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# The effects of phenobarbitone, leptazol, dexamphetamine, iproniazid, imipramine, LSD, chlorpromazine, reserpine and hydroxyzine on the *in vivo* levels of adenine nucleotides and phosphocreatine in the rat brain

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1. Enzymic and ion-exchange chromatographic analyses were used to measure adenosine triphosphate (ATP), diphosphate (ADP) and monophosphate (AMP) in brain extracts from rats treated with a wide range of centrally acting drugs. Phosphocreatine (PC) was assayed by the acid molybdate method.

2. An anaesthetic dose of phenobarbitone caused an increase in brain levels of ATP and PC, and a reduction in ADP and AMP. A convulsant dose of leptazol gave rise to precisely opposite effects. Subanaesthetic (hypnotic) and subconvulsive doses of the two drugs, respectively, produced no alterations in brain nucleotide levels.

3. Among the psychotropic drugs, dexamphetamine, LSD and hydroxyzine, at the doses used, caused no changes in brain levels of the adenine nucleotides. Iproniazid and imipramine caused slight increases in the ATP level and ATP/ADP ratio, respectively. Chlorpromazine failed to give rise to any effect in the nucleotides 3 hr after administration, but produced a rise in brain ATP after 6 hr. Reserpine, on the other hand, caused a fall in the ATP/ADP ratio 6 hr after injection.

4. These results indicate that some psychotropic drugs can cause small changes in the rat brain ATP/ADP ratio but do not support claims by certain workers that such changes correlate closely with the behavioural effects of these drugs.

Many reports, some of which are contradictory, have been published concerning the effects of psychotropic drugs on the *in vivo* levels of adenosine mono-, di- and triphosphates (AMP, ADP and ATP, respectively) in the brain. The results of one extensive study have suggested that there is a correlation between the behavioural effects and the changes in the brain ATP/ADP ratio induced by a large number of centrally acting drugs (Lewis & Van Petten, 1962, 1963; Kaul & Lewis, 1963a, b; Kaul, Lewis & Livingstone, 1965; Lewis, Ritchie & Van Petten, 1965). The enzymic assay (Kalckar, 1947) used by Lewis and coworkers has recently been

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shown (Wilson & Thomson, 1969) to give erroneous estimates of the adenine nucleotides in rat brain extracts.

In the present work, alternative methods of enzymic and ion-exchange chromatographic assay are used to investigate the effects of a wide range of centrally acting drugs in an effort to confirm or disprove the above theory. In addition to representatives of the antidepressive, tranquillizing and hallucinogenic drugs investigated by Lewis and coworkers, a non-specific depressant, phenobarbitone, and stimulant, leptazol, are also tested.

## Methods

The drugs used included phenobarbitone sodium, leptazol, dexamphetamine sulphate, iproniazid phosphate, imipramine hydrochloride, lysergic acid diethylamide (LSD), chlorpromazine hydrochloride, reserpine and hydroxyzine; all doses refer to these substances. All drugs (except in series 11) were dissolved in sterile 0·9% (w/v) saline and administered by intraperitoneal injection of 0·2 ml. drug solution/100 g body weight; an equivalent volume of sterile 0·9% (w/v) saline served as the control. In series 11, 25% (w/v) ascorbic acid was used as drug solvent and as control solution, the volume of drug or control solution injected being, in this series, 0·4 ml./100 g body weight.

Male Wistar rats (75–95 g) were placed in groups of two (series 2, 3, 5, 8, 13) or three (all other series) of equal body weight. One rat in each group served as a control. The order of treatment and of all subsequent procedures was randomized (Lewis & Van Petten, 1962, 1963). The data obtained were evaluated by the analysis of variance appropriate to the design used.

After injection of the drug or control solution, each rat was put into a small cylindrical cage, except in series 11, where the rats were allowed access to food and water in communal cages (one cage for each treatment group of three). The cages were kept in a quiet air-conditioned room at 20° C (28° C in the case of series 11).

The rats were killed and their brains frozen, removed and weighed as described by Lewis & Van Petten (1962). In series 1, 3, 4 and 5, the frozen brains were pulverized and extracted by the method of Lewis & Van Petten (1962). In all other series, the extraction was carried out immediately (<5 sec) after transferring the pulverized frozen brain from solid $CO_2$ to wet ice (Wilson & Thomson, 1969), instead of allowing it to " thaw " for 2 min as in the former procedure. The brain levels of the adenine nucleotides were estimated by the Boehringer method or by ion-exchange chromatography, as described by Wilson & Thomson (1969). It should be noted that the estimates of ATP and ADP by the Boehringer method include triphosphates and diphosphates, respectively, of guanosine and uridine (GTP + UTP and GDP + UDP, respectively).

With the ion-exchange chromatographic analysis, the total nicotinamide adenine dinucleotides (NAD) and the uridine diphosphate coenzymes (UDP-Co), consisting of the total UDP-glucose and UDP-*N*-acetylglucosamine, were also measured in a similar fashion to the adenine nucleotides. In order to estimate GTP and UTP, the extinction readings on the fractions of eluate in which these nucleotides occurred (that is, peak VIII, see Wilson & Thomson, 1969) were repeated at 290 m$\mu$. From the total number of optical density units at each wavelength (260 and 290 m$\mu$), the composition was resolved by means of simultaneous equations (Vierordt's method).

TABLE 1. In vivo *effects of leptazol, phenobarbitone, chlorpromazine, dexamphetamine and LSD on the nucleotide and PC levels of the rat brain*

| Series | Treatment | Dose (mg/kg) | Time of treatment (hr) | *N* | Concentration (μmoles/g frozen brain) PC | AMP | [ADP] | [ATP] | AMP+[ADP]+[ATP] | Ratio [ATP]/[ADP] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Control | | 0·5 | 9 | 2·79±0·09 | — | — | 2·49±0·07 | — | — |
| | Leptazol | 20 | 0·5 | 9 | 2·93±0·10 | — | — | 2·48±0·07 | — | — |
| | Leptazol | 50 | 0·5 | 9 | 2·82±0·10 | — | — | 2·42±0·06 | — | — |
| 2 | Control | | 7 min | 8 | 2·32±0·16 | 0·22±0·01 | 0·67±0·02 | 2·93±0·04 | 3·81±0·03 | 4·40±0·13 |
| | Leptazol | 90 | (see text) | 8 | †1·39±0·12 | †0·33±0·02 | †0·87±0·04 | †2·35±0·07 | †3·55±0·03 | †2·77±0·21 |
| 3 | Control | | 2 | 10 | 2·58±0·12 | 0·36±0·04 | 0·81±0·04 | 2·37±0·08 | 3·54±0·04 | 3·03±0·23 |
| | Phenobarbitone | 100 | 2 | 10 | *3·02±0·15 | 0·30±0·03 | 0·78±0·04 | 2·46±0·09 | 3·53±0·05 | 3·27±0·23 |
| 4 | Control | | 3 | 9 | 3·09±0·14 | — | — | 2·52±0·05 | — | — |
| | Chlorpromazine | 25 | 3 | 9 | *3·43±0·16 | — | — | 2·63±0·08 | — | — |
| | Dexamphetamine | 2·5 | 3 | 9 | 3·03±0·16 | — | — | 2·45±0·08 | — | — |
| 5 | Control | | 3 | 10 | — | — | — | 2·66±0·04 | — | — |
| | Dexamphetamine | 2·5 | 3 | 10 | — | — | — | 2·65±0·04 | — | — |
| 6 | Control | | 3 | 9 | — | 0·23±0·01 | 0·68±0·01 | 2·79±0·04 | 3·70±0·04 | 4·12±0·09 |
| | Dexamphetamine | 2·5 | 3 | 9 | — | 0·24±0·01 | 0·68±0·01 | 2·77±0·03 | 3·69±0·04 | 4·11±0·06 |
| | Dexamphetamine | 10 | 3 | 9 | — | 0·22±0·01 | 0·67±0·02 | 2·70±0·05 | 3·59±0·06 | 4·04±0·14 |
| 7 | Control | | 1·5 | 9 | — | 0·22±0·01 | 0·66±0·03 | 2·67±0·05 | 3·55±0·03 | 4·14±0·25 |
| | Dexamphetamine | 10 | 1·5 | 9 | — | 0·22±0·02 | 0·67±0·02 | 2·70±0·08 | 3·59±0·05 | 4·08±0·24 |
| | Phenobarbitone | 100 | 1·5 | 9 | — | *0·18±0·02 | *0·60±0·03 | *2·88±0·03 | 3·65±0·05 | *4·92±0·24 |
| 8 | Control | | 1·5 | 10 | — | 0·22±0·01 | 0·66±0·01 | 2·81±0·05 | 3·69±0·03 | 4·31±0·12 |
| | LSD | 0·1 | 1·5 | 10 | — | 0·22±0·01 | 0·65±0·01 | 2·86±0·06 | 3·73±0·05 | 4·42±0·15 |

In series 1–8, the Boehringer assay was used and hence [ADP] includes GDP+UDP and [ATP] includes GTP+UTP. Each value is the mean of the number of determinations (*N*) shown, ±S.E. of mean. Significance of difference from control: *0·05>*P*>0·01; †0·001>*P*.

The determination of GTP and UTP by this method is relatively imprecise because the spectra of the two nucleotides are not sufficiently different to provide extinction ratios particularly favourable to the method (see Glenn, 1960). There was, however, reasonable agreement between estimates obtained in this way from readings at 260 and 290 mμ, and estimates similarly derived from observations at 260 and 295 mμ.

For the estimation of inorganic phosphate (Pi) and phosphocreatine (PC), 1 ml. of brain extract (in 0·3 M $HC1O_4$) was diluted to 10 ml. with deionized water and assayed by the macromodification (Lewis & Van Petten, 1962) of the method of Furchgott & De Gubareff (1956).

**Results**

The brain levels of PC (in series 1, 3 and 4 only) and adenine nucleotides (as measured by the Boehringer method) determined after different periods of treatment with various doses of the drugs are shown in Table 1.

Subconvulsive doses of leptazol (20 and 50 mg/kg acting for 30 min, series 1) caused the rats to be somewhat more excitable than the controls but did not alter the brain levels of ATP + GTP + UTP or PC. In series 2, leptazol at 90 mg/kg caused convulsions within a few minutes of injection, the drug-treated animals in this series being killed exactly 30 sec after the start of the tonic convulsive phase. Controls were killed 7 min after injection. Leptazol convulsions significantly lowered the total nucleotide triphosphates and PC in the brain and raised the AMP and total nucleotide diphosphate levels. The ratio nucleotide triphosphates/diphosphates was also significantly lower in the convulsed than in the control rats.

Phenobarbitone at a dose of 100 mg/kg was about the threshold for anaesthesia in rats. Two hours after injection (series 3), the rats were still deeply sedated, but the limb withdrawal reflex was present. This drug treatment resulted in no significant alteration in the brain levels of the nucleotides measured, but the concentration of PC was raised. In a subsequent experiment with the same dose of phenobarbitone, anaesthesia was complete in all the animals when they were killed 1·5 hr after injection (series 7). Here, significant increases occurred in the brain level of nucleotide triphosphates and in the ratio triphosphates/diphosphates. The concentrations of nucleotide diphosphates and of AMP were significantly lowered by phenobarbitone anaesthesia.

Chlorpromazine (25 mg/kg, series 4) produced no change in the concentration of nucleotide triphosphates in the brains of rats killed 3 hr after injection, but did significantly raise the PC level. Dexamphetamine was repeatedly investigated at dose levels of 2·5 mg/kg, with a treatment time of 3 hr (series 4, 5 and 6), and 10 mg/kg, with treatment times of 3 hr (series 6) and 1·5 hr (series 7). At none of these doses or time intervals was any significant alteration observed in the brain AMP, nucleotide diphosphate or triphosphate concentrations, or in the PC level on the one occasion on which this was measured (in series 4). 1·5 hr after treatment with LSD (0·1 mg/kg, series 8), the brain concentrations of AMP, nucleotide disphosphates and triphosphates and also the ratio of triphosphates to diphosphates were no different from those of the controls. In the series of experiments listed in Table 1 in which PC was measured, Pi was also assayed, but in no case was a significant difference from the control level (about 6 μmoles/g frozen brain) observed.

TABLE 2. In vivo *effects of dexamphetamine, chlorpromazine, imipramine, iproniazid, reserpine, hydroxyzine, phenobarbitone and LSD on the nucleotide levels of the rat brain*

| Series | Treatment | Dose (mg/kg) | Time (hr) | Concentration in μmoles/g frozen brain | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NAD | UTP | GTP | AMP | ADP | ATP | AMP+ADP +ATP | Ratio ATP/ADP |
| | Control | — | | 0·35±0·01 | — | — | 0·31±0·03 | 0·51±0·03 | 2·10±0·05 | 2·92±0·07 | 4·21±0·25 |
| 9 | Dexamphetamine | 2·5 | 3 | 0·37±0·01 | — | — | 0·33±0·06 | 0·56±0·03 | 2·11±0·06 | 2·99±0·08 | 3·88±0·24 |
| | Chlorpromazine | 25 | | 0·36±0·02 | — | — | 0·23±0·03 | 0·50±0·03 | 2·16±0·07 | 2·89±0·07 | 4·44±0·28 |
| | Control | — | | 0·43±0·01 | 0·19±0·02 | 0·40±0·01 | 0·37±0·03 | 0·58±0·03 | 1·95±0·04 | 2·90±0·05 | 3·45±0·20 |
| 10 | Imipramine | 50 | 3 | 0·42±0·01 | 0·19±0·02 | 0·39±0·02 | 0·32±0·05 | 0·53±0·02 | 2·01±0·08 | 2·86±0·11 | *3·89±0·25 |
| | Iproniazid | 20 | | 0·42±0·01 | 0·20±0·02 | 0·41±0·01 | 0·29±0·03 | 0·56±0·02 | *2·12±0·03 | 2·97±0·04 | 3·80±0·13 |
| | Control | — | | 0·41±0·01 | 0·17±0·01 | 0·43±0·02 | 0·28±0·03 | 0·54±0·03 | 2·04±0·06 | 2·86±0·04 | 3·94±0·30 |
| 11 | Chlorpromazine | 25 | 6 | 0·40±0·01 | *0·22±0·02 | 0·47±0·01 | 0·33±0·04 | 0·49±0·01 | *2·21±0·04 | *3·04±0·05 | 4·54±0·17 |
| | Reserpine | 5 | | 0·40±0·01 | 0·20±0·02 | 0·42±0·01 | †0·44±0·05 | *0·61±0·03 | 1·95±0·05 | 3·00±0·06 | *3·23±0·20 |
| | Control | — | | 0·36±0·01 | 0·21±0·01 | 0·45±0·02 | 0·20±0·01 | 0·50±0·01 | 2·09±0·04 | 2·78±0·03 | 4·25±0·17 |
| 12 | Hydroxyzine | 60 | 1·5 | 0·35±0·01 | 0·20±0·02 | 0·44±0·02 | 0·19±0·01 | 0·51±0·02 | 2·11±0·06 | 2·80±0·05 | 4·23±0·23 |
| | Phenobarbitone | 170 | | 0·36±0·01 | 0·24±0·02 | *0·50±0·02 | *0·17±0·01 | †0·43±0·02 | ‡2·30±0·03 | *2·90±0·03 | ‡5·42±0·23 |
| | Control | — | 1·5 | 0·35 | 0·17 | 0·53 | 0·21 | 0·58 | 2·16 | 2·95 | 3·72 |
| 13 | LSD | 0·1 | | 0·34 | 0·12 | 0·50 | 0·31 | 0·52 | 2·14 | 2·97 | 4·12 |

Each value is the mean (±S.E. of mean) of nine determinations, except in series 13, where each value is a mean of two determinations. Significance of difference from control: *$0{\cdot}05>P>0{\cdot}01$; †$0{\cdot}01>P>0{\cdot}001$; ‡$0{\cdot}001>P$.

Table 2 shows the rat brain levels of NAD, GTP, UTP, AMP, ADP, ATP and the total adenine nucleotides, together with the ratios ATP/ADP, as estimated by ion-exchange chromatography. Reinvestigation by this method of the effects of dexamphetamine (2·5 mg/kg) and chlorpromazine (25 mg/kg) after a treatment interval of 3 hr (series 9) indicated no significant change in the concentrations of any of the metabolites measured. Small, but statistically significant increases occurred (series 10) in the ratio ATP/ADP 3 hr after the injection of imipramine (50 mg/kg) and in the ATP concentration after iproniazid (20 mg/kg); brain levels of other metabolites showed no changes as a result of these drugs.

The two major tranquillizers, chlorpromazine (25 mg/kg) and reserpine (5 mg/kg) had roughly opposite effects on the brain adenine nucleotides 6 hr after the injection of the drugs (series 11). Chlorpromazine caused a significant rise in ATP concentration which was reflected in a significant increase in the total adenine nucleotide level. AMP, ADP and the ATP/ADP ratio were unaffected. Reserpine, on the other hand, caused an increase in both AMP and ADP, and a decrease in the ATP/ADP ratio, while the slight lowering of the ATP concentration was statistically insignificant. The UTP concentration was significantly higher in the brains of rats treated with chlorpromazine at this time interval, although the GTP level was not significantly increased. Reserpine did not alter the levels of GTP or UTP, and neither drug affected the NAD concentration.

Hydroxyzine (60 mg/kg, series 12), after a treatment time of 1·5 hr, produced no changes in the brain concentrations of any of the nucleotides nor in the ATP/ADP ratio. A fully anaesthetic dose (170 mg/kg) of phenobarbitone (series 12) after the same time interval significantly raised the ATP, GTP and total adenine nucleotide concentrations and the ATP/ADP ratio, and significantly decreased the levels of AMP and ADP. The NAD concentration remained unaffected by this dose of phenobarbitone. In series 13, only two pairs of rats were tested for the effects of LSD (0·1 mg/kg, 1·5 hr), but no obvious change in the brain ADP, ATP, GTP or UTP concentrations occurred. No significant changes in brain NAD or UDP-Co concentrations occurred as a result of any of the above drug treatments. The control level of the latter was about 0·22 $\mu$moles/g frozen brain.

## Discussion

The large number of factors which contribute towards the variation amongst estimates of the high-energy metabolites of the brain have been discussed in detail by other workers (Weiner, 1961; Lewis & Van Petten, 1963; Pecháň & Marko, 1964).

*Extraction.* Rapid extraction of pulverized brain tissue by means of aqueous perchloric acid has been shown to prevent satisfactorily breakdown of ATP (Wilson & Thomson, 1969). Freeze-drying of brain tissue before extraction appears to permit the recovery of a small additional fraction of ATP (Brattgård, Løvtrup-Rein & Moss, 1966; Wilson & Thomson, 1969). While the rapid extraction technique used in series 2 and 6–13 merely improved the preservation of the nucleotides and the reproducibility of the results, freeze-drying the pulverized brain altered slightly the actual amounts of nucleotides recovered from the brain tissue. The latter was not therefore used in the present investigation because it would have confused the comparison of the results obtained with the work of Lewis and his colleagues.

*Leptazol and phenobarbitone.* The observations reported here confirm earlier reports that leptazol convulsions lower cerebral high-energy phosphate reserves (Coper, 1956 ; Koransky, 1956 ; Hirayama, 1963 ; Pecháň & Marko, 1963). Where, however, the drug caused no gross increase in functional activity (in subconvulsive doses) there is no suggestion in the results that the high-energy phosphate balance was disturbed. Anaesthesia due to phenobarbitone caused brain levels of ATP to remain unaltered (as found by Lowry & Passonneau, 1964) or to be slightly increased (in agreement with Fleming & Lacourt, 1965 ; Goldberg, Passonneau & Lowry, 1965). PC, where measured, showed a larger increase than ATP (Lowry & Passonneau, 1962 ; Fleming & Lacourt, 1965 ; Goldberg *et al.*, 1965). There appears, from the present results (series 3, 7 and 12), to be a correlation between full anaesthesia and a significant increase in brain ATP and decrease in ADP content. The absence of any change in rat brain ATP following a sedative dose of phenobarbitone was also reported by Gey, Rutishauser & Pletscher (1965).

The marked changes in brain activity brought about by leptazol and phenobarbitone are known to be accompanied by alterations in the rate of cerebral energy utilization: the energy requirement of the mouse brain during convulsions is 5- to 7-fold greater than normal (King, Schoepfle, Lowry, Passonneau & Wilson, 1967) and the rate of ATP utilization in mouse brain after decapitation is considerably (42%) lowered by pretreatment with an anaesthetic dose of phenobarbitone (Lowry & Passonneau, 1962). The changes in high-energy phosphate levels due to leptazol convulsions are thus caused by the inability of the brain to maintain this very rapid supply of energy. The main conclusion to be drawn from these results is that these drugs do not alter brain nucleotide levels until gross changes occur in the functional activity of the brain. The above pattern of alterations in brain nucleotide concentrations due to anaesthesia and convulsions is now quite well established. For the other drugs under examination, however, much contradictory evidence exists.

*Central stimulants, antidepressives and hallucinogens.* No comparable results on the effects of dexamphetamine have been published, other than those of Lewis & Van Petten (1962). Methylamphetamine has earlier been claimed to produce a large rise in the brain ATP concentration in rabbits (Palladin & Rybina, 1953) and of mice (Heim, Leuschner & Estler, 1957). A recent investigation (Estler & Ammon, 1967) using improved techniques revealed, however, that this drug (at 3 mg/kg) causes no significant change in mouse brain nucleotide triphosphates or diphosphates. This supports the present finding that dexamphetamine does not alter rat brain adenine nucleotide levels.

The above results indicate that LSD (at 0·1 mg/kg) similarly lacks any effect on the rat brain nucleotides. Krawczynski (1961) also failed to find any significant alteration in cerebral ATP on administration of LSD (0·25 mg/kg) to rats.

The present results show that the dose of iproniazid used (20 mg/kg) gave rise to a small (less than 10%) increase in rat brain ATP concentration. Very few comparable results have been published. Iproniazid (100 mg/kg) caused a slight fall in ATP content of the rabbit cerebrum (Kurs'kii & Zryakov, 1964), while the cerebellar nucleotides were unaffected. Another monoamine oxidase (MAO) inhibitor, pheniprazine (25 mg/kg) was reported by Bernsohn, Possley & Custod (1960) to produce a 25% reduction in rat brain ATP level. There is no evidence at present to indicate whether the observed effect on brain ATP is connected with either MAO inhibitory or antidepressive activity.

The effect of imipramine was to increase the ATP/ADP ratio in the rat brain by 13%. It should be noted that this change represents an alteration in the state of phosphorylation of only 2·5% of the brain ATP. Pecháň (1965) used the extraction procedure of Minard & Davis (1962a) and ion-exchange analysis to investigate imipramine at the same dose and time interval, and obtained similar results again: a significant increase only in the ATP/ADP ratio.

*Major and minor tranquillizers.* Chlorpromazine (25 mg/kg), injected 3 hr before the animals were killed, causes no change in the brain nucleotides as measured by both the Boehringer and ion-exchange procedures. A similar lack of effect on the rat brain nucleotides was observed 0·5 hr after 10 mg/kg (Minard & Davis, 1962b) and 2 hr after 25 mg/kg of the same drug (Bernsohn *et al.*, 1960). Gey *et al.* (1965) reported that the ATP level was unchanged 0·5 hr after 25 mg/kg. The cerebral adenine nucleotides of the mouse are also unchanged 1–2 hr after chlorpromazine at a dose of 10 mg/kg (Chowdhury, Skinner, Spector & Yap, 1968).

The present findings following 6 hr of chlorpromazine treatment are difficult to interpret in the absence of changes in brain nucleotides after shorter periods. The prevention of hypothermia during the 6 hr treatment with chlorpromazine cannot explain the observed effects, because drug-induced hypothermia in fact tends to increase brain ATP levels (Veksler, 1964).

A state of decreased phosphorylation of the brain adenine nucleotides after treatment with reserpine was observed in the present study. Brain ATP levels in mice, on the other hand, appear to be slightly increased by reserpine (Balzer, Holtz & Palm, 1961). This may represent a species difference or may be due to hypothermia. A further complicating aspect is introduced by the observation (Gey *et al.*, 1965) that while reserpine (2 mg/kg) acting for 3 hr induced no change in the ATP level in the brains of fed rats, there was a small yet significant reduction in this level if the rats had been starved for 16 hr. This may be connected with the present findings, where reserpine-treated rats lay sedated for 6 hr without showing interest in food or drink.

The changes observed here in brain adenine nucleotide concentrations may also be related to the accumulation of cerebral glycogen (Svorad, 1959; Balzer *et al.*, 1961) and glucose (Rutishauser, 1963) and to the decreases in brain hexose-6-monophosphates (Gey *et al.*, 1965) and pyruvate (Rutishauser, 1963) which have been reported in rats during reserpine treatment.

Extremely little information is available on the metabolic effects of the minor tranquillizers in general and hydroxyzine in particular. Some inhibition of *in vitro* oxidative phosphorylation at a concentration of $3 \times 10^{-4}$M has been reported (Nishi, Koketsu, Cerf & Abood, 1959), but the present results do not suggest any interference of this drug in brain energy metabolism *in vivo*.

Due to their consistent observations using the Kalckar (1947) assay that central stimulants and antidepressives caused an increase in the ATP/ADP ratio in rat brain and that tranquillizers gave rise to a decrease in this ratio, Lewis and coworkers concluded that all these classes of drugs were possibly acting " primarily on phosphate metabolism " (Kaul & Lewis, 1963a). The consistency of these findings is supported neither by the results of other workers, nor by those reported here. It therefore seems unlikely that changes in brain levels of the adenine nucleotides are either typical or, where they occur, primary effects of psychotropic drugs.

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