*4*). Oxygen uptake and carbon dioxide production were followed by mass spectrometry. The observed $G(-O_2)$ values correspond to an energy requirement of only 1.5 and 2.0 ev per molecule of oxygen removed by gelatin and poly-D-L-alanine, respectively.

The magnitude of these G values prompted us to give particular attention to the quantitative basis of such reactions. Dose is determined by extrapolation on the basis of the ratio of the electron densities of the solids and the Fricke dosimeter (*5*). The validity of the extrapolation for the low-density solids and for the geometries used here was established experimentally in a series of control experiments in which the density and the volume of the irradiated samples were varied from about 0.025 to 0.6 g/cm^3 and from 1 to 80 cm^3, respectively. The possibility that not all of the observed chemical change arises as a result of the direct action of the radiation on the polypeptides was also examined in some detail. The fact that the degradation yields in oxygen are independent of pressure from 650 mm down to 20 mm as shown in Table 2 indicates that energy absorption by oxygen is not a major contributing factor. At 20 mm pressure, the oxygen in the gas phase plus that absorbed on the solid peptide represents less than 1 percent of the mass of the total sample (*6*). The possibility that part of the energy absorbed by the pyrex cell is transferred to the peptide through excited states of oxygen is negated by the observed pressure independence and by the observation that addition of a glass-fiber plug above the peptide sample does not result in any change in the degradation yields. There is also the question whether or not the amide and carbonyl yields given in Table 1 include some contributions from "dark reactions" induced in the hydrolysis. Both peroxides and carbonyl compounds, for example, are known to degrade amino acids by way of the Strecker reaction, with the formation of ammonia under certain con-

Table 1. Product yields in the γ-radiolysis of gelatin and poly-D,L-alanine in the solid state. *A*, irradiated in a vacuum and then exposed to oxygen prior to chemical manipulation. *B*, irradiated in oxygen at a pressure of 50 mm-Hg. The dose was 1.44×10^{20} ev/g. G = molecules per 100 ev absorbed energy.

	G-values			
	$>C=O$	NH_3	CO_2	$-O_2$
	A. Evacuated			
Gelatin	2.3 ± 0.3	8.1 ± 0.5	0.63 ± 0.03	
Poly-D,L-alanine	4.8 ± .3	5.6 ± .3	.41 ± .01	
	B. Oxygen atmosphere			
Gelatin	17.9 ± 0.9	42.1 ± 1.8	15.7 ± 3.0	72.7 ± 0.5
Poly-D,L-alanine	24.3 ± .7	30.9 ± 1.7	33.8 ± .5	52.3 ± .9