A PHARMACOLOGIC STUDY OF SOME NUCLEOSIDES AND NUCLEOTIDES

BY PAUL V. BUDAY*, C. JELLEFF CARR† AND TOM S. MIYA

From the Department of Pharmacology, Purdue University, School of Pharmacy, Lafayette, Indiana

Received January 17, 1961

Adenosine, adenosine-5′-phosphoric acid (AMP), adenosine-5′-triphosphate (ATP), guanosine-5′-triphosphate (GTP), uridine, uridine-5′-triphosphate (UTP), and cytidine diphosphate-5′-choline (CDPCh) were studied *in vitro* and *in vivo*. The intraventricular injection of high doses of adenosine, ATP, and methacholine into rats elicited clonic-tonic convulsions or an akinetic state, or both. Perfusion of frog hearts *in situ* with equimolar concentrations of the "high-energy" compounds, with the exception of ATP, failed to exert any positive inotropic effects. ATP did not potentiate the inotropic actions of (−)-adrenaline or (−)-noradrenaline. The cholinergic-like properties of adenosine and ATP were partially antagonised by concomitant perfusion with methylene blue. ATP, but not adenosine, obliterated transitorily, the cardio-toxicity of pilocarpine. The inhibition of the isolated clam heart by AMP and ATP qualitatively resembled the effects of acetylcholine (Ach). Antagonism of the cardio-depressant properties of AMP and ATP by benzoquinonium and 5-hydroxytryptamine (5-HT) also supports previous evidence that the adenyl compounds act similarly to the cholinergic mediator. ATP produced marked contractions and a tonotropic effect on the oestrogenised, isolated, quiescent rat uterus; these effects were destroyed by low concentrations of (−)-adrenaline and papaverine. Adenosine, AMP, and CDPCh in equimolarities elicited no response from this test preparation.

THE energy-rich nucleotides have been found to catalyse and serve as substrates for numerous biotransformations which use energy liberated by catabolism. Some, in addition, have been implicated in the mode of action of drugs. Their pharmacodynamic effects and their ability to modify the action of other pharmacologic agents was studied *in vivo* and *in vitro* to confirm previous reports, and to compare these actions with those of some recently available nucleotides.

The nucleosides and nucleotides used were adenosine, adenosine-5′-phosphoric acid, adenosine-5′-triphosphate, disodium, guanosine, guanosine-5′-triphosphate, disodium, uridine, uridine-5′-triphosphate, trisodium, and cytidine diphosphate-5′-choline, monosodium.

EXPERIMENTAL METHODS

Cerebral Intraventricular Injections

Intraventricular injections of adenosine, ATP, and methacholine chloride were made into conscious albino rats (55–140 g.) after the method of

* Present Address: Department of Pharmacology, University of Rhode Island, Kingston, R.I., U.S.A.

† Present Address: Psychopharmacology Service Center, National Institute of Mental Health, Bethesda 14, Md., U.S.A.

Haley and McCormick (1957), employing a 26 G–3/8 in. hypodermic needle. The injection site was determined by preliminary injections of diluted India ink. Gross observations of the ink particle localisation showed that the injections were being made into the right lateral ventricle of the brain. All drugs were dissolved in normal physiological saline solution and the volume injected was 0·05 ml. Gentle heating was required to dissolve the nucleoside and the ATP at the higher doses. Both adenyl compounds were preserved either in the crystalline form, or in fresh solution frozen, at − 15°.

Perfusion Studies

In situ frog heart perfusions were performed from December to May employing *Rana pipiens* (L.) measuring 7·5–8·8 cm. in length, following the technique of Sollmann and Barlow (1926). The hearts were perfused with a modified Howell-Ringer solution (pH 7·2) at ambient room temperature (24–27°). All drugs were dissolved in the perfusate to obtain the final anhydrous molarities.

Isolated Clam Heart

Isolated hearts (ventricles) of the lamellibranch clam, *Venus mercenaria* (L.), were suspended in a 11 ml. tissue chamber. The techniques employed generally followed the recommendations of Wait (1943), Welsh and Taub (1948), and Tower and McEachern (1948). The mollusca were satisfactorily preserved for 6 to 8 days by storage at 9°. An artificial "sea water" containing the following salts in g./l. was employed: NaCl 30·00, KCl 0·90, $CaCl_2$ (anhydrous) 1·10, $MgSO_4.7H_2O$ 4·95, $NaHCO_3$ 0·03, and dextrose (anhydrous) 0·25. The bathing fluid (pH 7·5) was aerated and maintained between 17 and 20°. All drugs, with the exception of guanosine which was dissolved in bicarbonate-free fluid, were dissolved in double glass-distilled water, and added to the muscle chamber in 0·05 ml. volumes. Isotonic contractions were recorded kymographically. This investigation was restricted to early June to mid-July to minimise possible seasonal variations in drug responses (Prosser, 1940).

Isolated Rat Uterus

Uteri were excised from albino rats (120–235 g.) previously oestrogen-primed by subcutaneous injection of diethylstilboestrol in cottonseed oil 50 μg./100 g. at 2–24 hr. intervals before killing on the 72nd hr. The isolated horns were suspended in a muscle chamber (14 ml. capacity) containing de Jalon solution (1945), and the system was maintained at a temperature of 28–29°. Isotonic contractions were recorded by means of a writing lever and smoked kymograph. All drugs were introduced into the bath in 0·05 ml. volumes.

RESULTS

Intraventricular Injections

The administration of high doses (1,000 and 1,500 μg.) of ATP into rats elicited severe, generally unilateral, clonic-tonic convulsions. These

seizures, at times, were characterised by a spiral body movement ("rolling pin" motion) and persisted for from 15 to 90 sec. A condition of anergia or akinesis with a concomitant asthenia of the limbs then appeared. This latter state persisted for 4 to 26 min. Lower concentrations (25–500 μg.) of ATP and adenosine (6 rats at each dosage level) elicited solely the akinetic state and weakness. Methacholine chloride in 125 μg. doses (5 rats) evoked, in all but one instance where convulsions occurred, a catatonic or akinetic reaction. These animals appeared completely

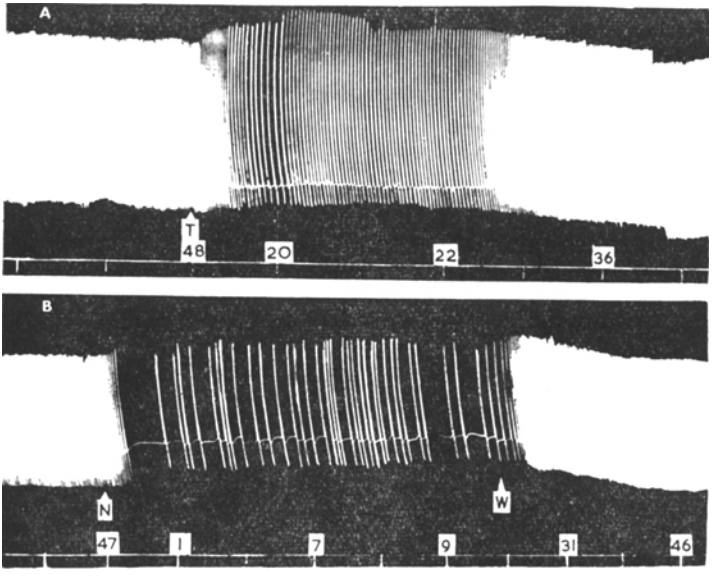

FIG. 1. Perfusion of *in situ* frog hearts with ATP and adenosine.
A. At T perfusion with ATP ($8{\cdot}26 \times 10^{-6}$M) started. Numbers above time line indicate heart rate in beats/min. Time marker, 1 min.
B. At N perfusion with adenosine $8{\cdot}26 \times 10^{-6}$M begun. At W perfusion of nucleoside stopped and washout with Howell-Ringer solution begun.

composed within $5\frac{1}{2}$ min. Control saline injections produced no comparable effects although the animals exhibited transient clockwise circling. This latter effect also supervened in the adenyl-treated animals.

Frog Heart Perfusions

ATP as low as $8{\cdot}26 \times 10^{-7}$ M produced an increased systolic force with a concurrent bradycardia. High concentrations ($8{\cdot}26 \times 10^{-6} - 3{\cdot}31 \times 10^{-5}$ M) elicited a profound, but transitory, heart block. Auricular, followed by ventricular diastolic stoppage occurred, but a spontaneous recovery of the myocardium was often noted, an action resembling "vagal escape". Perfusion with fresh Howell-Ringer solution readily reversed these effects. In general the reactions observed confirm the isolated amphibian and mammalian auricular and intact animal studies of Wedd and Fenn (1933), Drury (1936), and the frog heart perfusion investigations of Ostern and Parnas (1932), Gillespie (1934), Meyer (1951),

and Porro (1952). GTP and UTP in $3{\cdot}31 \times 10^{-5}$ and $1{\cdot}32 \times 10^{-4}$ M effected only slight augmentations in rate and little or no positive inotropism. CDPCh in $3{\cdot}31 \times 10^{-5}$ and $6{\cdot}62 \times 10^{-5}$ M, and sodium pyrophosphate in $8{\cdot}26 \times 10^{-6}$ and $3{\cdot}31 \times 10^{-5}$ M elicited no demonstrable responses. Adenosine in $8{\cdot}26 \times 10^{-7}$ — $3{\cdot}31 \times 10^{-5}$ M manifested a bradycardia and a diastolic stoppage in the higher concentrations, with only minor enhancement of cardiac excursions. These results with adenosine parallel the mammalian heart effects previously reported (Drury and

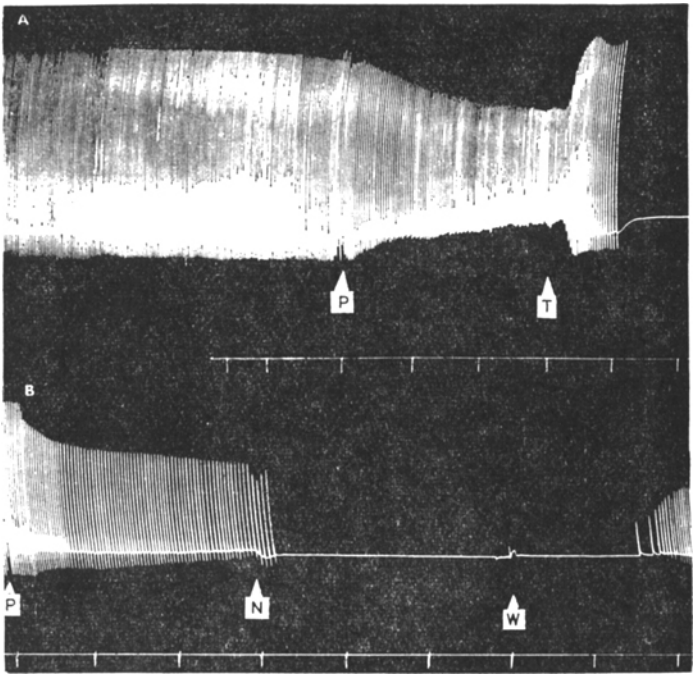

FIG. 2. Cardio-toxicity of pilocarpine on the *in situ* frog heart modified by ATP and adenosine.
A. At P perfusion with pilocarpine nitrate ($7{\cdot}37 \times 10^{-4}$M). At T perfusion begun with ATP in $3{\cdot}31 \times 10^{-5}$M. Time marker, 1 min.
B. At P perfusion with pilocarpine nitrate ($7{\cdot}37 \times 10^{-4}$M) begun. At N perfusion with adenosine $3{\cdot}31 \times 10^{-5}$M started. At W perfusion of nucleoside terminated and washout with Howell-Ringer solution begun.

Szent-Gyorgyi, 1929; Chevillard and Guerin, 1955; and Rand and others, 1955). Fig. 1 shows typical responses of the *in situ* frog heart to adenosine and ATP.

A 4 min. concomitant perfusion of methylene blue ($3{\cdot}0 \times 10^{-5}$ M) through 7 amphibian hearts inhibited (2–61 per cent) the bradycardia characteristically induced by $8{\cdot}26 \times 10^{-6}$ M concentrations of the adenyl derivatives when contrasted with the subsequent perfusion of the latter agents through the thoroughly washed, but blue stained heart. The phenazothionium dye when perfused concomitantly with ATP did not suppress the normal inotropic effect.

The cardio-accelerations induced by (—)-adrenaline HCl ($5{\cdot}46 \times 10^{-9}$ M) and (—)-noradrenaline bitartrate ($6{\cdot}26 \times 10^{-9}$ M) were readily inhibited (7–100 per cent) by the concomitant perfusion of each of the amines with $3{\cdot}37 \times 10^{-5}$ M adenosine. The perfusion of a just subthreshold concentration ($1{\cdot}09 \times 10^{-12}$ M) of (—)-adrenaline or (—)-noradrenaline ($1{\cdot}25 \times 10^{-11}$ M) with a threshold level ($4{\cdot}13 \times 10^{-7}$ M) of ATP failed to show, with 6 hearts, any demonstrable potentiation of amplitude.

The cardio-depressant properties of pilocarpine nitrate ($7{\cdot}37 \times 10^{-4}$ M) were sharply and readily, but only temporarily, reversed by ATP ($8{\cdot}26 \times 10^{-6}$ or $3{\cdot}31 \times 10^{-5}$ M), but not by equimolarities of the adenyl nucleoside. Fig. 2 graphically compares their antagonistic properties towards pilocarpine.

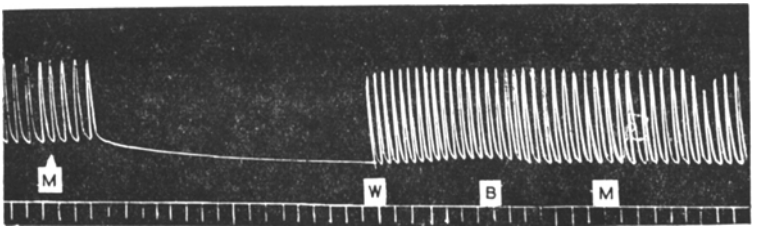

FIG. 3. Blocking action of benzoquinonium on the isolated clam heart. At M addition of AMP to bath to give $4{\cdot}48 \times 10^{-4}$M. At W washing out of the bath. At B addition of benzoquinonium chloride to give $4{\cdot}48 \times 10^{-5}$M. Time marker, 10 sec.

Isolated Clam Heart Experiments

ATP, $2{\cdot}24 \times 10^{-4} - 1{\cdot}34 \times 10^{-3}$ M, after a short latency, produced a gradual inhibition of amplitude and a diastolic arrest. Depression of rate was small. Occasionally, the ventricular stoppage was abrupt and was not preceded by progressive depression. In certain instances a transitory and erratic, partial or complete, escape occurred. AMP in equimolarities caused a similar effect. Uridine and guanosine, even in high concentrations ($4{\cdot}48 \times 10^{-4} - 1{\cdot}79 \times 10^{-3}$ M), produced only feeble inhibitions of rate and amplitude. No tachyphylaxis was noted with any of these compounds.

The addition of benzoquinonium chloride, an Ach-blocking agent on the clam heart (Luduena and Brown, 1952), to the muscle bath to make a final concentration of $4{\cdot}84 \times 10^{-5}$ M (1 min. before addition of either AMP or ATP in $4{\cdot}48 \times 10^{-4}$ M) either increased the latency period preceding depression or wholly prevented this quasi-muscarinic action of the nucleotides. Fig. 3 shows a typical kymogram of this complete protection.

5-HT (creatinine sulphate complex) in $1{\cdot}18 \times 10^{-6}$ M was found, as in earlier studies (Welsh, 1953, 1954, 1957), to be a potent excitant to the clam heart, the stimulation consisting of a singular increase in cardiac

excursion and, in some instances, of a pronounced tonotropic effect. Four of 5 hearts when partially or completely inhibited by ATP ($4{\cdot}48$–$8{\cdot}96 \times 10^{-4}$ M) were subsequently and completely protected from a second depressant concentration by 5-HT.

Using 6 clam ventricles a semi-quantitative comparison of the inhibitory properties of Ach and ATP showed that a mean concentration of $9{\cdot}37 \times 10^{-11}$ and $6{\cdot}72 \times 10^{-4}$ M respectively was required to educe complete inhibition of the isolated ventricles within a 1 min. period (Fig. 4).

Rat Uterus Experiments

ATP in serial molarities ($2{\cdot}24 \times 10^{-5}$ — $2{\cdot}24 \times 10^{-4}$) elicited an almost immediate spasm of the isolated, quiescent uterus, which was followed by contractions of irregular magnitude. These contractile responses

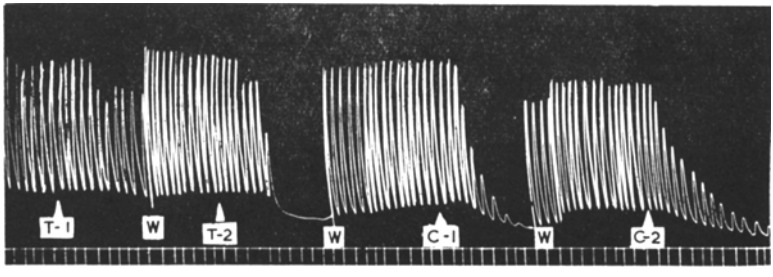

FIG. 4. Comparative actions of ATP and Ach on the isolated clam heart. At T-1 addition of ATP to bath to give $4{\cdot}48 \times 10^{-4}$M. At W washing out of the bath. At T-2 addition of ATP to bath to give $8{\cdot}96 \times 10^{-4}$M. At C-1 addition of Ach to bath to give 2×10^{-10}M. At C-2 addition of Ach to bath to give 1×10^{-10}M. Time marker, 10 sec.

were only partially obliterated by thorough washings with de Jalon solution thus suggesting that the phosphoriboside enhanced the irritability of the smooth muscle tissue. In contrast, GTP ($7{\cdot}48 \times 10^{-5}$ M) usually produced only a minor, single contraction with no display of a tonotropism. CDPCh ($7{\cdot}48 \times 10^{-5}$ M), adenosine, and AMP ($7{\cdot}48 \times 10^{-5}$ and $2{\cdot}24 \times 10^{-4}$ M), even after 12 min. of contact, demonstrated no uteromotor effects.

Ten uteri under an 8 min. stimulation by ATP ($7{\cdot}48 \times 10^{-5}$ M) were readily and completely inhibited by minute concentrations ($4{\cdot}05$ and $8{\cdot}10 \times 10^{-11}$ M) of (—)-adrenaline; papaverine HCl ($2{\cdot}65 \times 10^{-5}$ and $6{\cdot}63 \times 10^{-6}$ M) showed a similar, but slower, effect on another series of *in vitro* preparations. Fig. 5 shows the former antagonism.

DISCUSSION

Although some cerebral injury was inevitable with concurrent, but transitory effects, due to the intraventricular injection method, the akinetic condition induced by adenosine and ATP confirms the studies of Feldberg and Sherwood (1954) in the cat. This reaction and the asthenia after convulsive doses of the adenyl compounds might be interpreted as

fatigue, but this seems untenable, in part, in light of the fact that these effects supervened in response to the lower, non-convulsive doses.

The direct central actions of Ach were first described by Dikshit (1934), and later by Schnedorf and Ivy (1939) and Haley and McCormick (1957). These reports indicated the production of a sleep-like or akinetic state. It would appear most inferential from the literature and from the results reported here that the direct central actions of the adenyl compounds resemble those elicited by some cholinergic agents.

UTP, GTP, and CDPCh, although all possessing "high-energy" bonds, were incapable of significantly affecting either the rate or amplitude of the

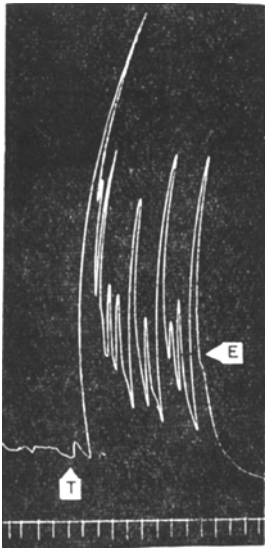

FIG. 5. The effects of ATP on the isolated, quiescent, oestrogenised rat uterus antagonised by adrenaline. At T addition of ATP to bath to give $7{\cdot}48 \times 10^{-5}$M. At E addition of (—)-adrenaline bitartrate to bath to give $4{\cdot}05 \times 10^{-11}$M. Time marker, 1 min.

frog heart. In contrast, ATP was noted, as other amphibian and mammalian studies have shown (Chevillard and Guerin, 1955; Green and Stoner, 1950; Kanda and others, 1954; Meyer, 1951; Sekiya, 1953) to initiate a positive inotropic effect soon succeeded by a bradycardia or diastolic arrest. The lack of biologic activity of the non-adenyl compounds implies a stringent biochemical-myokinetic specificity of the myocardium. The vagomimetic properties of adenosine, without significant attendant inotropic effects, were first described by Drury and Szent-Gyorgyi (1929) and are well documented (Drury, 1932; Drury, 1936; Green and Stoner, 1950; Wedd and Fenn, 1933). The cardiovascular actions of the adenyl series are due apparently to the ribose residue (Sydow and Ahlquist, 1954) and to the presence of a 6-NH_2 group on the purine moiety (Clarke and others, 1952; Green and Stoner, 1950), with phosphate esterification enhancing and modifying the activity (Green and Stoner, 1950).

Cook (1926) demonstrated, on the isolated frog heart, that the vagomimetic characteristics of Ach were antagonised by simultaneous perfusion with methylene blue. This finding suggested that the neurohormone's site of action was restricted to the cell membrane or to a surface receptor. Thus the partial antagonism of the myocardial actions of adenosine and ATP by this same dye suggests that the adenyl derivatives also exert their cholinergic-like effects, at least in part, through a reversible alteration of the plasmatic membrane. This adds substance to the unproven speculation (Gillespie, 1934) that the rapidity of action of the adenyl agents might be explained by their ability to alter cellular colloids. Adenosine's antagonism of the cardio-acceleratory properties of certain sympathomimetic amines further adds to the evidence that the active members of the adenyl family possess cholinergic actions.

The inability of ATP to enhance the positive inotropic actions of (−)-adrenaline and (−)-noradrenaline was a rather surprising finding since both amines affect intermediary metabolism in muscle. Our results do not agree with those of Edwards and Booker (1959) who found ATP to enhance the myocardial action of adrenaline on the guinea-pig isolated heart. Perhaps species and perfusion differences underlie this incongruity. The ephemeral, but conspicuous, antagonism of ATP towards pilocarpine induced cardio-toxicity indicates that the former substance possesses the ability to exert its typical inotropic effects independent of intracellularly fixed alkaloid, perhaps, in contrast to adenosine, by antagonising the decreased myocardial oxygen consumption as engendered by the parasympathomimetic agent (David, 1930).

The Ach-like actions of AMP and ATP were clearly demonstrated on the isolated clam heart inferring the latters' ability to produce a cardioplegic action on the diffuse myogenic pacemaker or conduction mechanisms of this organism. The spontaneous and irregular recovery of the heart at various times while under the depressant influence of ATP seems to add credence to this hypothesis. Although the inhibitory actions of ATP differed from preparation to preparation, and on the same specimen, its generally rapid action, and ready removal by washing suggests again that ATP, like Ach, is acting upon or near the surface of the cell membrane. The blocking action of benzoquinonium towards the purine nucleotides also lends support to this conclusion, since the depolarising activity of the bis-onium salts may be explained by the adsorption of the polymethylene chain via Van der Waal's forces to the cellular surface (Barlow, 1955). However, it is most unlikely that a specific competitive antagonism between the adenyl compounds and benzoquinonium exists.

The fact that 5-HT application to the molluscan heart before the addition of ATP prevents the resultant cholinergic-like effects indicates that these two compounds are pharmacodynamically antagonistic. The inertness of uridine and guanosine implies that these nucleosides play little, if any, role in molluscan myokinetics.

In contrast to the reported uterine stimulatory properties of adenosine (Bennet and Drury, 1931; Moulton and others, 1957) and AMP (Deuticke, 1932) on the guinea-pig, adenosine and AMP were found to be biologically

inactive on the quiescent, isolated rat uterus. ATP on the other hand, as in the guinea-pig (Deuticke, 1932), caused pronounced contractile and tonic effects. These observations seem to implicate the high-energy linkages as responsible for the pharmacologic differences between the energy-rich and non-energy-rich derivatives. Previous investigators (Jurascheck, 1932; Flossner, 1934) reported that guanylic acid and guanosine were stimulatory to the guinea-pig uterus, while others claim the nucleotide (von Euler, 1932) and nucleoside (Moulton and others, 1957) to be inert. Drury (1932) found the former compound to be relaxant. The feeble and ephemeral action of GTP compared to ATP again seems to indicate the proper position of the NH_2 group on the purine ring is fundamental to activity and dominates the possession of high-energy bonds by a nucleotide. The inertness of CDPCh was conspicuous and puzzling, for cytidylic acid has been reported (Jono, 1936) to be briefly stimulatory to the rat isolated uterus.

Csapo (1950) has shown with *in vitro* mammalian actomyosin threads that contraction occurs slowly in the presence of ATP. Because of the antagonism of ATP's uterotonic effects by low concentrations of papaverine and (−)-adrenaline it is conceivable that these spasmolytics directly interfere with the contractile protein-ATP reaction, although the possibility still exists that the antagonism is non-specific and may involve, at least for adrenaline, an action on an independent structure which counteracts contractile stimuli (Jensen and Lund, 1960).

Acknowledgements. The authors acknowledge the generosity of the Sigma Chemical Company, St. Louis, Missouri, for graciously supplying certain of the nucleotides employed in this study. Thanks are also given to Dr. K. E. Hamlin of Abbott Laboratories, Chicago, Illinois, for the gift of 5-HT. This investigation was supported, in part, by a Graduate Fellowship from the American Foundation for Pharmaceutical Education.

REFERENCES

Barlow, R. B. (1955). *Introduction to Chemical Pharmacology*, p. 186, New York, John Wiley and Sons, Inc.
Bennet, D. W. and Drury, A. N. (1931). *J. Physiol.*, **72**, 288–320.
Chevillard, L. and Guerin, J. (1955). *C. R. Soc. Biol., Paris*, **149**, 235–238.
Clarke, D. A., Davoll, J., Philips, F. S., and Brown, G. B. (1952). *J. Pharmacol.*, **106**, 291–302.
Cook, R. B. (1926). *J. Physiol.*, **62**, 160–165.
Csapo, A. (1950). *Amer. J. Physiol.*, **160**, 46–52.
David, J. C. (1930). *J. Pharmacol.*, **40**, 229–234.
de Jalon, P. G., Bayo, S. B., and de Jalon, M. G. (1945). *Farmacoter. act.*, **2**, 313.
Deuticke, H. J. (1932). *Pflug. Arch. ges. Physiol.*, **230**, 537–555.
Dikshit, B. B. (1934). *J. Physiol.*, **83**, 42P.
Drury, A. N. (1932). *Ibid.*, **74**, 147–155.
Drury, A. N. (1936). *Physiol. Rev.*, **16**, 292–325.
Drury, A. N. and Szent-Gyorgyi, A. (1929). *J. Physiol.*, **68**, 213–237.
Edwards, D. R. and Booker, W. M. (1959). *Fed. Proc.*, **18**, 41.
Feldberg, W. and Sherwood, S. L. (1954). *J. Physiol.*, **123**, 148–167.
Flossner, O. (1934). *Arch. exp. Path. Pharmak.*, **174**, 245–254.
Gillespie, J. H. (1934). *J. Physiol.*, **80**, 345–359.
Green, H. N. and Stoner, H. B. (1950). *Biological Actions of the Adenine Nucleotides*, pp. 65–103, London, H. K. Lewis and Co., Ltd.
Haley, T. J. and McCormick, W. G. (1957). *Brit. J. Pharmacol.*, **12**, 12–15.
Jensen, K. B. and Lund, R. B. (1960). *Acta pharm. tox., Kbh.*, **17**, 173–181.

Jono, Y. (1936). *J. orient. Med.*, **24**, 1185–1191.
Jurascheck, H. (1932). *Arch. exp. Path. Pharmak.*, **167**, 451–458.
Kanda, Z., Sekiya, A., and Inoue, K. (1954). *Nagoya J. med. Sci.*, **17**, 85–90.
Luduena, F. P. and Brown, T. G., Jr. (1952). *J. Pharmacol.*, **105**, 232–239.
Meyer, A. E. (1951). *Arch. exp. Path. Pharmak.*, **213**, 154–161.
Moulton, R., Spector, W. G., and Willoughby, D. A. (1957). *Brit. J. Pharmacol.*, **12**, 365–370.
Ostern, P. and Parnas, J. K. (1932). *Biochem. Z.*, **248**, 389–397.
Porro, A. (1952). *Boll. Soc. Biol. sper.*, **28**, 1560–1562.
Prosser, C. L. (1940). *Biol. Bull., Wood's Hole*, **78**, 92–102.
Rand, M. J., Stafford, A., and Thorp, R. H. (1955). *Aust. J. exp. Biol. med. Sci.*, **33**, 663–670.
Schnedorf, J. G. and Ivy, A. C. (1939). *Amer. J. Physiol.*, **125**, 491–505.
Sekiya, A. (1953). *Folia pharm. jap.*, **49**, 268–281.
Sollmann, T. and Barlow, O. W. (1926). *J. Pharmacol.*, **29**, 233–255.
Sydow, V. and Ahlquist, R. P. (1954). *J. Amer. pharm. Ass., Sci. Ed.*, **43**, 166–170.
Tower, D. B. and McEachern, D. (1948). *Canad. J. Res.*, **26**, (Sect. E) 183–187.
von Euler, U. S. (1932). *Arch. exp. Path. Pharmak.*, **167**, 171–176.
Wait, R. B. (1943). *Biol. Bull., Wood's Hole*, **85**, 79–85.
Wedd, A. M. and Fenn, W. O. (1933). *J. Pharmacol.*, **47**, 365–375.
Welsh, J. H. (1953). *Arch. exp. Path. Pharmak.*, **219**, 23–29.
Welsh, J. H. (1954). *Fed. Proc.*, **13**, 162.
Welsh, J. H. (1957). *Ann. N.Y. Acad. Sci.*, **66**, 618–630.
Welsh, J. H. and Taub, R. (1948). *Biol. Bull., Wood's Hole*, **95**, 346–353.