ejection is altered in its transmission to peripheral branches partly by the superposition of a standing wave created, according to common belief, by reflection from peripheral branching arteries. The period of this wave depends on the volume of blood in the aortic system and the volume elasticity characteristics of the aortic compression chamber. Normally such superposition is responsible for augmentation of the primary wave in the femoral artery and the occurrence of a marked dicrotic wave.

Previous work has shown that when distensibility is increased over the entire length of aorta (distributed distensibility) the natural period of the system becomes longer and the transmission rate of pressure waves is reduced. As a result the dicrotic wave in the femoral artery is flattened or abolished.

Experiments recently carried out in our laboratory by Robert E. Whittlesey revealed that when the volume elasticity of the aorta was apparently doubled by insertion of a large compression chamber (limited distensibility) the aortic and femoral pressure pulses showed just the opposite of the anticipated effects. The following conclusions were reached:

1. The insertion of a compression chamber into the aorta does not produce a common system but two systems with independent natural frequencies.

2. Such a chamber affects pressures in the arterial system by abstraction of blood during the latter portion of systole and early diastole. This initiates a negative pressure wave and, through reverberation, a positive pressure wave in the arterial system proper. The natural frequency of the arterial system remains virtually unchanged. These negative and positive standing waves are superimposed on the aortic and femoral pulses and respectively account for the marked lowering of pressure at the beginning of diastole and an intensification of the dicrotic wave.

3. The reduction in pulse amplitude is not due to damping but to an effect of increasing the capacity of the whole arterial system.

Rice in Asia

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Throughout southern Asia, except northwest India and western Pakistan, rice is prevailingly the staple food of the masses, supplying about 80% of the daily calories. The life of the people centers largely in the production of rice, which requires an incredible amount of hand labor.

With progressive industrialization of the East, rice is increasingly milled to whiteness to render it more palatable and better adapted to storage during long transport from distant farm to city kitchen. Thus the tendency to beriberi is aggravated.

Large-scale experimental fortification of rice has been practiced in Bataan, Philippines, for the past 3 years. Whereas formerly there were 150–300 deaths from beriberi each year in this 93,000 population, no single death from this disease has been reported for the past 18 months.

The question of applicability of such a measure in other parts of Asia occasioned a recent tour of all principal rice-eating lands, where observations were made of the various factors involved. A major obscurity is the extent of beriberi in Asia, where few countries have trustworthy mortality figures. Accordingly, it is hoped that further trial introduction of the fortified product will give a better measure of the present extent of this need as compared with needs based on numerous other dietary shortcomings. Also food, politics, and peace are not unrelated, and interesting observations were made on these aspects of Asian life.

Technical Papers

Induction of Mutations with β-Propiolactone[1]

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In a search for new chemical mutagens, compounds are being screened on the basis of tests using *Vicia faba* and *Neurospora crassa*. Results are reported here with the compound β-propiolactone, which was found to be effective in (1) inducing chromosomal aberrations in mitotic divisions of root tip cells in *V. faba*; and (2) causing reversions to growth-factor-independence in biochemical mutant strains of *N. crassa* and inducing gene mutations which confer resistance to the antibiotic effects of canavanine on *Neurospora*.

β-propiolactone[2] is the simplest lactone, and consists of a 4-membered ring containing 1 oxygen and 3 carbon atoms. The structural formula is $CH_2-CH_2-C=O$ (with O bridging the first CH_2 and the C in the ring).

Compounds with 4-membered rings are usually characterized by great chemical activity because of the tendency of the ring to open. The highly strained ring of β-propiolactone is known to open under the influence of many types of reagents to form a large variety of compounds. β-propiolactone is a liquid (bp, 155° C at 760 mm) and is soluble in water (37% by vol, at 25° C). The compound is available in quantity owing to its use in the field of industrial synthetic organic chemistry.

Preliminary trials with *V. faba* root tips, at different concentration-time levels, showed that immersion in a 0.05% aqueous solution of β-propiolactone for 20

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[2] Appreciation is expressed to the B. F. Goodrich Chemical Company, Cleveland, Ohio, for supplying β-propiolactone in lots for experimental purposes.

TABLE 1

FREQUENCY OF CHROMOSOMAL ABERRATIONS IN *Vicia faba* ROOT TIPS TREATED WITH β-PROPIOLACTONE

Treatment: concentration, time	Recovery time (hr)	No. anaphases examined	No. chromosome aberrations	Anaphases with aberrations (%)
(1) *Preliminary trial*				
0.05%, 20 min	24	81	3	3.70
	48	100	16	16.00
Control	24	100	0	0
	48	100	0	0
(2) *Confirmatory trial*				
0.05%, 20 min	48	813	117	14.39
Control	48	700	1	0.14

min was a threshold sublethal dosage at which chromosomal aberrations were produced. Root tips were fixed immediately after treatment and at subsequent intervals of 6, 24, and 48 hr, during which they were grown in moist vermiculite. No aberrations were found in cells fixed immediately or 6 hr after treatment. In cells that were fixed 24 or 48 hr after treatment, aberrations were observed to occur in the frequencies shown in Table 1.

The most frequent aberrations observed were 1 or 2 large fragments that lagged at anaphase (Fig. 1).

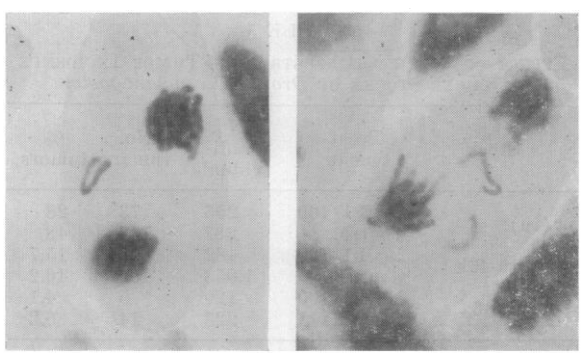

FIG. 1. Two anaphase figures showing chromosomal aberrations in root tip cells of *V. faba* (× 900) that had been treated 48 hr previously with β-propiolactone.

The size of the fragments indicated that the breaks occurred most frequently near the centromere, possibly in heterochromatic regions, and in some figures there appeared to be inactivation rather than loss of the centromere region.

Two other, more complex lactones have been reported to cause chromosomal abnormalities. Ostergren (*1*) found that coumarin (O-hydroxycinnamic acid lactone) produced chromosome bridges and breaks in *Allium cepa*. Erickson and Rosen (*2*) reported that protoanemonium, $C_5H_4O_2$, produced cytological disturbances in root tip cells of *Zea mays*, though crucial evidence on recovered cells was lacking.

Induction of gene mutations in *Neurospora* is being investigated by methods essentially the same as those utilized by Kölmark and Westergaard (*3*) and by Dickey, Cleland, and Lotz (*4*). Reversions to growth-factor-independence are being studied both in adenineless and inositolless colonial strains. Treated spores are plated onto minimal agar medium, and the appearance of growing colonies is taken as initial evidence of mutation. The colonial gene has also been incorporated into a canavanine-sensitive strain (*5*). In experiments with the colonial canavanine-sensitive strain, treated spores are plated onto media containing canavanine in order to detect mutations for resistance. In all our experiments controls are established by plating untreated spores from the same cultures which have served as the source of the treated material. Except for exposure to the mutagen, control spores are carried through all of the processes involved in treatment, and for the same time periods.

TABLE 2

MUTAGENIC EFFECT OF TREATMENT WITH 0.01% β-PROPIOLACTONE FOR 12 HR ON 2 STRAINS OF *Neurospora*

Treatment	Strain 70,007–37,401 (inositolless)			Strain 70,007–38,701 (adenineless)		
	No. conidia plated	No. colonies formed after plating	No. mutations per 10^6 conidia	No. conidia plated	No. colonies formed after plating	No. mutations per 10^6 conidia
Controls (untreated)	52.7×10^6	0	0	7.2×10^6	0	0
UV*, 1 min	28.4×10^6	158	5.56	3.3×10^6	5	1.51
UV, 2 min	28.4×10^6	243	8.56	3.3×10^6	8	2.42
β-Propiolactone	48.8×10^6	7	0.14	5.5×10^6	84	15.27

* The source of ultraviolet light was a G-E germicidal lamp, approximate calibration 75 ± 10 ergs/mm²/sec.

Table 2 shows the results of an experiment in which inositolless and adenineless spores were treated with 0.01% β-propiolactone for 12 hr. As part of the same experiment, spores of the mutant strains were exposed to ultraviolet light. The results of this latter treatment, together with the values obtained with untreated spores, serve as indications of the effectiveness of treatment with the lactone.

Recognizing that relatively long treatment periods complicate the analysis of mutation experiments, we have attempted to obtain evidence for the mutagenicity of β-propiolactone over shorter periods of treatment. Table 3 records the results of a recent experiment in which samples of spores of the adenineless strain were treated for 1 hr at three concentration levels and at two temperatures. The results show that effective mutagenic action can be obtained with β-propiolactone, even when the treatment periods are relatively short.

TABLE 3

EFFECT OF 1-HR TREATMENT WITH β-PROPIOLACTONE ON *Neurospora* STRAIN 70,007–38,701 (adenineless)

	25° C			35° C		
	No. conidia tested	No. colonies formed after plating	No. mutations per 10^6 conidia plated	No. conidia tested	No. colonies formed after plating	No. mutations per 10^6 conidia plated
Control	1.75×10^6	0	0	2.00×10^6	0	0
0.01%	2.63×10^6	6	2.28	2.50×10^6	10	4.00
0.02%	2.75×10^6	10	3.64	2.75×10^6	15	5.45
0.03%	3.13×10^6	12	3.83	2.38×10^6	17	7.14

Mutagenicity of β-propiolactone against different loci in *Neurospora* seems now to be well established. Besides the evidence from reversions of the adenineless and inositolless strains, we find that treatment with the lactone substantially increases the number of canavanine-resistant colonies which appear when canavanine-sensitive spores are plated onto canavanine-containing medium. However, the spontaneous appearance of canavanine-resistant colonies is relatively high. And, in addition, some instances of appearance of growth spots on canavanine medium appear not to be due to gene mutation. We have, therefore, placed more reliance on tests with the adenineless and inositolless strains. It seems clear that mutation accounts for most of the reversions to growth-factor independence in these strains. Colonies that appear after plating have all been picked, established in separate culture, and then tested on minimal medium. All such cultures have shown the ability to grow on minimal medium. We have also made crosses of these cultures back to strains carrying the original mutant gene, but of opposite mating type. Ascospore isolates have been made from approximately one fifth of these crosses, and from all of the crosses wild-type progeny have been recovered.

Two phenomena of interest have been observed in these mutation experiments. One is the apparent effect of temperature on the mutagenic action of β-propiolactone, as indicated in Table 3. The other is the fact that in experiments so far, β-propiolactone has appeared to be considerably more effective in creating mutations in the adenineless strain than in the inositolless strain, although our ultraviolet treatments show the opposite effect. Both of these phenomena are under further investigation.

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Influence of Diethylstilbestrol on *Drosophila melanogaster* Tumors

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The capacity of estrogenic substances to induce, stimulate, or inhibit tumor development in several species of vertebrates suggested an investigation as to whether these substances exhibit similar properties in relation to invertebrate tumors.

Three different hereditary, benign-tumor-bearing *Drosophila melanogaster* stocks were raised in food cultures containing diethylstilbestrol. The stocks used were st sr; tu-36a, described and analyzed by Russell (*1*), which presents 4–5% tumors at laboratory temperature (20°–25° C); bw(tu), with a tumoral frequency which fluctuates around 50%, according to Hartung (*2*); se e^{11}; tu-49th, which was formed in our laboratory and which has a tumoral incidence of 40–50% (*3*).

Sterile eggs of the three stocks were cultivated in shell vials containing a sterile medium composed of water, agar, ammonium sulfate, magnesium sulfate, tartaric acid, monopotassic phosphate, and dead brewer's yeast, after the formula suggested by Birch (*4*); 1% diethylstilbestrol was suspended in the medium. Control flies were cultivated in an identical medium without the drug.

TABLE 1

EFFECT OF DIETHYLSTILBESTROL ON TUMOR INCIDENCE IN THREE STOCKS OF *Drosophila melanogaster*

Stock	Treatment	No. individuals	No. tumors	% tumors
bw(tu)	DSB 1/100	255	73	28
	Controls	287	138	48
se e^{11}; tu-49h	DSB 1/100	482	76	15.7
	Controls	1,058	489	46.2
st sr; tu-36a	DSB 1/100	120	5	4.1
	Controls	223	14	6.2

The *D. melanogaster* eggs collected according to the method suggested by Schweitzer (*5*), were sterilized for 20 min in a 1/10,000 solution of sodium mercury thiosalicilate (Merthiolate "Lilly") in 70° alcohol. After being rinsed in 70° alcohol or saline solution, approximately 50 eggs were put into shell vials containing the medium. The treated and the control eggs were cultivated under the same environmental conditions.

As a result of the experiments (Table 1), it was observed that the flies raised in the medium containing diethylstilbestrol presented a lower percentage of tumors than those cultivated in the control vials. This lower number, nevertheless, was statistically significant only in the stocks of high tumoral incidence: bw (tu) and se e^{11}; tu-49h.

The experiments indicate that diethylstilbestrol,