garded with caution, since investigations of venom composition are usually made with pools of extractions from a large number of snakes, and the individual venom composition is thus hidden in the species' venom pool (*6*).

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30 December 1958

High-Energy Phosphates during Long-Term Hibernation

Abstract. Adenosine triphosphate (ATP) and phosphocreatine (PC) show contrasting levels in muscle and liver after short and long periods of hibernation. In prolonged hibernation cardiac and skeletal muscle PC continues to maintain ATP, but at lower levels. In the liver, control levels for these compounds are regained.

During the course of experiments with hibernating ground squirrels, *Citellus tridecemlineatus,* we were fortunate in having nine animals hibernate for a month without interruption. Since past reports (*1*) have pertained to an uninterrupted hibernation period of 3 to 5 days (short-term hibernation), we decided to compare those results with results obtained from the animals that hibernated a month (long-term hibernation).

The control animals were kept in an environmental temperature of 25° to 27°C and were anesthetized with Nembutal before sacrifice. The hibernating animals were kept in a cold room at 3° to 5°C, and tissue was removed for analysis within 30 seconds after the animal was first handled. At the time of sacrifice the thoracic cage was opened, and the heart was frozen *in situ* with mixture of ether and Dry Ice and removed. The same procedure was applied to the liver and the muscle from the hind limbs. For phosphate analysis 0.85- to 1.00-g samples of tissue were extracted with cold 10-percent trichloroacetic acid at 0°C, and the determinations were based on the method of Fiske and Subarrow (*2*). Inorganic phosphate (IP) was precipitated with calcium at an alkaline *p*H (*a*); inorganic phosphate, including phosphocreatine, was hydrolyzed with molybdic acid at room temperature for 30 minutes (*b*); and total acid-soluble phosphate was hydrolyzed by heating at 100°C in 1*N* HCl for 8 minutes (*c*). Accordingly, the following values are obtained: IP = *a*; PC = *b* − *a*; and APP = *c* − *b*. Adenosine polyphosphate (APP), which is actually a combination of adenosine triphosphate and adenosine diphosphate, is reported in this paper as adenosine triphosphate (ATP). Glycogen was determined by the procedure of Kemp and Van Heijningen (*3*).

The results show that when cardiac muscle of the long-term hibernators is compared either with that of the controls or that of the short-term hibernators, ATP ($P < .05$) and PC ($P < .01$) decrease significantly and to approximately the same degree (Fig. 1). Apparently the longer the heart beats at the extremely slow hibernating rate of 15 to 25 beats per minute, the smaller the high-energy phosphate content. Since the ATP/PC ratio is greatest (5/1) at this time, most of the high-energy phosphate present is ATP, the "active" form which is supplying energy, at the expense of PC, to the slow but continually beating heart.

Although skeletal muscle shows a significant ($P < .01$) decrease of 49 percent in both ATP and PC when long-term and short-term hibernation are compared, the ATP/PC ratio is the same, 1/1. The quantities are smaller, but when one takes into consideration the lack of movement over a longer period of time, this is plausible.

In the liver ATP is decreased 55 percent ($P < .01$) and PC is increased 107 percent ($P < .02$) when the long-term hibernators are compared with the short-term ones. However, when the short-term hibernators are compared with the controls the reverse is true; ATP is increased 120 percent ($P < .01$) and PC is decreased 62 percent ($P < .01$). It appears that the high-energy phosphate compounds of the long-term hibernators have reached an equilibrium comparable to that of the controls.

Glycogen values of the long-term hibernators, when compared with those of the short-term hibernators, showed the following decreases: cardiac muscle, 36 percent; skeletal muscle, 3 percent; liver, 9 percent. None of these changes are significant.

An increase in the hibernating period from 5 to 30 days decreases both ATP and PC in cardiac muscle. Phosphocreatine shows the greater decrease because it is maintaining ATP which is considered the "active" form (*4*). Although the high-energy phosphate content decreases in skeletal muscle, the ATP/PC ratio remains approximately the same. The slowly beating heart is using ATP and depleting PC while the stationary limb muscle maintains metabolic function at the 1/1 ratio of short-term hibernation with lower levels of the compounds. The increase in liver in organic phosphate during long-term hibernation is difficult to explain but may be due to an increased breakdown of organic phosphate compounds resulting from the interruption of the activity of certain enzyme systems. With metabolic transformations apparently at a minimum, ATP and PC have resumed control levels.

In the regulation of metabolism, gly-

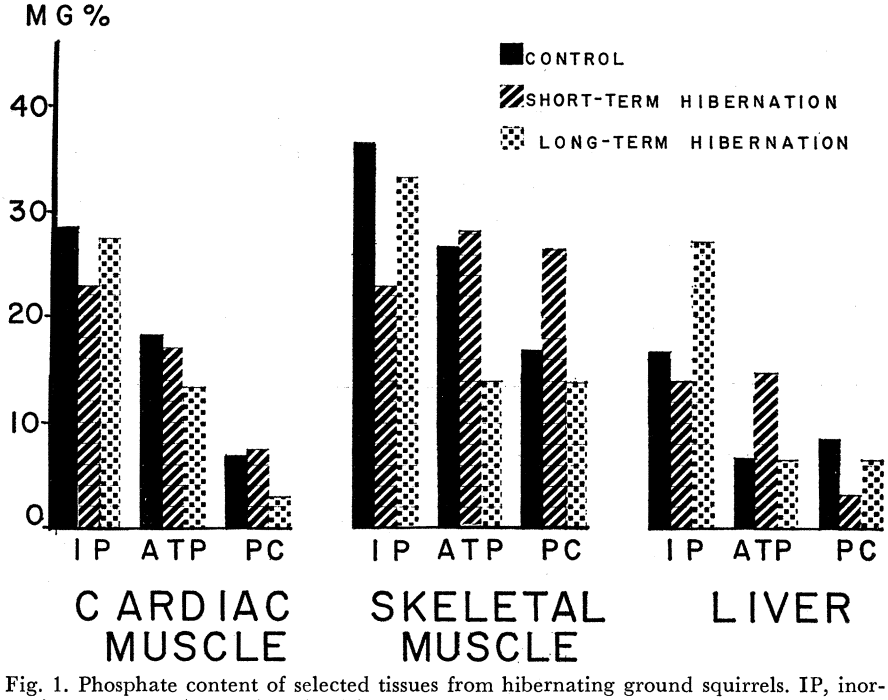

Fig. 1. Phosphate content of selected tissues from hibernating ground squirrels. IP, inorganic phosphate; ATP, adenosine triphosphate; PC, phosphocreatine.

colysis is inversely dependent upon the concentration of ATP (*5*). Since none of the tissues have undergone sudden changes necessitating quick energy, thus lowering ATP and stimulating glycolysis, glycogen levels do not show any significant differences (*6*).

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26 January 1959

Factors Influencing the Effect of β-Propiolactone on Chromosomes of Vicia faba

Abstract. The effect of β-propiolactone on chromosomes is independent of *p*H, oxygen tension, and some metabolic inhibitors. It is influenced by temperature, concentration, and pretreatments with dinitrophenol. Interphase sensitivity and localization of breaks are discussed in terms of the action of β-propiolactone and the relationship of β-propiolactone to other radiomimetic compounds.

Several studies (for example, Kihlman's, *1*) have indicated that with few exceptions each of a now long list of radiomimetic compounds differs from the others in its mode of action (that is, in interphase sensitivity and preferential breakage) or in its dependence on oxygen tension, temperature, and metabolic activity. In general, most radiomimetic chemicals can be grouped into two classes: (i) weakly reactive compounds such as maleic hydrazide and 8-ethoxycaffeine whose observed effects are much enhanced and depressed by factors that alter the metabolic state of the cell at the time of exposure; (ii) strongly reactive compounds whose observed effects seem to be somewhat independent of experimentally altered exposure conditions, acting as if by the law of mass action. It seems, then, that the less reactive chemicals are dependent on, and the more reactive chemicals independent of, oxidative metabolism systems for their observed effect. The work reported in this paper (*2*) indicates that β-propiolactone (BPL) is a substance belonging to the more reactive group, although differences in action are discernible.

β-Propiolactone is the simplest of a number of related compounds. It is used industrially as a plasticizer. Our interest in an understanding of its action, apart from determining its place in our study of the comparative effects of mutagens, stems partly from its promise as a chemotherapeutic agent in oncology. Its mutagenic properties have been demonstrated in yeast, bacteria, and *Neurospora*, while Smith and Lotfy (*3*) have demonstrated its effectiveness as a radiomimetic agent, testing it on the chromosomes of *Vicia* and *Allium*.

Our work is unlike that of Smith and Lotfy in two basic respects: metaphase rather than anaphase chromosomes were scored and environmental conditions were altered to test their influence on the effect of β-propiolactone in an attempt to elucidate the mechanism by which it acts in producing chromosomal aberrations. The lateral root-tip chromosomes of *Vicia* were used. Smith and Lotfy employed primary roots in their study, but the erratic and often high frequency of spontaneous aberrations in the primary roots makes the use of laterals, roots, in which spontaneous aberrations are generally absent or are present in a frequency of less than 1 percent, advantageous in this type of work. There is also the added advantage that chromosomes from roots of the same bean, presumably with the same genetic constitution, can be treated and tested.

The techniques of culture, treatment and recovery, and slide preparation have been previously described (*4*). Fresh preparations of BPL were always used in treatments because BPL has a tendency to break down in solution. A variety of BPL concentrations were tested, but $7 \times 10^{-3} M$ seemed to be the most effective since it resulted in a high frequency of aberrations and a low frequency of cell death. Only experiments in which this concentration was used are reported. The roots were treated at 17°C for 30 minutes and were allowed to recover for 48 hours at 25°C before fixation.

β-Propiolactone seems to act on chromosomes most effectively when the chromosomes are in early interphase. Few aberrations are observed after 24 hours' recovery, a peak frequency is reached after 48 hours, a somewhat lower frequency occurs after 72 hours, and an appreciable decline sets in by 96 hours. The prolonged delay of the peak frequency is due largely to the depressing effect of BPL on the mitotic rate.

The first burst of mitosis following the suppression of cell division coincides with the peak frequency, but whether BPL causes cells in prophase to regress to earlier stages is not known, although

Table 1. Effect of temperature on the activity of BPL.

Temperature (°C)	No. of cells	Isochromatids (%)	Exchanges (%)
5	100	22	3
17	100	43	10
25	100	43	29
37	Lethal		

no polyploid cells result from the suppression. It can be assumed, therefore, that the principal delay occurs at a stage prior to DNA synthesis and chromosome replication. When a low frequency of aberrations was encountered, it was paralleled by a low mitotic rate and a high frequency of dead cells. Lower concentrations of BPL interfered less with the mitotic rate but did not appreciably affect the time of appearance of aberrations. Our findings in this regard are in accord with those of Smith and Lotfy. We do not agree, however, on the time that definitive chromosome aberrations occur. One can see abnormal cells so far as the presence of sticky bridges and lagging chromosomes is concerned. This is especially true with regard to the satellites of the long chromosomes which often lag at anaphase to the extent that they appear to be fragments. Some of the latter abnormalities are seen at 24 hours, but they are very few in number, mitosis being severely inhibited at this time. The results reported here are similar to those reported by Smith and Srb (*5*) so far as time of appearance of aberrations is concerned.

The aberrations induced were entirely of the chromatid rather than the chromosome type. As is usual, isochromatid aberrations were predominant; interchanges were about one-third as frequent as isochromatids, and single chromatid deletions—never a large class of experimentally induced aberrations in *Vicia*—were conspicuously absent. The presence of fragment types, as contrasted to the anaphase bridges reported by Smith and Lotfy, cannot be confirmed by these experiments. We disagree therefore with Smith and Lotfy's conclusion that sister and nonsister fusion of broken ends is

Table 2. Effect of dinitrophenol (DNP) ($1 \times 10^{-4} M$ at 17°C for 2 hours) on BPL activity.

Treatment	No. of cells	Isochromatids (%)	Exchanges (%)
BPL	200	41.5	24.5
DNP + BPL	100	96	22
BPL + DNP	100	98	51