The Effects of Electroshock on the Acid-soluble Phosphates of Rat Brain

FREDERICK N. MINARD AND RAYMOND V. DAVIS*

From the Department of Biochemistry, Research Division, Abbott Laboratories, North Chicago, Illinois

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Although electroshock is an established therapy for mental depression and schizophrenia, its effects on the biochemistry of the central nervous system are comparatively unknown. In the present paper we report the changes observed in the acid-soluble phosphates of the whole brain when rats are subjected to convulsive seizures by electroshock.

Previous investigations have been made on the effect of electroshock on nucleotides and related metabolites of the brain, and with a few exceptions a lowering of adenosine triphosphate and phosphocreatine levels has been consistently observed (e.g. 1–3). In the present investigation the work of Dawson and Richter (1), which showed the sequence of changes in these phosphates following electroshock, was extended to include many other metabolites.

An improved method for the extraction of energy-rich phosphates from brain is described in this paper. Because the many reports in the literature do not agree on the levels of nucleotides in normal control brains, we felt that this variation may be due to the different methods used for preparation of the brain for analysis. On treatment of brain tissue according to the method described here, levels of adenosine nucleotides and phosphocreatine were obtained which are probably nearer to actual levels in vivo than those usually obtained by extraction at 0° with aqueous perchloric acid. Furthermore, the level of adenosine monophosphate is indicative of metabolic stress, since it varied from almost zero in brains of control animals to appreciable levels in those of animals subjected to convulsive seizures and other stresses. A preliminary report of this work has been presented (4).

EXPERIMENTAL PROCEDURE

Preparation of Acid-soluble Extracts—To avoid the degradation of brain metabolites that was found to occur so readily at temperatures around 0°, details of the following procedure were consistently followed: Sprague-Dawley male rats, weighing 200 to 350 g, were used in all the experiments. Their brains were frozen by immersing the animals headfirst in liquid nitrogen for at least 5 minutes. The entire brain, with occasional reimmersion of the head in liquid nitrogen, was removed with a chisel and forceps precooled in Dry-Ice. As pieces of brain were obtained, they were placed in a tared test tube which was kept in a mixture of Dry-Ice and acetone. Finally, the weight of the brain was determined by difference. The tissue was next pulverized in a tall, stainless steel mortar and pestle with a few milliliters of a 10% solution of trichloroacetic acid in acetone to prevent splattering of the tissue, and the resulting slurry was transferred to a glass-Teflon homogenerizer with 10 to 15 ml more of the trichloroacetic acid in acetone. All procedures, including the homogenization step, were carried out in vessels immersed in a Dry-Ice and acetone mixture.

After homogenization the tissue was transferred to an evaporating dish resting in crushed ice, and 10 to 15 ml of a cold aqueous 10% solution of trichloroacetic acid were added. The acetone was evaporated by a fine stream of nitrogen, and after centrifugation the acid was removed from the supernatant by four successive extractions, each with 1 volume of ether. The residual ether was evaporated from the extract by a fine stream of nitrogen, and the resulting solution of nucleotides was neutralized to pH 6.3 to 6.9 with a small amount of 1N potassium hydroxide, before chromatography on a column of Dowex 1-formate. All procedures after the homogenization were performed at 0–5°.

Separation and Analysis of Phosphates—Separation of the nucleotides on a column of Dowex 1 × 10-formate, (200 to 400 mesh, 0.9-cm diameter × 13-cm length) was based on the method of Hurlbert, Schmitz, Brumm, and Potter (5), and the resin was prepared as they described. However, the elution systems they utilized were reduced to two, namely, (a) 400 ml of water in the mixing flask and 450 ml of 3.75 N formic acid in the reservoir, and (b) 1.0 liter of 2.00 N formic acid in the mixing flask and, in the reservoir, 800 ml of a solution containing 0.80 N ammonium formate in 4.0 N formic acid. The size of the eluted samples was maintained at 60 drops by a photodetection drop counter, and their absorbancy at 260 μ was determined with a spectrophotometer. Since the volume of each drop from the column varied with the solvent gradient, calibrated centrifuge tubes were used as every 10th collecting tube, and thus the volume of any fraction could be determined by plotting the volume of every 10th fraction against the fraction number. From this plot the total absorbancy units (AU) for any particular nucleotide peak was readily calculated by a summation of the products of each tube’s volume in milliliters and its absorbancy at 260 μ.

Phosphate was determined by Bartlett’s micromodification (6) of the Fiske-SubbaRow method (6a). Total creatine was determined in the acid-soluble extracts, after hydrolysis of the phosphocreatine, according to the method of Ennor and Rosenberg (7): p-chloromercuribenzoate was included in each assay mixture as recommended by the authors. In addition, a known amount of creatine was added to another portion of each extract.

* Present address: Department of Biochemistry, Medical School, University of Missouri, Columbia, Missouri.
TABLE I

Comparison of extraction procedures using brains of normal control rats

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Acetone-trichloroacetic acid at $-78^\circ$</th>
<th>Perchloric acid at $0^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$moles/g brain, fresh weight</td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>$0.07 \pm 0.02 (4)^*$</td>
<td>$0.49 \pm 0.01 (2)^*$</td>
</tr>
<tr>
<td>ADP</td>
<td>$0.24 \pm 0.02 (4)$</td>
<td>$0.88 \pm 0.08 (2)$</td>
</tr>
<tr>
<td>ATP</td>
<td>$2.06 \pm 0.04 (4)$</td>
<td>$1.14 \pm 0.04 (2)$</td>
</tr>
<tr>
<td>ATP AMP ratio</td>
<td>$45 \pm 12 (4)$</td>
<td>$2.4 \pm 0.0 (2)$</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>$3.48 \pm 0.13 (4)$</td>
<td>$2.01 \pm 0.0 (2)$</td>
</tr>
<tr>
<td>DPN</td>
<td>$0.32 \pm 0.0042 (58)^*$</td>
<td>$0.25 \pm 0.01 (2)$</td>
</tr>
<tr>
<td>$\Sigma (\text{AMP} + \text{ADP} + \text{ATP})$</td>
<td>$2.34 \pm 0.014 (58)^*$</td>
<td>$2.30 \pm 0.01 (2)$</td>
</tr>
<tr>
<td>Total $AU_{260}$</td>
<td>$49.8 \pm 0.27 (45)^*$</td>
<td>$46.4 \pm 0.6 (2)$</td>
</tr>
</tbody>
</table>

* Number of brains is given in parentheses.
† Experimental and control brains.

RESULTS

Extraction, Separation, and Identification of Phosphates—For our purpose, the preferred extraction method was considered to be the one giving the highest ratio of ATP-AMP, since the results in later sections show that it is a more sensitive indicator of ATP degradation and utilization than the more commonly used ratio of ATP-ADP. Larger values for ATP-AMP were obtained by an initial extraction of brain with trichloroacetic acid in acetone at $-78^\circ$ than by extraction at $0^\circ$ with aqueous perchloric acid, according to the method of Hurlbert et al. (5). A comparison of the two extraction methods is given in Table I, where it may be seen that although higher levels of ATP and phosphocreatine were obtained with the use of acetone-trichloroacetic acid, the amounts of DPN, total adenine mononucleotides, and also the summation of absorbancy units from the columns were approximately the same. Of special interest in Table I is the remarkable constancy of the three latter values

![Figure 1](http://www.jbc.org/)  

FIG. 1. Separation of the acid-soluble phosphates of a normal rat brain upon Dowex I-formate. The acid-soluble fraction was obtained by the method using acetone-trichloroacetic acid at $-78^\circ$. PC, phosphocreatine.

and the per cent recovery of the added creatine was used as a correction factor in the final calculation.

Electroshock seizures were induced by stimulation with 60-cycle alternating current through silver-tipped stainless steel electrodes applied to the crown of the rat; EKG Sol1 was used to insure a reproducible contact with the corneas. The duration of each shock was 1 second, and the voltage, milliamperes, and exact time to immersion in liquid nitrogen were accurately determined. Values are generally expressed as a mean $\pm$ S.E.M. (the standard error of the mean). Each point in the figures corresponds to a single animal.

1 Obtained from Burton, Parsons and Company, Washington, D.C.
however, more discrete solvent changes. The elution positions of the adenine and guanine nucleotides, UTP, DPN, P_i, PP_i, and phosphocreatine from the standard column of Dowex 1-formate were confirmed by chromatography of known samples. Dowex was further identified by formation of the cyanide derivative (8). The identity of the nucleic acid bases in the peaks derived from brain extracts was also determined by paper chromatography subsequent to hydrolysis in 1 N hydrochloric acid. To characterize further each adenine nucleotide, peaks of AMP, ADP, and ATP were pooled from three to six brains and freed of formic acid by evaporation to dryness in a vacuum, and the resulting residues were hydrolyzed in 1 N hydrochloric acid for 1 hour at 100°. Before evaporation, ammonium ion was removed from the ATP peak by passage over a column of Dowex 50-H+ (6). Each hydrolysate was chromatographed on a column of Dowex 1-chloride by elution with a gradient of 2-amino-2-methyl-1,3-propanediol and hydrochloric acid; this system readily separates adenine, guanine, CMP, UMP, and TMP. The results are given in Table II, where adenine is seen to be the predominant base recovered from the peaks of the adenine mononucleotides. Although the recovery of absorbancy units from the ATP peak was low, a similar recovery was obtained when a known sample of ATP was dissolved in formic acid-ammonium formate and analyzed according to the same procedure. In addition, nearly theoretical values for micromoles of P_i per micromole of nucleotide were obtained for the peaks of AMP, ATP, and DPN from numerous individual brains (Table II). The relatively high value of 2.3 for this ratio from the ADP peak is probably due to the presence of sugar phosphates, which are known to be eluted with ADP (9). Although phosphocreatine was routinely determined by phosphate analysis, its presence was confirmed by creatine analysis, and in the one peak examined an almost theoretical 1:1 molar ratio of creatine to P_i was found.

To correct for any acid-catalyzed hydrolysis of phosphates during isolation, simulated tissue extractions were performed at -78° with known quantities of AMP, ATP, and phosphocreatine, and the resulting solutions were chromatographed upon the standard column of Dowex 1-formate. Recovery of these phosphates from duplicate experiments was as follows: AMP, 90 and 92%; ATP, 88 and 89%; phosphocreatine, 58 and 60%. Since ATP was hydrolyzed only slightly during its isolation, as evidenced by negligible amounts of AMP and ADP appearing during chromatography (the total was less than 4% of the ATP), the low recovery values were considered to be due either to experimental error or to an impure sample, and therefore corrections of the actual data were not made. Corrections were not made in the recovery of AMP either. However, each column-derived value of phosphocreatine was multiplied by the factor 1.059 to give the corrected values reported in this paper.

Variation of Adenine Nucleotide and Phosphocreatine Levels after Maximal Electroshock Seizure—To investigate the period after a seizure, rats were given a standard electroshock of 95 ma for 1 second and then immersed in liquid nitrogen at varying times from the start of the shock. This stimulus was always sufficient to elicit a maximal electroshock seizure (10) which consisted of a tonic flexion, followed by a tonic extension; the extensor phase began at approximately 3.8 seconds after the start of the electroshock and continued for approximately 7.0 seconds. When the brains were analyzed according to the method given under "Experimental Procedure," the data in Fig. 2 were obtained. The changes in phosphocreatine are a confirmation of the earlier experiments of Dawson and Richter (1). Of particular interest in this figure are the inverse changes of AMP and ATP and also the relative constancy of ADP; GMP and GTP-UTP, which are not included in the figure, varied directly with the corresponding adenine nucleotides. All of these metabolites returned to nearly control levels by 60 to 75 seconds after the electroshock. DPN, as noted previously (Table I), remained almost constant in these and each of the other experiments. Analysis of the peak of inorganic phosphate showed that its change paralleled that of AMP in that it increased after electroshock and then decreased to normal. The levels of P_i were more variable than those of the organic phosphates and this was due, perhaps, to chips of bone inadvertently included during removal of the frozen brain. A known sample of pyrophosphate was shown to be eluted between the peaks of ATP and GTP-UTP, but its presence could not be detected after chromatography of either a control brain or a brain taken 10 seconds after an electroshock.

Variation of Adenine Nucleotides and Phosphocreatine Levels with Amperage—In Fig. 3 are shown the changes in these phosphates which occurred after rats were given a 1-second electroshock at different amperages and then immersed in liquid nitrogen 10 seconds after the start of the shock. A 10-second period was chosen because of the maximal effects occurring at this time in a maximal electroshock seizure elicited by a stimulus of 95 ma (Fig. 2).

A progression of seizures was observed as the intensity of the electrostimulus was increased, varying from a running clonic at 8 to 15 ma to a tonic flexor at intermediate amperages and finally to a full tonic extensor seizure at 95 ma. Only the adenine nucleotides and phosphocreatine are shown in Fig. 3, but the other nucleotides changed according to the pattern of the previous experiments. It may be seen that very unusual peaks and valleys in the levels of these metabolites are associated with running clonic seizures in the 8- to 15-ma region. The varia-

Table II

<table>
<thead>
<tr>
<th>Nucleotide peak</th>
<th>Base content of peak*</th>
<th>%</th>
<th>%</th>
<th>Pumoles P_i/µmole nucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>99</td>
<td>1</td>
<td>98</td>
<td>0.9 ± 0.2 (3)†</td>
</tr>
<tr>
<td>ADP</td>
<td>97</td>
<td>3</td>
<td>88</td>
<td>2.3 ± 0.2 (3)</td>
</tr>
<tr>
<td>ATP</td>
<td>96</td>
<td>4</td>
<td>85</td>
<td>3.2 ± 0.2 (2)</td>
</tr>
<tr>
<td>DPN</td>
<td></td>
<td></td>
<td></td>
<td>2.0 ± 0.1 (5)</td>
</tr>
</tbody>
</table>

Table II

Purity of nucleotide peaks obtained by Dowex 1-formate chromatography of acid-soluble fraction of brain

* Peaks combined from three to six brains were hydrolyzed in 1 N hydrochloric acid and chromatographed upon a column of Dowex 1-chloride with a gradient of 2-amino-2-methyl-1,3-propanediol and hydrochloric acid.

† Number of individual peaks used to obtain mean of each ratio is given in parentheses.

1 F. N. Minard and R. V. Davis, unpublished material.
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ADENOSINE TRIPHOSPHATE

0.0; 20 I 40 I 60 I 80 I 100 I 200 I 300

SECONDS AFTER ELECTROSHOCK

FIG. 2. Levels of AMP, ADP, ATP, and phosphocreatine in brain after a maximal electroshock seizure. The 1-second stimulus of 95 ma began at zero time. Animals were immersed in liquid nitrogen at times shown.

ELECTROSHOCK—mAMPERES

ADENOSINE TRIPHOSPHATE

0.0; 20 I 40 I 60 I 80 I 100 I 200 I 300

FIG. 3. Variation of AMP, ADP, ATP, and phosphocreatine levels in brain with the intensity of electroshock. Each shock was 1 second in duration and each animal was immersed in liquid nitrogen 10 seconds after the start of the shock.

Effects of Anoxic Atmosphere on Levels of Adenine Nucleotides and Phosphocreatine—Because anoxia is a concomitant of electroshock seizures (11), its relationship to the biochemical changes must be considered. In an attempt to answer this question, rats were placed individually in a 29-liter vented plastic chamber through which a mixture of 95% nitrogen and 5% carbon dioxide was rapidly passing. Each of the animals tested retained its balance and none of them went into a seizure. After 10 seconds in the chamber, two of the animals were plunged into a reservoir of liquid nitrogen inside the chamber; the third rat was removed from the chamber at 10 seconds and was rapidly immersed in an immediately adjacent container of liquid nitrogen. Analysis of the brains gave the following results in micromoles per gram of brain: AMP, 0.21 ± 0.04; ADP, 0.38 ± 0.05; ATP, 1.81 ± 0.02; phosphocreatine, 1.66 ± 0.17. Comparison of these values with those in Table I for control animals shows the rapid degradation of labile phosphates due to an anoxic atmosphere. Greater deviations from normal control values were observed in an animal subjected to 25 seconds of anoxia. Hyperventilation caused by 5% carbon dioxide in the gaseous mixture probably increased the rate of these changes. The above results with anoxia are similar to those observed by Samson, Balfour, and Dahl (12).

Variation of Creatine Kinase Reaction with Level of AMP—The enzyme creatine kinase, which catalyzes Reaction 1, has been shown to occur in brain (13). A function, $K'_{ek}$ (Equation 2), can be expressed

$$\text{ATP} + \text{C} \rightleftharpoons \text{ADP} + \text{PC}$$  \hspace{1cm} (1)

$$K'_{ek} = \frac{(\text{ADP})(\text{PC})}{(\text{ATP})(\text{C})}$$  \hspace{1cm} (2)
(in micromoles per gram of brain, fresh weight) for this reaction, and a determination of its value would reveal how readily a condition of equilibrium is maintained for the reaction in brains subjected to stress. At equilibrium conditions, \( K'_{CK} \) becomes identical to the thermodynamic equilibrium constant.

Values for ADP, ATP, and phosphocreatine were obtained by column chromatography of the brain extracts. Total creatine was determined in the original brain extracts and was found to have a mean of 9.49 ± 0.12 μmoles per g, fresh weight, in 13 brains selected at random from the control and test groups. Since total creatine was thus reasonably uniform among these 13 brains, the mean value, 9.49 μmoles per g, was used in the calculation of \( K'_{CK} \) for a total of 54 brains. The free creatine in each brain was calculated by subtraction of the number of micromoles of phosphocreatine from the number of micromoles (9.49) of total creatine. Fig. 4 shows that when the resulting values of \( K'_{CK} \) are plotted against the corresponding values of AMP, \( K'_{CK} \) is found to decrease with increasing levels of AMP. The line in Fig. 4 was determined by the method of least squares.

The value enclosed by a square is from an animal which was frozen immediately after becoming unconscious during a swimming stress, and the encircled points correspond to animals used for experiments concerning the effects of depressant drugs upon the brain (14, 15); the heads of these animals were frozen 15 or 30 seconds after being severed from the body. The other values were obtained from animals convulsed by electroshock and Metrazol, or treated with anoxia and depressant drugs. Because the values of \( K'_{CK} \) obtained from the 43 brains of intact animals are largely separate from those animals whose heads were severed before freezing, or which were unconscious, they were used to calculate a mean value of 0.052 ± 0.0018 for \( K'_{CK} \). This should be considered, however, only an approximation to the value of normal \( K'_{CK} \) in normal rat brain because of both the obvious scattering of the data and the tendency for \( K'_{CK} \) to decrease with increasing metabolic stress upon the brain.

**Variation of Adenylate Kinase Reaction with Level of AMP—**

Another important reaction that is catalyzed by a single enzyme can be examined with the data reported in this paper. Adenylate kinase is known to be present in brain tissue (16), and it catalyzes Reaction 3; a function, Equation 4, can be expressed for this reaction (in micromoles per gram of brain, fresh weight) that under equilibrium conditions becomes identical to the thermodynamic equilibrium constant.

\[
\text{ATP} + \text{AMP} \rightarrow 2 \text{ADP} \tag{3}
\]

\[
K'_{AK} = \frac{(\text{ADP})^2}{(\text{ATP})(\text{AMP})} \tag{4}
\]

The values of \( K'_{AK} \) calculated from the data of 54 brains are plotted in Fig. 5 against their levels of AMP. It should be noted that the data were obtained from the same animals described in the previous section on creatine kinase. As shown in Fig. 5, there is an orderly and pronounced decrease in \( K'_{AK} \) with increasing levels of AMP. In contrast to the results obtained with creatine kinase, however, a value for \( K'_{AK} \) in normal brains could not be defined because of its pronounced tendency to increase as the level of AMP approaches zero. This appears to agree with the mathematical requirements of Equation 4, i.e. \( K'_{AK} \) approaches infinity as AMP approaches zero, if a true equilibrium process does not exist.

**Variation in Levels of ADP, ATP, and Phosphocreatine with Level of AMP—**

During accumulation of the data reported in this paper, it became evident that regardless of the type of stress imposed upon the animals predictable changes occurred in the levels of these phosphates. This is demonstrated in Fig. 6, in which the levels of ADP, ATP, and phosphocreatine in whole brain are plotted against the corresponding levels of AMP. The data are a compilation of those obtained from all the previous experiments and accordingly they represent brains taken from control animals and also from animals subjected to anoxia, electroshock and Metrazol seizures, swimming stress, chlorpromazine, ether, and phenobarbital. Again, the value enclosed by a square is from an animal which was frozen immediately after becoming unconscious during a swimming stress, and the encircled points correspond to animals used for experiments con-
ATP degradation that may occur either during stress in vivo or paper. Such a correction was not applied to the data reported in this exactly agrees with the two latter values. As discussed earlier, in ATP which occurs during its isolation (as discussed in an in our hands, a degradation of ADP and a corresponding increase in AMP. The value of 2.34 pmoles per g, fresh weight, for the in our section), a value of 2.54 pmoles per g is obtained which other recent values of 3.94 (17) and 1.70 pmoles per g (18) previously reported for this sum, but it does agree reasonably well with the other recent values of 2.55 (19) and 2.6 pmoles per g (20). Moreover, if a correction should be made for an apparent loss in ATP which occurs during its isolation (as discussed in an earlier section), a value of 2.54 pmoles per g is obtained which exactly agrees with the two latter values. As discussed earlier, such a correction was not applied to the data reported in this paper.

The ATP-AMP ratio seems to be a sensitive indicator of any ATP degradation that may occur either during stress in vivo or during the isolation procedure. The value of 43 reported here for this ratio is considerably larger than the values calculated from the data of previous investigators—1.2 (18), 4.9 (17), 7.7 (19), and 10.0 (20)—all of whom used an aqueous perchloric acid extraction at 0°. Probably our higher value for this ratio is due to the inactivation of degradative enzymes by acid and acetone prior to their contact with an aqueous system.

Examination of the data presented in the experimental section suggests that the level of AMP may be considered a measure of stress upon the brain. If this is true, the values for adenine nucleotides reported in Table I for control brains very likely approximate the actual levels in vivo. In fact, the level of AMP found in brains of the normal control rats was so low that better freezing and extraction techniques, and also a careful isolation of the rats themselves, may reveal an insignificant level of AMP in such brains. Although no attempt was made to keep the control rats from ordinary laboratory stimuli, analysis of brains from rats freed of these stimuli by sedation with chlorpromazine, ether, or phenobarbital showed that the level of AMP in each of these brains was slightly lower than those of normal controls (14, 15), suggesting that laboratory conditions themselves may have elevated the level of AMP.

We cannot state definitely whether the changes observed in nucleotides are a direct consequence of the electroshock or of the subsequent seizure. A major difficulty of putting such relationships on a time basis rests in the cooling rate of the brain in situ. Richter and Dawson (21) found that when 35-g rats were immersed in liquid nitrogen, a period of 9 to 20 seconds elapsed before the interior of the brains reached 0°. In addition, LePage (22) observed that a 300-g rat required 40 seconds for freezing, as evidenced by a thermocouple inserted into the stomach. In four experiments with our rats, each weighing 300 g, in which a thermistor was placed against the inside of the skull by insertion through the foramen magnum, a mean time of 40 ± 17 seconds was required for the temperature to fall to 0° from 37° after immersion of the rats in liquid nitrogen. When the curves of Fig. 2 are examined with a reasonable freezing lag in mind, the difficulties of correlating metabolite levels with either electroshock or seizure readily become evident, especially since organized metabolism ceases at some unknown time before 0° is reached. However, because seizures induced by agents other than electroshock are also known to lower levels of ATP and phosphocreatine in brain (2), the effects reported here are very likely a result of the seizure itself, i.e. they are not artifacts of the electrostimulus.

The reduction in the encephalic levels of high energy phosphates during seizures reflects the intense conversion of energy occurring at this time. However, hypoxia localized in the brain itself may also be involved in these changes by preventing a normal resynthesis of ATP and phosphocreatine (2, 11). According to this hypothesis, the oxygen demand of brain during a seizure, because of its stimulated metabolism, is far greater than the blood can supply, and until the oxygen tension is restored either by termination of the seizure or by an increased cerebral blood flow, the level of high energy phosphate cannot be regenerated.

Hypoxia may exert an effect on the high energy phosphates of brain in another way. After a 10-second exposure to an anoxic atmosphere, there was a significant decrease in the high energy phosphates of brain, and decapitation also decreased the level of these phosphates within a few seconds (14, 15). These
effects may be due to stimulation of the brain by a sudden hypo-

oxia (23). According to this latter hypothesis, the hypoxia

that has been observed during seizure symptoms (11) may exert

an active role in decreasing the ATP and phosphocreatine levels
during a seizure.

The pronounced changes in $K'_{CR}$ and $K'_{CK}$ with stress (as
evidenced by increased levels of AMP) suggest that equilibrium

or steady state conditions can be upset in the brain without
causing death of the animal. The value of 0.052 ± 0.0018 for
$K'_{CR}$, calculated from the data of 43 brains from both control
and intact experimental animals, agrees with that reported for
muscle tissue, $K'_{CR} = 0.053$ (24), and for the thermodynamic

equilibrium constant of crystalline creatine kinase at pH 7.4,
$K'_{CK} = 0.01$ to 0.05 (25); it does not agree as closely with two
values calculated from data in the literature for rat brain:
$K'_{CR} = 0.12$ (17) and $K'_{CK} = 0.38$ (2).

The rigid interrelationships in the phosphate metabolism

of whole brain were rather surprising. Actually the data in Fig. 6
suggest that the levels of ADP, ATP, and phosphocreatine can be
estimated in a brain subjected to any treatment simply from
a determination of its level of AMP. Although the data were not
included here, changes in GMP and GTP-UTP were always
similar to those of the corresponding adenine nucleotides. This
suggests, and is emphasized by the curves in Fig. 6, that the
entire energy metabolism of brain is controlled by a set of con-
titions so rigid that it can respond in but one way to severe met-
abolic insults. The ordered changes among the adenine nucleo-
tides are a consequence of the constant sum of AMP, ADP,
and ATP and the slight changes in ADP which together force
an inverse relationship between AMP and ATP.

The definite relationships shown in Fig. 6 are of interest in

gard to the hypothesis of Lindberg and Ernster (26), Siekervitz

and Potter (27), and also of IsbSen, Ccen, and McKee (28),
that AMP is involved with metabolic control mechanisms. Further-
more, comparison of Fig. 2 with Fig. 8 of IcbSen et al. (28) and
Figs. 6 and 7 of Hess and Chance (29) shows the very similar
changes occurring in brain after electroshock and in ascites cells
after addition of glucose. In ascites cells the ATP becomes
lowered by its reaction with glucose, whereas in brain the exact
mechanism for the lowering in ATP is unknown. Consequently,
it could also be caused by an increased reaction with glucose.

Because the absolute levels of the phosphates changed so
significantly under the influence of anoxia and convulsive sei-
zure studies with the aid of isotopes were not con-

sidered. A similar set of interrelationships among the adenosine
nucleotides during mild stimulation or sedation might be re-
vealed with the use of isotopes; i.e., during metabolism of brain
in which the levels of nucleotides and phosphocreatine remain
unchanged, the turnover rates of ATP and AMP may be di-

ectly related.

**SUMMARY**

The effect of electroshock on the acid-soluble nucleotides and
phosphocreatine of rat brain was studied. When brains of rats
frozen in liquid nitrogen were extracted at -78° with a solution
of trichloroacetic acid in acetone and the nucleotides were sepa-
rated by column chromatography on Dowex 1-formate, adeno-
sine triphosphate (ATP) to adenosine monophosphate (AMP)
ratio values averaging 43 were obtained in the whole brains of
control animals. Although the sum of AMP, adenosine diphos-
phate (ADP), and ATP was constant in the 58 control and ex-
perimental brains, having a mean of 2.34 ± 0.014 amoles per g,
fresh weight, there were important changes in the individual
phosphate. When tonic extensor seizures were induced in rats by
a 95-ma, 1-second stimulus, ATP and phosphocreatine reached
minimal levels 10 seconds later. ATP was again at its
normal level 75 seconds after the shock; phosphocreatine was
slower to reach its normal level. AMP varied inversely with
ATP, and ADP remained relatively unchanged. Two minima
in the levels of ATP and phosphocreatine were observed when
the intensity of the electrostimulus was varied; the depression
of ATP and phosphocreatine levels was found to be as severe during
clonic seizures at 10 to 12 ma as in tonic-extensor seizures at
95 ma.

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