to utilize succinate, despite the fact that the substrate concentration greatly exceeded 0.15 mM, the K_m value[10] for beef-heart succinoxidase at 20 °C. If, as in the case of fumarase[11], the K_m decreases with temperature, and if species differences are not significant, the affinity of the enzyme complex for succinate might be expected to be of an even higher order at 4 °C.

Previous studies of 30 hearts have established the value of following the fate of lactate produced metabolically during perfusion[2-4]. Regardless of the state of preservation of the tissues, small quantities (20–300 µmoles) of lactate always appeared in the perfusates during the first 4–6 h. In the cases of well-preserved organs, these amounts had invariably fallen by about 40% within the next 18–20 h; conversely, lactate levels in the perfusates of deteriorating hearts rose by a factor of between two and fourteen within the same period of time[4]. These observations constitute a sensitive and early indication of the state of viability of the tissues. However, when hearts are presented with such large amounts of exogenous lactate, as in the present experiments, the position is far less clear. The fact that an organ continued to metabolise lactate at an appreciable rate for nearly 48 h, even though other criteria[3] had already shown conclusively that the heart was no longer viable, indicates that the presence of a large quantity of added lactate, possibly due to a mass-action effect, does not give information regarding the state of the tissue at a sufficiently early stage.

Although the number of hearts studied so far is small, the clear-cut nature of the results and the pressing need for non-invasive tests of viability have dictated the early presentation of these findings. In selecting the original level of succinate, the aim was to present the tissues with an amount of substrate the oxygen equivalent (30 mmoles) of which was adequate to cover the oxygen requirement of the tissue for 3–4 days. Considerations are necessarily different when a concentration appropriate for monitoring loss of viability is to be selected. The greatest fall in the succinate level yet obtained has been 0.6 mM in 24 h; not all deteriorating tissues would necessarily cause a decrease of this magnitude. Although, in other experiments, a monitoring concentration of 2 mM has been employed, 0.5–1 mM would appear to be a more suitable range if the significance of differences less than 0.6 mM are not to be masked by experimental error, especially if the interval between sampling times needs to be shortened. The phenomenon of lactate production and utilization by the isolated perfused heart and its relationship to organ viability is sufficiently well documented[3,4] to stand. In future investigations the question as to whether or not the fate of added succinate is affected by the introduction of comparatively large amounts of exogenous lactate must be given careful attention.

The problem of whether an organ is genuinely viable or not is of cardinal importance in preservation. The ideal would be to perform rapid and simple tests exclusively on samples of perfusate, thereby presenting no hazard to the organ. While orthotopic transplantation must remain the ultimate test of viability, the procedure is particularly difficult to carry out with success[12]. Detailed discussions of the criteria of viability applied in this investigation have appeared elsewhere[3,6,7].

Résumé. Dans le cœur isolé du chien, perfusé à 4 °C, le succinate de sodium ajouté n'est pas utilisé par des tissus vivants, mais il est métabolisé par le cœur qui a perdu sa viabilité. Le destin du lactate exogène ne donne pas d'informations assez claires sur la viabilité des tissus, tandis que c'est le cas du lactate produit par métabolisme.

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Inhibition of Beef Heart and Rat Brain Nucleoside-3′:5′-Monophosphate Phosphodiesterase by 3β-14-Dihydroxy-21-oxo-23-desoxo-5-β-card-20(22)-enolide 3-acetate (AY-17,605) and Structurally Related Compounds

The importance of cyclic adenosine-3′:5′-monophosphate (cyclic-AMP) as an intracellular mediator of various hormone actions is well documented[1]. Nucleoside-3′:5′-monophosphate phosphodiesterase (PDE) hydrolyzes cyclic-AMP to AMP and appears to be of relevance as a control mechanism for the intracellular levels of cyclic-AMP. Various compounds which have been demonstrated to be inhibitors of PDE have also been shown to affect, i.e. increase, hormonal actions. Recently, a member of a novel series of compounds which are isomeric to the natural cardiac glycosides has been found to exhibit properties of cardiotonic agents[2]. These isomers have the steroid nucleus attached to the α-carbon instead of the β-carbon of the α, β-unsaturated lactone (Figure). The inhibitory activities of compounds of this series on beef heart PDE and rat brain PDE have been determined in the present studies.

Materials and methods. The measurements of PDE activity were carried out essentially as described previously[3,4]. The beef heart PDE (Boehringer Mannheim) was a dialyzed preparation; the rat brain PDE was prepared as described previously[3]. The protein concentration was determined by the method of Lowry et al.[5]. The enzyme assay contained beef heart PDE (0.12 mg protein/ml) or rat brain PDE (0.095 mg protein/ml), snake venom (1 mg/ml) and bovine albumin (2 mg/ml) in 60 mM *Tris*-HCl buffer, pH 7.8 with 5 mM mercaptoethanol. The PDE was omitted in the blanks. 50 µl of the enzyme preparation were added to a glass scintillation vial containing 5 µl of the test compound in dimethyl sul-

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phoxide and 50 µl water and the vial preincubated at 30 °C for 5 min. 50 µl ^{3}H-cyclic-AMP in 120 m*M* *Tris*-HCl buffer, pH 7.8, containing 120 m*M* $MgCl_2$ were added and the reaction was terminated after 1 min in the case of the beef heart PDE and 1.5 min with the rat brain PDE by the addition of 1 ml of a 50% suspension of Dowex 1. After 10 min, 15 ml of scintillation solvent[3] were added and the samples counted.

The test compounds were also examined to insure that they did not affect the snake venom nucleotidase or interfere in the radioactivity determination.

The substances used were beef heart PDE, Boehringer Mannheim (15153 EPAY, control No. 7151212); RUSSELL's viper venom, Sigma Chemical Co.; [8-^{3}H] cyclic adenosine-3′:5′-monophosphate (14.3–14.5 C/mmole), Schwarz-Mann; Dowex 1-X2 minus 400 mesh chloride form, Bio-Rad Laboratories; digitoxin, Gedeon Richter Ltd; theophylline monoethanolamine, K. and K. Laboratories; the AY compounds (Figure) were synthesized by Dr. J.M. FERLAND, Ayerst Laboratories.

By varying the substrate and/or inhibitor concentrations the K_m for each phosphodiesterase was determined by the method of LINEWEAVER and BURK[6] and the K_I for inhibitors by the method of DIXON[7]. By plotting % inhibition versus the logarithm of inhibitor concentration the concentration at which 50% inhibition of the hydrolysis occurred was determined (I_{50}). Each point represents the mean of triplicate determinations. The curves represent the best fit, obtained by the method of least squares.

Results and discussion. The concentration of theophylline which caused a 50% inhibition of beef heart PDE was 108 µ*M* (Table A). Digitoxin was 3 times less active than theophylline. Formation of the 3-hemisuccinyl derivative, i.e. digitoxigenin hemisuccinate (AY-17,611), did not alter the activity. Presence of the α-attachment to the lactone in the latter derivative (AY-22,252) resulted in a 2-fold increase in the respective activity.

In the isomeric lactone derivatives replacement of the 3-hemisuccinyl by a 3-acetyl moiety yielded a derivative (AY-17,605) 3 times more active and thus 8 times more potent than digitoxin. The activity decreased to the level of that of digitoxin when the replacement was by a 3β-D-glucopyranosyl group (AY-22,241). The most potent derivative AY-17,605 was about twice as active as theophylline.

The findings indicate the importance of the nature of the substituent on position 3 in the isomeric lactone derivatives of digitoxin with the small acetyl group yielding the highest activity and the activity decreasing with the larger 3-hemisuccinyl and 3β-D-glucopyranosyl groups. Also, presence of the α-attachment to the lactone

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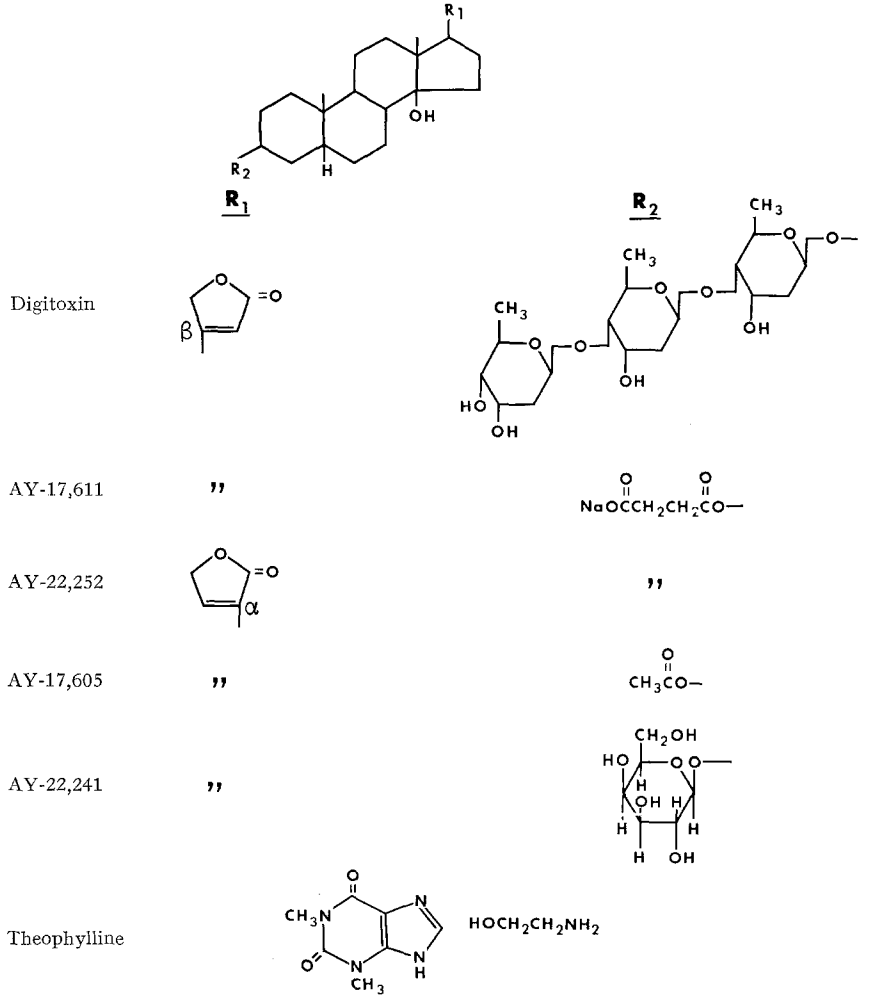

Structures of compounds studied

Inhibition of Nucleoside-3′:5′-Monophosphate Phosphodiesterase

A) Beef heart

Compound	I_{50} (μM)
AY-17,605	44
Theophylline	108
AY-22,252	150
AY-17,611	320
Digitoxin	350
AY-22,241	540

Final concentration of cyclic-AMP: $1.16 \times 10^{-7} M$

B) Rat brain

Compound	% Inhibition at $1 \times 10^{-4} M$
Theophylline	35
AY-17,605	24
Digitoxin	9
AY-22,252	7
AY-17,611	7
AY-22,241	3

Final concentration of cyclic-AMP: $0.58 \times 10^{-7} M$

yields a more active derivative than the natural lactone in the case examined.

When the various compounds were examined at 1×10^{-4} M for their inhibitory effect on the rat brain PDE, theophylline exhibited a moderate inhibition (35%) (Table B). None of the other compounds, i.e. AY-17,605, digitoxin, AY-22,252, AY-17,611 and AY-22,241, exhibited a greater activity.

The LINEWEAVER-BURK plot showed the K_m for the beef heart PDE was 33.3×10^{-7} M and 6.25×10^{-7} M for the rat brain PDE. With the beef heart PDE, and the cyclic-AMP at substrate concentrations of 0.875 and 1.75×10^{-7} M, AY-17,605 exhibited a K_I of 0.64×10^{-4} M as determined from the Dixon plot and its inhibition was of the non-competitive type. The K_I for theophylline was 1.3×10^{-4} M and the inhibition was also of a non-competitive nature.

The type of inhibition with theophylline apparently can vary according to the nature of the enzyme preparation utilized and the assay conditions employed as theophylline has also been reported to exhibit a non-competitive type in studies with dog heart and frog erythrocytes[8,9], competitive type with rat brain and beef heart[10,11] and mixed type with beef heart and rat erythrocytes[4,12].

In comparison with theophylline, the above factors appear to be of importance with regard to the inhibitory activity of AY-17,605 as indicated by the findings that AY-17,605 was more potent than theophylline with respect to the beef heart PDE but not to the rat brain PDE.

AY-17,605 could act to prevent the degradation of cyclic-AMP through its inhibitory action on heart PDE with higher levels of cyclic-AMP thereby resulting. This activity of AY-17,605 might be of importance with respect to its actions as a cardiotonic agent. Of interest in regard to the present findings was the demonstration that a different type of compound, i.e. 4-(3,4-dimethoxybenzyl)-2-imidazolidinone (RO 7-2956), which exhibited actions of a cardiotonic agent[13], was also an inhibitor of PDE[12], i.e. rat erythrocyte PDE.

Résumé. Des composés isomères aux glycosides cardiaques naturels ont démontré l'inhibition in vitro de l'enzyme nucleoside-3′:5′-monophosphate phosphodiesterase (PDE) d'origine cœur du bœuf exercée sur le cyclic-AMP, le composé le plus actif étant un dérivé de la lactone isomérique contenant une fraction 3-acetyl (AY-17,605); le K_I du composé AY-17,605 était de 0.64×10^{-4} M et le procédé inhibiteur était d'une nature non-compétitive. Aucun des composés n'a exhibé une activité supérieure autre qu'une inhibition modérée du PDE dans le cerveau du rat.

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Amino Acids in the Excreta of the Tsetse Fly, *Glossina palpalis*

The excretion of nitrogenous waste products has been investigated in many insects. Our knowledge on the subject has been reviewed by WIGGLESWORTH[1] and CRAIG[2]. But little information is available regarding the excretory products of hematophagous insects[3], particularly the tsetse fly[4]. The present work has been undertaken to determine the number and quantity of amino acids in the faeces of *Glossina palpalis*, and to provide a basis for further metabolic investigations.

Materials and methods. The flies used in this study were *Glossina palpalis* obtained as pupae from the Nigerian Institute for Trypanosomiasis Research, Kaduna, where enclosed colonies have been maintained for many generations. The pupae were reared at the Tsetse Research Laboratory, Langford, Bristol, and maintenance techniques of emergent flies were essentially the same as those described by NASH et al.[5]. Environmental conditions were maintained at about 25 °C and 80% r.h. Flies were kept in Geigy 10 type of cages and were fed on lop-eared rabbit blood. Immediately after feeding, the fly cages were

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