ENERGY METABOLISM
INTRODUCTION

The movement and distribution of ions across cell membranes is greatly influenced by the presence in biological systems of charged macromolecules which cannot cross membrane barriers. The pumping of ions by cells which form an interface with the external environment (e.g., intestine, kidney) also allows complex organisms to control the ionic composition of the extracellular medium. Ion pumps are also used for the selective secretion of the ionic components of exocrine secretions.

In order to maintain ionic gradients, ions must be moved in a thermodynamically unfavourable direction. This process consumes energy, the amount being determined by the concentration gradient (non-ionized permeants) or electrochemical potential (ions). Energy is supplied by cellular energy metabolism. Many systems derive the energy for the creation of ion gradients (e.g., Na\(^+\) or Ca\(^{2+}\)) directly from the hydrolysis of ATP.
Calcium ATPase shows a high affinity for Ca^{2+}, both for activation of ATPase activity and for ion-pumping. Both ATP and the electrochemical gradients of ions represent energy stores which can be exploited by the cell. The ion gradients are essential for the occurrence of many bioelectrical phenomena and to supply energy for the active accumulation of other molecules, in both prokaryotic and eukaryotic cells.

Cell membranes, especially those of homeotherms such as mammals and birds, generally perform their normal functions within a narrow temperature range, and excursions outside this range often cause a marked decline in activity. Moreover, membrane enzyme activities frequently show patterns of temperature dependence different in form from those typical of soluble enzymes.

When membranes are extensively damaged through modifications to the lipid phase their passive permeability to ions rises and membrane activities are modified. Amongst the most cytotoxic of these changes are increased inward leakage of Ca^{2+} and Na^+. Cells tend to become ATP depleted as a result of a futile expenditure of energy on trying to pump the ions out.

To confirm and concur the enhancement and decrement of various parameters studied earlier in this study, an attempt is made to analyse the energetics, correlation and its relevance to the results. Moreover the ions, sodium, potassium, calcium
and magnesium, under investigation play a vital and prominent role to assess the damage and recovery of various components in the tissue.

**METHODOLOGY**

**Ions**

The weighed organs were wet ashed in 50:50 (v/v) concentrated perchloric acid and nitric acid (Dall, 1967). After keeping the wet ash solutions for half an hour, until the organs were completely dissolved, they were evaporated at 100°C to 200°C temperature. The residues were dissolved in glass distilled water and made upto 10.0ml. It was filtered through Wattman No. 1 filter paper. Further appropriate dilutions were made prior to the estimation of sodium, potassium, calcium and magnesium ions with the help of Atomic Absorption Spectrophotometer (Perkin -Elmer model 2380). Standard solutions of sodium, potassium, calcium and magnesium were prepared by using analar grade chemicals. The values were expressed as μg./g wet wt. of tissue.

**ATPase activities**

Sodium, Potassium, Calcium, Magnesium and total ATPase activity were estimated separately in the liver and pancreas of mice under this study. Thus activities were assayed according to the method of Tirri et. al., (1973), with slight modification. 1% tissue homogenates were prepared in ice-cold
0.25M sucrose solution containing 1.0μM of Ethylenediaminetetra-acetic acid (EDTA) (pH 7.5) and 0.01M imidazole. The homogenates were fractionated into two parts where one part was centrifuged at 1,400 rpm and the supernatant thus obtained was used as enzyme source for Mg^{2+} ATPase, while the other part containing crude homogenate was used for the assay of total ATPase activity.

**Mg^{2+} ATPase activity**

After due standardization of enzyme kinetic parameters, the reaction mixtures with the following composition was used which provides optimal conditions for enzyme activity, 100.0 μM of Tris-ATP (substrate), 10.0μM of MgCl₂ (co-factor), 10.0 μM of ouabine (potent inhibitor of Na⁺-K⁺ ATPase) and 0.3ml of supernatant as enzyme source.

**Total ATPase activity**

100.0μM of Tris-ATP (substrate), 25.0μM of MgCl₂, NaCl and KCl(co-factor) respectively and 0.3ml of crude homogenate as enzyme sources.

The reaction mixtures were incubated at 37°C for exactly 15 minutes and then the reaction was arrested by adding 2.0ml of cold 10% TCA. The inorganic phosphates liberated were estimated by the method of Fiske & Subba Row (1925). The absorbance was measured at 660nm. Endogenous blanks were
prepared to find out endogenous inorganic phosphate. Another blank was prepared without using co-factor to detect the sodium salt stimulated activity as the substrate used was a sodium salt of ATP. ATPase activity was expressed as µM pi formed/g. protein/hr.

\[ \text{Na}^+ - \text{K}^+ \text{ ATPase activity} = \text{Total ATPase activity} - \text{Mg}^{2+} - \text{ATPase activity}. \]

The activity in presence of Ouabine (1.0mM) and Ethyleneglycol - bis (B-aminoethyl ether) N,N,N,N, - tetra-acetic acid (EGTA)(10.0mM) was taken as Mg\(^{2+}\)-ATPase activity and the difference in the activity in absence of ouabine and in the presence of EGTA(10.0mM) was taken as Ca\(^{2+}\)-ATPase activity.

**RESULTS**

**Liver**

**Total ATPase**

The activity is illustrated in figure (73) and table (25). At 5W the activity is increased on all the days of sacrifice. A similar tendency is seen in 10W and 15W exposed liver. A maximum increase of 96% and a minimum of 7% is recorded.
**Na**<sup>-K</sup> <sub>ATPase</sub>

Figure (77) and table (26) showed the activity of the enzyme. 5W and 10W exposed liver exhibited the elevated activity of the enzyme. At 15W intensity the enzyme activity showed a significant and progressive increase on all the days of sacrifice with maximum activity on day 10 of 180 seconds (98%).

**Ca**<sup>2+</sup> <sub>ATPase</sub>

Table (27) records the effect of ultrasound on hepatic Ca<sup>2+</sup> - ATPase activity under experimental conditions. A similar apparent increase in enzymatic activity is obtained. The maximum activity of 80% is seen on day 10 of 180 seconds, in 15W.

**Mg**<sup>2+</sup> <sub>ATPase</sub>

Mg<sup>2+</sup> ATPase activity apart from a slight rise on day zero (Table 28) showed a maximum increase of 92% on day 10 of 180 seconds in 15W. The activity is greatly enhanced when compared with controls. The variation ranged more in 180 seconds regime than in 60 seconds regime as seen from figure.
Pancreas

Total ATPase

Figure (105) compares the specific activities of total ATPase in pancreas of controls and those treated with ultrasound. A significant increase to a maximum of 99% is recorded.

Na\(^+\)-K\(^+\) ATPase

The activity is shown in figure (108). There is an elevation with a maximum of 96% and a minimum of 13% is seen. The elevation is higher than that was found in liver.

Ca\(^{2+}\) ATPase

A rapid enhancement is seen in the activity as shown from figure (117) and table (35). The activity shoots up to 80% on day 10 of 180 seconds in 15W.

Mg\(^{2+}\) ATPase

An increased activity is seen (table 36) and figure (126). The variation ranged from a minimum of 10% to a maximum of 85% on day zero of 60 seconds, 5W and on day 10 of 180 seconds, 15W respectively.

Totally the activity of all these enzymes is very high when compared with sham controls. The variation ranged more in 180 seconds regime than in 60 seconds regime.
Liver

**Sodium Ion Concentration**

Sodium ion concentration is decreased on day zero and day one and exhibited an increase on day 5 and day 10 of 5W. In 10W, it decreased on day zero and day one and a slight recovery was observed on day 5 and day 10. A gradual increase is recorded in 15W on all the days as seen from table (29) and graph (79).

**Potassium Ion Concentration**

Figure (81) and table (30) shows the ionic strength. An elevation in the concentration of potassium ions is observed on day zero and day one of 5W. Reduction is observed in day 5 and day 10 of 60 seconds. It reached normalcy on day 5 of 120 seconds and 180 seconds and showed an increase on day 10 in 5W exposure. 10W exposure recorded a gradual increase in the concentration on day zero and day one of 60 seconds. 15W showed a decrease in the ionic strength on day zero and day one and a gradual and constant increase on all other days.

**Calcium Ion Concentration**

Figure (87) and table (31) explains the strength of calcium ion pools. It showed an increase on all the days for all the wattages. A maximum enhancement of 47% on day 5 of 180 seconds in 15W and a minimum of 5% on day zero
of 60 seconds in 5W is recorded. The variations ranged more in 180 seconds regime than 60 seconds regime.

**Magnesium ion Concentration**

Magnesium ion concentration followed the same pattern as calcium ion concentration. It showed a maximum increase of 55% on day 5 of 180 seconds in 15W and a minimum of 6% on day zero of 60 seconds in 5W.

**Pancreas**

**Sodium ion Concentration**

Sodium ion pool is reduced on day zero and day one of 5W. A slight recovery is observed on day 5 of the 3 exposures and day 10 of 60 seconds. It is further reduced on day 10 of both 90 seconds and 180 seconds of 5W.

In 10W, a significant decrease is seen on day zero and day one with a slight increase on day 5 and day 10. A constant and gradual increase is recorded on all days of 15W.

**Potassium ion Concentration**

The ionic strength is increased as seen from the figure (111) and table (38). The enhancement is seen on all the days of both 5W and 10W. The maximum enhancement reaches upto 122% on 180 seconds of 5W on day one.
**TABLE : 25**

LIVER TOTAL ATPase

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**Total ATPase activity is expressed as um of Pi liberated/mg protein/hr.**

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent percent change over the control.
LIVER Na⁺-K⁺ ATPase

**Na⁺-K⁺ ATPase activity is expressed as μmol of Pi liberated/mg protein/hr.**

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent percent change over the control.

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**LIVER Ca++ ATPase**

Ca++ ATPase activity is expressed as um of Pi liberated/mg protein/hr.

Each value is a mean of 2D samples.

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**Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.**

Values in parentheses represent per cent change over the control.
**LIVER Mg**<sup>++</sup> ATPa$e**

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**Mg**<sup>++</sup> ATPa$e activity is expressed as μm of Pi liberated/mg protein/hr.

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.
** Sodium ion concentration is expressed as μ moles/gm. wt.**

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.
**Potassium ion concentration is expressed as μ mol/gm. wt.**

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.
TABLE: 31

LIVER CALCIUM ION CONCENTRATION

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** Calcium ion concentration is expressed as µ moles/g wet weight. Each value is a mean of 20 samples. **

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent percent change over the control.
**TABLE : 32**

**LIVER MAGNESIUM ION CONCENTRATION**

**Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.**

Values in parentheses represent per cent change over the control.

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<td>(35.04)</td>
<td>(46.30)</td>
<td>(33.85)</td>
<td>(39.30)</td>
<td>(48.64)</td>
<td>(35.69)</td>
<td>(41.96)</td>
<td>(54.51)</td>
</tr>
<tr>
<td>10</td>
<td>496.67</td>
<td>513.33</td>
<td>556.67</td>
<td>586.67</td>
<td>596.67</td>
<td>573.33</td>
<td>503.33</td>
<td>516.67</td>
<td>576.67</td>
</tr>
</tbody>
</table>

**Magnesium ion concentration is expressed as μ moles/gm.wt.**

Each value is a mean of 20 samples.
**Total ATPase activity is expressed as μmol of Pi liberated/mg protein/hr.**

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent percent change over the control.

<table>
<thead>
<tr>
<th>Day of sacrifice</th>
<th>DURATION OF EXPOSURE</th>
<th>INTENSITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 sec</td>
<td>120 sec</td>
</tr>
<tr>
<td>Control</td>
<td>3.68a</td>
<td>3.68a</td>
</tr>
<tr>
<td>0</td>
<td>4.17b</td>
<td>4.40b</td>
</tr>
<tr>
<td></td>
<td>(13.32)</td>
<td>(19.57)</td>
</tr>
<tr>
<td>1</td>
<td>4.72c</td>
<td>4.99c</td>
</tr>
<tr>
<td></td>
<td>(28.26)</td>
<td>(35.60)</td>
</tr>
<tr>
<td>5</td>
<td>5.23d</td>
<td>5.72d</td>
</tr>
<tr>
<td></td>
<td>(42.12)</td>
<td>(55.43)</td>
</tr>
<tr>
<td>10</td>
<td>5.30d</td>
<td>6.07e</td>
</tr>
<tr>
<td></td>
<td>(44.02)</td>
<td>(64.95)</td>
</tr>
</tbody>
</table>

**TABLE : 33**

PANCREATIC TOTAL ATPase
PANCREATIC Na⁺-K⁺ ATPase

Hs + -K⁺ ATPase activity is expressed as μm of Pi liberated/mg protein/hr.

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent percent change over the control.

<table>
<thead>
<tr>
<th>Day of sacrifice</th>
<th>5W</th>
<th>10W</th>
<th>15W</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 sec</td>
<td>120 sec</td>
<td>180 sec</td>
</tr>
<tr>
<td>Control</td>
<td>1.13a</td>
<td>1.13a</td>
<td>1.13a</td>
</tr>
<tr>
<td>0</td>
<td>1.29b</td>
<td>1.34b</td>
<td>1.41b</td>
</tr>
<tr>
<td></td>
<td>(13.27)</td>
<td>(18.58)</td>
<td>(24.78)</td>
</tr>
<tr>
<td>1</td>
<td>1.41c</td>
<td>1.49c</td>
<td>1.57c</td>
</tr>
<tr>
<td></td>
<td>(24.78)</td>
<td>(31.86)</td>
<td>(38.94)</td>
</tr>
<tr>
<td>5</td>
<td>1.57d</td>
<td>1.64d</td>
<td>1.76d</td>
</tr>
<tr>
<td></td>
<td>(38.94)</td>
<td>(45.13)</td>
<td>(55.75)</td>
</tr>
<tr>
<td>10</td>
<td>1.64d</td>
<td>1.76e</td>
<td>1.81d</td>
</tr>
<tr>
<td></td>
<td>(45.13)</td>
<td>(55.75)</td>
<td>(60.18)</td>
</tr>
</tbody>
</table>

** Na⁺-K⁺ ATPase activity is expressed as μm of Pi liberated/mg protein/hr.**

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent percent change over the control.
** Ca++ ATPase activity is expressed as μmol of Pi liberated/mg protein/hr.

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.

<table>
<thead>
<tr>
<th>Day of sacrifice</th>
<th>5W</th>
<th>10W</th>
<th>15W</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.20a</td>
<td>1.20a</td>
<td>1.20a</td>
</tr>
<tr>
<td>60 sec</td>
<td>1.31b</td>
<td>1.39b</td>
<td>1.49b</td>
</tr>
<tr>
<td>120 sec</td>
<td>(9.17)</td>
<td>(15.03)</td>
<td>(24.17)</td>
</tr>
<tr>
<td>180 sec</td>
<td>1.34b</td>
<td>1.41b</td>
<td>1.62b</td>
</tr>
<tr>
<td>60 sec</td>
<td>(14.63)</td>
<td>(31.71)</td>
<td>(25.60)</td>
</tr>
<tr>
<td>120 sec</td>
<td>1.57b</td>
<td>1.64b</td>
<td>1.72b</td>
</tr>
<tr>
<td>180 sec</td>
<td>(31.20)</td>
<td>(37.60)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.41b</td>
<td>1.49b</td>
<td>1.59c</td>
</tr>
<tr>
<td>60 sec</td>
<td>1.49c</td>
<td>1.62c</td>
<td>1.71c</td>
</tr>
<tr>
<td>120 sec</td>
<td>(31.71)</td>
<td>(39.02)</td>
<td>(31.20)</td>
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<tr>
<td>180 sec</td>
<td>1.64c</td>
<td>1.74c</td>
<td>1.82c</td>
</tr>
<tr>
<td>60 sec</td>
<td>(42.40)</td>
<td>(45.60)</td>
<td></td>
</tr>
<tr>
<td>120 sec</td>
<td>(45.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180 sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.62c</td>
<td>1.76c</td>
<td>1.86d</td>
</tr>
<tr>
<td>60 sec</td>
<td>1.76d</td>
<td>1.82d</td>
<td>1.94d</td>
</tr>
<tr>
<td>120 sec</td>
<td>(43.09)</td>
<td>(47.97)</td>
<td>(57.72)</td>
</tr>
<tr>
<td>180 sec</td>
<td>1.94d</td>
<td>2.02d</td>
<td>2.12d</td>
</tr>
<tr>
<td>60 sec</td>
<td>(55.20)</td>
<td>(61.60)</td>
<td>(69.60)</td>
</tr>
<tr>
<td>120 sec</td>
<td>(69.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180 sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.76d</td>
<td>1.86c</td>
<td>1.96e</td>
</tr>
<tr>
<td>60 sec</td>
<td>1.82e</td>
<td>1.89d</td>
<td>1.99d</td>
</tr>
<tr>
<td>120 sec</td>
<td>(47.97)</td>
<td>(52.85)</td>
<td>(61.79)</td>
</tr>
<tr>
<td>180 sec</td>
<td>2.10e</td>
<td>2.18e</td>
<td>2.25e</td>
</tr>
<tr>
<td>60 sec</td>
<td>(58.00)</td>
<td>(74.40)</td>
<td>(80.00)</td>
</tr>
<tr>
<td>120 sec</td>
<td>(80.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180 sec</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE : 35

PANCREATIC Ca++ ATPase

** Ca++ ATPase activity is expressed as μmol of Pi liberated/mg protein/hr.

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.
### TABLE 36

**PANCREATIC Mg²⁺ ATPase**

<table>
<thead>
<tr>
<th></th>
<th>INTENSITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 W</td>
</tr>
<tr>
<td><strong>Day of</strong></td>
<td><strong>DURATION OF EXPOSURE</strong></td>
</tr>
<tr>
<td>of sacrifice</td>
<td>60 sec</td>
</tr>
<tr>
<td></td>
<td>60 sec</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>5 W</th>
<th>10 W</th>
<th>15 W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>1.00a</td>
<td>1.00a</td>
<td>1.00a</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>1.10b</td>
<td>1.18b</td>
<td>1.25b</td>
</tr>
<tr>
<td></td>
<td>(10.00)</td>
<td>(18.00)</td>
<td>(25.00)</td>
</tr>
<tr>
<td><strong>1</strong></td>
<td>1.18b</td>
<td>1.25b</td>
<td>1.32b</td>
</tr>
<tr>
<td></td>
<td>(18.00)</td>
<td>(25.00)</td>
<td>(32.00)</td>
</tr>
<tr>
<td><strong>5</strong></td>
<td>1.34c</td>
<td>1.41c</td>
<td>1.49c</td>
</tr>
<tr>
<td></td>
<td>(34.00)</td>
<td>(41.00)</td>
<td>(49.00)</td>
</tr>
<tr>
<td><strong>10</strong></td>
<td>1.47d</td>
<td>1.57d</td>
<td>1.62d</td>
</tr>
<tr>
<td></td>
<td>(47.00)</td>
<td>(57.00)</td>
<td>(62.00)</td>
</tr>
</tbody>
</table>

**Mg²⁺ ATPase activity is expressed as μm of Pi liberated/mg protein/hr.**

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.
** Sodium ion concentration is expressed as μ moles/gm. wt.

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.
### PANCREATIC POTASSIUM ION CONCENTRATION

Potassium ion concentration is expressed as μ moles/gm wt.

Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent percent change over the control.

<table>
<thead>
<tr>
<th>Day of sacrifice</th>
<th>5 W (60 sec)</th>
<th>10 W (60 sec)</th>
<th>15 W (60 sec)</th>
<th>5 W (120 sec)</th>
<th>10 W (120 sec)</th>
<th>15 W (120 sec)</th>
<th>5 W (180 sec)</th>
<th>10 W (180 sec)</th>
<th>15 W (180 sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>178.67a</td>
<td>178.67a</td>
<td>178.67a</td>
<td>182.00a</td>
<td>182.00a</td>
<td>182.00a</td>
<td>176.67c</td>
<td>176.67c</td>
<td>176.67c</td>
</tr>
<tr>
<td>0</td>
<td>257.67c</td>
<td>273.33c</td>
<td>305.00c</td>
<td>265.33c</td>
<td>270.57c</td>
<td>296.67c</td>
<td>135.00a</td>
<td>128.33a</td>
<td>116.67a</td>
</tr>
<tr>
<td></td>
<td>(49.81)</td>
<td>(52.98)</td>
<td>(70.71)</td>
<td>(45.72)</td>
<td>(48.72)</td>
<td>(63.01)</td>
<td>(-23.59)</td>
<td>(-27.36)</td>
<td>(-33.96)</td>
</tr>
<tr>
<td>1</td>
<td>339.33e</td>
<td>331.67e</td>
<td>361.67d</td>
<td>313.33e</td>
<td>330.00e</td>
<td>366.67e</td>
<td>151.67b</td>
<td>163.33b</td>
<td>196.67c</td>
</tr>
<tr>
<td></td>
<td>(73.13)</td>
<td>(85.63)</td>
<td>(102.42)</td>
<td>(72.16)</td>
<td>(81.32)</td>
<td>(101.47)</td>
<td>(-14.15)</td>
<td>(-7.55)</td>
<td>(11.32)</td>
</tr>
<tr>
<td>5</td>
<td>281.67d</td>
<td>293.33d</td>
<td>306.67c</td>
<td>291.67d</td>
<td>298.33d</td>
<td>331.33d</td>
<td>184.00c</td>
<td>203.33d</td>
<td>256.67d</td>
</tr>
<tr>
<td></td>
<td>(57.66)</td>
<td>(64.17)</td>
<td>(71.64)</td>
<td>(60.26)</td>
<td>(63.92)</td>
<td>(82.05)</td>
<td>(4.15)</td>
<td>(15.09)</td>
<td>(45.28)</td>
</tr>
<tr>
<td>10</td>
<td>216.67b</td>
<td>203.33b</td>
<td>193.33b</td>
<td>236.67b</td>
<td>246.67b</td>
<td>255.67b</td>
<td>196.67d</td>
<td>233.33e</td>
<td>292.67e</td>
</tr>
<tr>
<td></td>
<td>(24.27)</td>
<td>(13.80)</td>
<td>(8.21)</td>
<td>(30.04)</td>
<td>(35.53)</td>
<td>(41.03)</td>
<td>(11.32)</td>
<td>(32.07)</td>
<td>(65.66)</td>
</tr>
</tbody>
</table>

** Potassium ion concentration is expressed as μ moles/gm wt.

** Each value is a mean of 20 samples.

** Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

** Values in parentheses represent percent change over the control.
** Calcium ion concentration is expressed as μ moles/gm. wt. 
Each value is a mean of 20 samples.

Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test.

Values in parentheses represent per cent change over the control.
** Magnesium ion concentration is expressed as μ moles/gm. wt. Each value is a mean of 20 samples. Means within a column followed by the same letter are not significantly different (p < 0.05) from each other according to DMR test. Values in parentheses represent per cent change over the control.
FIG. 73
Total ATPase-60s - Liver

Day of Sacrifice

FIG. 74
Total ATPase-120s - Liver

Day of Sacrifice
FIG. 75

Total ATPase-180s-
Liver

Day of Sacrifice

Percent Increase

0 1 5 10

5W 10W 15W
FIG. 76

5w-60 Seconds-Liver

FIG. 77

5w-120 Seconds-Liver
FIG. 78

5w-180s-Liver

Day of Sacrifice

Percent change

[Graph showing % change of Na, K, and Na-K ATPase over days of sacrifice]
FIG. 79

10w-60 Seconds - Liver

![Graph showing percent change over days of sacrifice for Na⁺, K⁺, and Na⁻ - K⁺ ATPase](image)

FIG. 80

10w-120 Seconds - Liver

![Graph showing percent change over days of sacrifice for Na⁺, K⁺, and Na⁻ - K⁺ ATPase](image)
FIG. 81.

10w-180s-Liver

Day of Sacrifice

Percent change

Na⁺ conc  K⁺ conc  Na⁺ - K⁺ AT pase
FIG. 82

15w-60 Seconds-Liver

![Graph showing percent change in Na⁺, K⁺, and Na⁺ - K ATPase activities over Day of Sacrifice.]

FIG. 83

15w-120 Seconds-Liver

![Graph showing percent change in Na⁺, K⁺, and Na⁺ - K ATPase activities over Day of Sacrifice.]
FIG. 84

15w-180s-Liver

Percent change

Day of Sacrifice

Na⁺ conc  K⁺ conc  Na⁺ - K⁺ AT pase
FIG. 85

5w-60Seconds-Liver

Day of Sacrifice

Ca^2+ conc       Ca^2+ ATPase

FIG. 86

5w-120Seconds-Liver

Day of Sacrifice

Ca^2+ conc       Ca^2+ ATPase
FIG. 87

5w-180s-Liver

Day of Sacrifice

Percent Increase

$\text{Ca}^{2+}$ conc $\text{Ca}^{2+}$ ATPase
FIG. 88
10w-60Seconds-Liver

![Graph showing percent increase in liver function over days of sacrifice for Ca$^{2+}$ concentration and Ca$^{2+}$ ATPase activity.]

FIG. 89
10w-120Seconds-Liver

![Graph showing percent increase in liver function over days of sacrifice for Ca$^{2+}$ concentration and Ca$^{2+}$ ATPase activity.]

Day of Sacrifice:
- 0
- 1
- 5
- 10

Percent Increase:
- Ca$^{2+}$ conc
- Ca$^{2+}$ ATPase
FIG. 90

10w-180s-Liver

Day of Sacrifice

Percent Increase

0 1 5 10

Day 0 1 5 10

Ca$^{2+}$ conc Ca$^{2+}$ ATPase
FIG. 91

15w-60Seconds-Liver

Day of Sacrifice

Ca⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺⁺阳性
FIG. 93

15w-180s-Liver

Day of Sacrifice

Percent Increase

0 20 40 60 80 100

0 1 5 10

Ca\textsuperscript{2+} conc  Ca\textsuperscript{2+} ATPase
FIG. 94

5w-60Seconds-Liver

FIG. 95

5w-120Seconds-Liver
FIG. 96

5w-180s-Liver

Day of Sacrifice

Percent Increase

Mg$^{2+}$ Conc

Mg$^{2+}$ ATPase
**FIG. 97**

10w-60Seconds-Liver

**FIG. 98**

10w-120Seconds-Liver
FIG. 99

10w-180s-Liver

Day of Sacrifice

Percent Increase

Mg\textsuperscript{2+} Conc

Mg\textsuperscript{2+} ATPase
15w-180s-Liver

Day of Sacrifice

Percent Increase

Mg\(^{2+}\) Conc
Mg\(^{2+}\) ATPase

FIG. 102
FIG. 103

Total ATPase-60s-
Pancreas

Day of Sacrifice

0 1 5 10

FIG. 104

Total ATPase-120s-
Pancreas

Day of Sacrifice

0 1 5 10

5W 10W 15W

5W 10W 15W
Total ATPase-180s-
Pancreas

Day of Sacrifice
0 1 5 10

Percent Increase
0 20 40 60 80 100 120

5W 10W 15W
FIG. 106

5w-60s-Pancreas

FIG. 107

5w-120s-Pancreas
5w-180s-Pancreas

![Graph showing the change in Na⁺, K⁺, and Na⁺-K⁺ ATPase over 10 days of sacrifice.](image-url)
FIG. 109

10w-60s-Pancreas

FIG. 110

10w-120s-Pancreas
FIG. 115

5w-60s-Pancreas

FIG. 116

5w-120s-Pancreas
FIG. 117

5w-180s-Pancreas

Day of Sacrifice

Ca^{2+} conc Ca^{2+} ATPase

Percent Increase

0  20  40  60  80  100  120  140
0  1  5  10

Day of Sacrifice
FIG. 118

10w-60s-Pancreas

Day of Sacrifice

FIG. 119

10w-120s-Pancreas

Day of Sacrifice
FIG. 120

10w-180s-Pancreas

Day of Sacrifice

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<td>60</td>
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FIG. 123

15w-180s-Pancreas

Day of Sacrifice

Percent Change

Ca$^{2+}$ conc

Ca$^{2+}$ ATPase
**FIG. 124**

5w-60s-Pancreas

![Bar graph showing the percentage increase of Mg^2+ Concentration and Mg^2+ ATPase activity over different days of sacrifice.](image)

**FIG. 125**

5w-120s-Pancreas

![Bar graph showing the percentage increase of Mg^2+ Concentration and Mg^2+ ATPase activity over different days of sacrifice.](image)
FIG. 126

5w-180s-Pancreas

Day of Sacrifice

Mg\(^{2+}\) Conc  Mg\(^{2+}\) ATPase

Percent Increase

0 20 40 60 80 100 120

0 1 5 10
FIG. 127

10w-60s-Pancreas

Day of Sacrifice

0 Mg^2+ Conc  Mg^2+ ATPase

FIG. 128

10w-120s-Pancreas

Day of Sacrifice

0 Mg^2+ Conc  Mg^2+ ATPase
FIG. 129

10w-180s-Pancreas

Day of Sacrifice

Percent Increase

0 20 40 60 80 100 120 140

Day of Sacrifice

Mg\(^{2+}\) Conc  Mg\(^{2+}\) ATPase
FIG. 132

15w-180s-Pancreas

Day of Sacrifice

Mg$^{2+}$ Concentration

Mg$^{2+}$ ATPase Activity
In 15W, day zero and day one samples showed a decrease in ion concentration with an exception on day one sample of 180 seconds. Marginal increase is seen on other days.

Calcium ion Concentration

Calcium ion concentration is increased and is shown in figure (117) and table (39). Maximum elevation is observed on day 5 of both 5W and 10W. (125.12% and 136%).

In 15W a significant reduction is observed on day zero and day one. However, day 10 of both 60 seconds and 120 seconds showed an increase. Day 5 and day 10 of 180 seconds recorded a drastic decrease.

Magnesium ion Concentration

A similar pattern is observed in magnesium ion concentration. Maximum enhancement is on day 5 (132%) of 180 seconds in 10W. The concentration is found decreased in all the days except day 10 in 15W.

DISCUSSION

\( \text{Na}^+, \text{K}^+; \text{Na}^+\text{-K}^+ \text{ATPase} \)

The internal environment is rich in \( K^+ \) and \( Mg^{2+} \) and the extracellular fluid is characterized by high \( Na^+ \) and \( Ca^{2+} \) content. In general, cells maintain a low intracellular \( Na^+ \) concentration and a high intracellular \( K^+ \) concentration; along
with a net negative electrical potential inside. The pump that maintains these gradients is an ATPase that is activated by \( \text{Na}^+ \) and \( \text{K}^+ \). Adenosine triphosphatases (ATPases) are integral membrane proteins and vital for regulating oxidative phosphorylation, ionic transport, muscle function and several other transport-dependent phenomena. ATPases require phospholipids for activity. \( \text{Na}^+\text{K}^+\text{ATPase} \) is a biochemical expression of active transport of \( \text{Na}^+ \) and \( \text{K}^+ \) in the cells (Skurn, 1975). It is an energy dependent enzyme, which maintain ionic gradients crucial to metabolite transport and osmotic gradients required for the maintenance of cell volume. Transport of \( \text{Na}^+ \) and \( \text{K}^+ \) is vital to a number of cellular processes such as the maintenance of electrochemical gradients across the cell membranes.

Ultrasound has been shown to increase membrane permeability (Coble and Dunn, 1976) and is associated with numerous structural and functional alterations (William, 1983).

A significant amount of ions and molecules are transported across the membranes of the cells involved in the repair process. Lehmann and Biegler (1954) concluded that the irreversible decrease in membrane potential, which they observed upon applying ultrasound to frog skin, was due to temperature increase.

When the abdominal skin of Rana pipiens was exposed to ultrasound, significant changes were observed in
transepithelial electrical potential, ionic conductance and the net current of the actively transported ions even at relatively modest levels of acoustic intensity (on the order of 100mW/cm²). Acoustic cavitation is playing a major role in the process (Dinno et al., 1989).

The release of K⁺ shortens on exposure to ultrasound (Selivanov et al., 1981). Lenart and Auslander (1980) obtained increases in the diffusion of sodium, calcium and potassium using 1MHz ultrasound in the intensity range 1.2-6.0W/cm². Lehmann (1955) demonstrated an increase in sodium transport in isolated frog skin with therapeutic levels of ultrasound by measuring changes in membrane potential caused by the ultrasound. The present study shows a significant difference in sodium ion concentration, specially on day zero, since it was decreased. However, the concentration reached normalcy and infact increased by day 5, in 10W exposures. In 15W exposure all the days showed an increase in sodium concentration. The potassium ion pool was found increased in the present study, which is in agreement with the findings of earlier workers. Coble and Dunn (1976) studied the sodium linked short circuit current and measured an increase which they could be related to the acoustic pressure on the membrane. Mortimer et al., (1984) studied membrane potentials in isolated papillary muscles and found that there was an increase in transport of both sodium and calcium into the cell for the duration of action potential during irradiation with therapeutic ultrasound. The increase in the ion pools
in the present study reveals that the pressure on membrane was exerted by ultrasound and hence, the membrane potential charged resulting in the increased transportation across in which both sodium and potassium passed into the cell which is in concurrence with the earlier results. However, Mortimer et al., (1984) observed the variation during period of irradiation, but in the present study, a similar situation exists post-irradiation also. It is known that many enzymes catalyzing the most varied conversions in the living body are activated by ions – Na and K ions raise ATPase activity. With rise in temperature, ATPase activity and superprecipitation rise (Pavliashvili, et al., 1982). Study of the dependence of ATPase activity on ionic strength for different cations showed that with rise in ionic strength ATPase activity rises, reaches on optimum and then falls. If the coupling of the Na and K ionic fluxes is shown, then \( \text{Na}^+, \text{K}^+ \text{ATPase} \) must be brought by \( \text{Na}^+ - \text{ATPase} \) (Marykyan et al., 1983). The \( \text{Na}^+, \text{K}^+ \text{ATPase} \) activity was increased in the present study, concurrent to the enhancement of ionic strength. As the active transport is energy dependent, the increase in activities of ATPases may be due to the increased production of ATP. Further, the hormones released in the liver during exposure to ultrasound also may influence the activities of ATPases leading to disturbance in ionic transport of cellular membranes. Further, these results are not dissimilar to the results of earlier workers.
The role of the cations, Na\(^+\) and K\(^+\) in regulating protein synthesis and cell growth is a complex subject that is not fully understood; important aspects of their transport and intracellular concentration include the permeability of the plasma membrane, the activity of the Na\(^+\)-K\(^+\) pump, and the viscosity of the membrane. In the present study, total protein content was decreased on all the days of all wattages. Several reports have addressed effects of ultrasound exposure on Na\(^+\)-K\(^+\)-ATPase mediated K\(^+\) transport; depletion of K ions in human erythrocytes in the absence of haemolysis (Lota and Darling, 1955); depression of K influx and enhancement of passive K efflux in rat thymocytes (Chapman, 1974; Tucker, 1976; Chapman et al., 1980); decrease in Na\(^+\)-K\(^+\)-activity in human erythrocytes (Pinamonti et al., 1982). The present results are partly similar to the studies of the above workers, since both the ions were found, in general, increased.

Ca\(^{2+}\) and Ca\(^{2+}\) -ATPase

The importance of divalent cations, especially Ca\(^{2+}\) in biological systems is well known, as many biological processes involve Ca\(^{2+}\) interaction with cell membranes (Muhleisen et al., 1983). Such Ca\(^{2+}\) interactions may involve Ca\(^{2+}\)- induced changes in the permeability of the membrane. Ca\(^{2+}\) has also been shown to play a dominant role in stimulus-secretion coupling in a wide variety of neural and endocrine cells (Pancreas is much less dependent on extracellular Ca\(^{2+}\)) and short term modulation
of intracellular free Ca$^{2+}$ concentration is largely determined by the activity of the intracellular organelles.

Mortimer and Dyson (1988) reported the initial increase in (Ca$^{2+}$) is the result of Ca$^{2+}$ mobilization from some intracellular compartment and does not reflect the influx of extracellular Ca$^{2+}$. Exposure of cultured fibroblasts to sonication intensities of 0.5-1.0W/cm$^2$ for 5 min. has been shown to augment intracellular calcium, a well known mediator of numerous cellular processes including protein synthesis. In the present study, the Ca$^{2+}$ was seen significantly increased even though the intensity was much higher to that of used by Mortimer and Dyson (1988).

In the cell, mitochondria may serve as the controller and the regulator of cytoplasmic calcium. Calcium influx, which is presumed to be passive, may be increased since a rise in cytoplasmic calcium has been shown to increase the membrane permeability to ions and other uncharged solutes (Lew, 1970; Romero and Whittam, 1971; Baker, 1972). Even in the present study, there is an indirect evidence for the increased permeability of the cell membrane. The variations in glycogen, glucose contents, in the Na, K ion pools and in the Ca ions emphasize the increased permeability of the cell membrane as a result of ultrasound irradiation.

Mg$^{2+}$ ATPase is an enzyme located in mitochondria, and is involved in not only the lysis of ATP but also has a significant role in the initiation of ATP synthesis.
(Lehninger, 1979). This enzyme is found in association with both Na\(^+\)-K\(^+\) and Na\(^+\)-NH\(_4\) ATPases. This enzyme is also essential to the integrity of the cellular membranes and to the intra-cellular cements.

The increase in Mg\(^{2+}\)-ATPase in all the 3 wattages indicate the triggered synthetic activities.