heating in aqueous N-NaOH (12·0 ml.). The solution, cooled to room temp., was mixed with a cold solution of $KMnO_4$ (2·107 g.≡3 atoms of O) in water (100 ml.). The mixture was kept at room temp. overnight and acidified with 2N-H_2SO_4, and SO_2 was passed to give a clear colourless solution, which was evaporated to about 50 ml. The resulting colourless crystalline solid (1·38 g.; m.p. 180–181° with effervescence) was collected, and ether extraction of the filtrate gave 0·94 g. of less pure material, m.p. 140–150°. The first crop was purified by crystallization from water, giving 3:4-*dimethoxy*-5-*methylphthalic acid* as colourless prisms, m.p. 184–185° (with effervescence) (Found: C, 54·6; H, 4·99; OMe, 26·2%; titration equiv., 123·6. $C_{11}H_{12}O_6$ requires C, 55·0; H, 5·03; 2 OMe, 25·8%; equiv. titrating as a dibasic acid, 120·1).

The above acid (114 mg.) was heated at 185–190° for 10 min., cooled and sublimed in high vacuum at 85–90°. The sublimate (96 mg., m.p. 114°) was crystallized from dry *iso*propyl ether, giving 3:4-*dimethoxy*-5-*methylphthalic anhydride* (61 mg.) as colourless needles, m.p. 118° (Found: C, 59·5; H, 4·49; OMe, 27·6. $C_{11}H_{10}O_5$ requires C, 59·4; H, 4·53; 2 OMe, 27·9%).

SUMMARY

1. Asperthecin has been shown to be either 3:4:5:6:7-pentahydroxy-2-hydroxymethylanthraquinone or 3:4:5:7:8-pentahydroxy-2-hydroxymethylanthraquinone.

2. It has been confirmed that full methylation of polyhydroxyanthraquinones with ethereal diazomethane is greatly facilitated by the addition of methanol to the methylation mixture.

3. The syntheses of 4-carboxy-3:6-dimethoxyphthalic anhydride, 5-carboxy-3:4-dimethoxyphthalic acid and its anhydride and 3:4-dimethoxy-5-methylphthalic acid and its anhydride are described.

4. 5-Carboxy-3:4-dimethoxyphthalic anhydride has been identified as an oxidation product of asperthecin tetra- and penta-methyl ethers.

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The Action of Di*iso*propyl Phosphorofluoridate and Other Anticholinesterases on Amino Acids

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The work described in this paper, which was carried out in 1950–52 and which has been the subject of a preliminary communication (Ashbolt & Rydon, 1952), was undertaken in an attempt to find some chemical parallel for the marked biochemical and toxicological difference between di*iso*propyl phosphorofluoridate (DFP) and its chlorine-containing analogue di*iso*propyl phosphorochloridate (DClP); as is well known, DFP is very toxic and a potent inhibitor of several esterases (notably cholinesterase, chymotrypsin and trypsin), whereas DClP is not markedly toxic and is not a powerful enzyme inhibitor. The discrepancy between the chemical and biochemical activities of the two compounds is very striking, since in general DClP is chemically the more reactive of the two (cf. Saunders & Stacey, 1948; Wagner-Jauregg, O'Neill & Summerson, 1951).

It is well established that the inhibition of chymotrypsin by DFP involves the di*iso*propylphosphorylation of a single amino acid side chain (Jansen, Nutting, Jang & Balls, 1949, 1950; Jansen, Nutting & Balls, 1949) and the same mechanism is highly probable for the inhibition of other enzymes by DFP. We decided accordingly to study the action of DFP and DClP on a number of amino acids under 'physiological' conditions (aqueous solution, pH about 7, 37°) in the hope of finding some difference between the two phosphorus compounds in this respect; a number of other anticholinesterases were included for the sake of completeness.

Mazur (1946) found that the rate of liberation of acid, as measured by carbon dioxide evolution, from

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aqueous sodium bicarbonate by DFP was unaffected by the presence of glycine, serine, arginine, glutamic acid, cysteine and tyrosine, and concluded that there was no reaction between DFP and any of these amino acids under the conditions of his experiments. This argument is, however, not sound, since acid may be liberated at the same rate by reaction with water and with amino acids. The presence or absence of reaction can be established only by direct estimation of the reactive grouping in the amino acid concerned, and this procedure has been used in the present work.

EXPERIMENTAL

Quantitative experiments

Analytical methods. A Hilger Spekker photoelectric absorptiometer, with Ilford Spectrum Filters, was used for all the colorimetric estimations. Fluoride ion was estimated by the thorium nitrate titration method of Milton, Liddell & Chivers (1947, 1949), with Chrome Azurol S as indicator. α-Amino groups were estimated colorimetrically (Yellow Filter no. 606) by the method of Moore & Stein (1948), with the modified ninhydrin reagent of Rydon & Smith (1955). Phenolic hydroxyl (tyrosine side chain) groups were estimated colorimetrically, either (Expts. 1 and 2 of Table 2) by the method of Thomas (1944) (Green Filter no. 604) or by the method of Folin & Ciocalteu (1927) (Red Filter no. 608). Alcoholic hydroxyl (serine side chain) groups were estimated volumetrically (Van Slyke, Hiller & MacFadyen, 1941).

Procedure. The requisite amount of the anticholinesterase was weighed into a 50 ml. calibrated flask. Water (about 40 ml.), at 40°, was added, followed at once by a solution of the requisite amounts of $NaHCO_3$ and the amino acid in a little water. The volume was then made up to 50 ml. with water and the flask placed in a thermostat, samples being withdrawn from time to time for analysis.

Table 1. *Liberation of fluoride ion from DFP in the presence of sodium bicarbonate at* 38·2 ± 0·2°

Initial concentrations (moles/l.)				
DFP	$NaHCO_3$	Tyrosine	Phenyl-alanine	$10^4 k_1$ (min.$^{-1}$)
0·0235	0·0856	0	0	6·0
0·0235	0·0856	0	0·0053	6·1
0·022	0·0856	0·0055	0	6·2

Table 2. *Reaction of anticholinesterases with amino acids*

Temperature: 37·0° (38·2° in Expts. 1 and 2). Abbreviations: *t*, reaction time (hr.); *A*, % α-NH_2 not reacting; *B*, % side-chain functional group not reacting; DFP, di*iso*propyl phosphorofluoridate; DClP, di*iso*propyl phosphorochloridate; TEPP, tetraethyl pyrophosphate; E 600, diethyl *p*-nitrophenyl phosphate; TFPA, *NN'*-tetramethylphosphorodiamidic fluoride, $(Me_2N)_2PO\cdot F$. All the recorded experiments were continued for at least 20 hr., but there was no significant further reaction after the times given below. No significant reaction was observed, under similar conditions, of DFP with phenylalanine, of DClP with serine and phenylalanine, of E 600 (concentrations about one-quarter of those used in the other experiments) with tyrosine, serine and phenylalanine, or of TFPA with tyrosine, serine and phenylalanine.

Expt. no.	Reactants								
1	Tyrosine, 0·0033 M	*t*	3	4	6	9			
	DFP, 0·013 M	*A*	100	—	93	—			
	$NaHCO_3$, 0·085 M	*B*	57	50	—	48			
2	Tyrosine, 0·0028 M	*t*	0·5	1	4	9			
	DClP, 0·0092 M	*A*	—	93	94	95			
	$NaHCO_3$, 0·085 M	*B*	100	—	99	95			
3	Tyrosine, 0·0032 M	*t*	0·5	1	1·5	3	4	5	6
	DFP, 0·013 M	*A*	—	—	95	94	—	95	—
	$NaHCO_3$, 0·053 M	*B*	85	75	71	—	58	—	57
4	Tyrosine, 0·0032 M								
	DClP, 0·013 M	*t*	0·5	1	1·5	4	6		
	$NaHCO_3$, 0·053 M	*B*	100	98	98	95	97		
5	Tyrosine, 0·0032 M	*t*	0·5	1	1·5	3	4	5	
	TEPP, 0·013 M	*A*	—	—	82	81	—	82	
	$NaHCO_3$, 0·052 M	*B*	77	77	74	—	74	—	
6	Serine, 0·003 M								
	DFP, 0·012 M	*t*	0·5	1·5	3·5	5·5			
	$NaHCO_3$, 0·048 M	*A*	98	99	97	98			
7	Serine, 0·0048 M								
	DFP, 0·021 M	*t*	0·5	1	2·5	4	7·5		
	$NaHCO_3$, 0·081 M	*B*	100	100	100	99	100		
8	Serine, 0·002 M	*t*	1	1·5	2	3	4	5	
	TEPP, 0·0078 M	*A*	98	93	—	92	—	94	
	$NaHCO_3$, 0·032 M	*B*	95	—	97	—	95	94	
9	Phenylalanine, 0·0035 M								
	TEPP, 0·013 M	*t*	2	4	6	9			
	$NaHCO_3$, 0·051 M	*A*	83	72	74	72			

Table 3. *R_F values of spots appearing on chromatograms of reaction products of amino acids and anticholinesterases*

Abbreviations are as for Table 2. Solvents: A, *n*-Butanol–acetic acid–water (4:1:5); B, *n*-butanol–pyridine–water (65:35:65); C, phenol saturated with water.

Amino acid	Developing solvent	Anti-cholinesterase					
		None	DFP	DClP	TEPP	E600	TFPA
Tyrosine (3 mM)	A	0·21	0·21 0·61	0·21	0·21 0·51	0·21	0·21
Serine (5 mM)	B	0·15	0·15	0·15	—	—	—
Serine (2 mM)	A	0·04	—	—	0·02 (trace) 0·05	0·05	0·05
Lysine (2 mM)	C	0·25	0·23	0·24	0·25* 0·71*	0·22	0·25
Cysteine (5 mM)	C	0·11	0·12	0·10	0·12	0·11	0·10
Hydroxyproline (5 mM)	C	0·55	0·56	0·55	0·55	0·55	0·54
Arginine (5 mM)	C	0·69	0·65	0·62	0·65	0·69	0·65
Tryptophan (5 mM)	C	0·71	0·69	0·70	0·70	0·70	0·71
Asparagine (5 mM)	C	0·32	0·32	0·33	0·32	0·30	0·32

* Neither spot was visible when the paper was dusted with copper carbonate before being sprayed with ninhydrin (Crumpler & Dent, 1949); this shows that both contain free α-amino groups.

Liberation of fluoride ion. The rate of liberation of fluoride ion satisfactorily obeys first-order kinetics, and the results are accordingly expressed, in Table 1, in terms of the first-order velocity constant, k_1.

Disappearance of amino acid functional groups. Owing to the considerable hydrolysis which accompanies reaction with the amino acids, the results cannot be expressed in simple kinetic terms; they are collected in Table 2.

Qualitative experiments

The amino acids, in the concentrations indicated in Table 3, were kept in aqueous solution at 37° for 24 hr. with the inhibitor (4 mol.) and $NaHCO_3$ (16 mol.). The resulting solutions were spotted on Whatman no. 1 filter paper and the chromatograms developed with the solvent mixtures shown in the table; after drying, the spots were revealed by spraying with ninhydrin followed by heating at 100°. The results are summarized in Table 3.

Isolation and synthesis of reaction products

Isolation. (*a*) L-Tyrosine (3 g.), DFP (25 ml.) and $NaHCO_3$ (25 g.) were stirred in water (100 ml.) at 45° for 11 days. The mixture was then filtered and the solid washed with water until paper chromatography showed the washings to be free of the required product. The filtrate and washings were then evaporated to dryness under reduced pressure and the residue was extracted thoroughly with absolute ethanol. The extract was evaporated to dryness under reduced pressure and the residue dissolved in water (55 ml.); this solution was run through a column (14 cm. × 2·3 cm.) of charcoal ('decolorizing charcoal', British Drug Houses Ltd.) (28 g.), deactivated with KCN (Schramm & Primosigh, 1943). Elution was carried out with 5% (w/v) phenol in 20% (v/v) acetic acid, and those fractions found by paper chromatography to contain the required product were evaporated and freed from phenol by steam-distillation under reduced pressure. Two precipitations from ethanol with ether, followed by two recrystallizations from water, afforded *diisopropyl* L-*tyrosine* O-*phosphate* (350 mg.; 61%) as stout needles, m.p. 167–168°, $[\alpha]_D^{23}$ − 3·0° in water (*c*, 0·8) (Found: C, 51·8; H, 6·9; N, 4·0. $C_{15}H_{24}O_6NP$ requires C, 52·2; H, 7·0; N, 4·1%).

(*b*) L-Tyrosine (1 g.) was stirred with DFP (4 ml.) in 8% (w/v) aqueous $NaHCO_3$ (150 ml.) at 40° for 3 days; more DFP (4 ml.) was then added and the mixture again stirred overnight. The product was filtered and the filtrate treated with $NaHCO_3$ (2·5 g.) and 1-fluoro-2:4-dinitrobenzene (2·5 g.) in ethanol (7 ml.). After being shaken for 3 hr. at room temp., the mixture was concentrated to 70 ml. and then diluted to 250 ml. with water and extracted thoroughly with ether. The residual solution was acidified and ether was added. The crystalline precipitate (1·5 g.; 53%) was collected by filtration and recrystallized, thrice from aqueous methanol and twice from anhydrous methanol. *Diisopropyl* N-*2:4-dinitrophenyl*-L-*tyrosine* O-*phosphate* forms needles, m.p. 159–160° (Found: C, 49·4; H, 5·1; N, 7·9. $C_{21}H_{26}O_{10}N_3P$ requires C, 49·3; H, 5·1; N, 8·2%). The same compound, m.p. and mixed m.p. 157–160°, was obtained by treatment of di*iso*propyl L-tyrosine *O*-phosphate with 1-fluoro-2:4-dinitrobenzene under similar conditions.

Synthesis. L-Tyrosine ethyl ester hydrochloride (6·8 g.) was shaken at 0° with $CHCl_3$ (100 ml.) and N-NaOH (34 ml.) until solution was complete. *p*-Bromobenzyl chloroformate (6·8 g.) and a solution of Na_2CO_3 (4·1 g.) in water (30 ml.) were then added, in alternate small portions, with vigorous shaking, during 20 min. More $CHCl_3$ (200 ml.) was added and the organic layer separated, washed with 2N-HCl, dried and evaporated. Recrystallization of the residue from $CHCl_3$–light petroleum (b.p. 60–80°) gave N-p-*bromobenzyloxycarbonyl*-L-*tyrosine ethyl ester* (9·4 g.; 82%) as needles, m.p. 117–119°; an analytical specimen, thrice recrystallized from $CHCl_3$–CCl_4 and once from light petroleum (b.p. 60–80°), had m.p. 120–121° (Found: C, 54·1; H, 4·9; N, 3·5. $C_{19}H_{20}O_5NBr$ requires C, 54·0; H, 4·7; N, 3·3%).

This ester (2 g.) was shaken at room temp. with N-NaOH (10 ml.). Solution was complete in 10 min. and 0·5N-HCl (40 ml.) was added after 15 min. The crystalline precipitate was dissolved in 3% sodium acetate (50 ml.) and reprecipitated by acidification. Three crystallizations from 30% (v/v) ethanol and one from ethyl acetate–light petroleum (b.p. 60–80°) gave N-p-*bromobenzyloxycarbonyl*-L-*tyrosine* (1·6 g.; 85%) as needles, m.p. 156–157° (Found: C, 51·7; H, 4·1; N, 3·4. $C_{17}H_{16}O_5BNr$ requires C, 51·8; H, 4·1; N, 3·6%).

This compound (2·3 g.), in water (70 ml.) containing Na_2CO_3 (15 g.), was stirred for 40 hr. at 40° with DFP

(20 ml.). The mixture was extracted with ether and the residual aqueous solution acidified. The precipitated oil was extracted with ether and the dried extract evaporated to dryness, yielding a gum (2·3 g.). A portion (1 g.) of this was dissolved in $CHCl_3$–light petroleum (b.p. 60–80°); on cooling the solution deposited *di*iso*propyl* N-p-*bromobenzyloxycarbonyl*-L-*tyrosine* O-*phosphate* (390 mg.; 29%), m.p. 85–95° [Found: C, 49·7; H, 5·0; N, 2·8%; equiv. (by titration), 550. $C_{23}H_{29}O_8NBrP$ requires C, 49·5; H, 5·2; N, 2·5%; equiv. 558]. The remaining gum (1·3 g.) was dissolved in methanol (10 ml.) and shaken in hydrogen in the presence of Adams's catalyst (100 mg.). Evolution of CO_2 became very slow after 4·5 hr. and the filtered solution was then evaporated to dryness. The residue failed to crystallize, but on paper chromatography with butanol–acetic acid–water (4:1:5) gave only one spot, R_F 0·63, inseparable from di*iso*propyl L-tyrosine *O*-phosphate. Treatment with fluoro-2:4-dinitrobenzene in aqueous ethanolic Na_2CO_3 as usual gave di*iso*propyl *N*-2:4-dinitrophenyl-L-tyrosine *O*-phosphate, m.p. 157–159 , not depressed on admixture with material prepared from DFP and L-tyrosine directly.

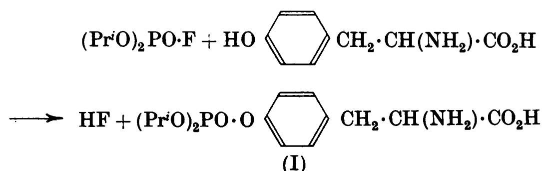
Hydrolysis. The rates of hydrolysis of di*iso*propyl L-tyrosine-*O*-phosphate in 0·012 M solution in N-NaOH at 37° and in 0·011 M-solution in N-H_2SO_4 at 100° were determined, the liberation of phenolic hydroxyl groups being followed colorimetrically by the method of Folin & Ciocalteu (1927). The reactions satisfactorily obeyed first-order kinetics, with the following constants: alkaline hydrolysis, $k_1 = 0·0046$ min.$^{-1}$; acid hydrolysis, $k_1 = 0·0016$ min.$^{-1}$.

N-(*Di*iso*propylphosphoro*)-DL-*phenylalanine*. DClP (2·9 g.) was added at 0° to DL-phenylalanine (2·0 g.) in ethanolic sodium ethoxide (from sodium, 0·6 g., and ethanol, 100 ml.). After 5 hr. at 0°, the solution was filtered and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in ice–water and extracted with ether; acidification of the aqueous residue, followed by extraction with ether, afforded N-(*di*iso*propylphosphoro*)-DL-*phenylalanine* (2·1 g.; 53%) as needles, m.p. 126–130°, raised to 133–134° by recrystallization from CCl_4–benzene and $CHCl_3$–hexane [Found: C, 55·0; H, 7·3; N, 4·3%; equiv. (by titration), 321. $C_{15}H_{24}O_5NP$ requires C, 54·7; H, 7·3; N, 4·3%; equiv., 329].

RESULTS

Our findings concerning the action of DFP and DClP, and three other anticholinesterases, on nine amino acids are summarized in Table 4.

It is clear that DFP differs markedly from DClP in that it alone reacts appreciably with the phenolic hydroxyl group of tyrosine under 'physiological' conditions; the nature of the reaction as a di*iso*propylphosphorylation of the phenolic hydroxyl group has been completely established by isolation of the product (I) and its identification by direct comparison with a synthetic specimen. [Jandorf, Michel, Schaffer, Egan & Summerson (1955) claim that Jandorf, Wagner-Jauregg, O'Neill & Stolberg (1952) have also obtained 'evidence for a phosphorylation of tyrosine by alkylphosphorohalidates in model systems', but reference to the latter paper reveals no direct evidence to support this claim.]



Tyrosine, alone of the nine amino acids studied, competes successfully with water for DFP at pH 7–8. The rate of liberation of fluoride ion is not noticeably affected by the presence of tyrosine (Table 1), a circumstance which explains the failure of Mazur (1946) to observe the reaction. A minor reaction with DFP is the di*iso*propylphosphorylation of the α-amino group which occurs, to a small extent, with both tyrosine and phenylalanine; this property is also possessed, but to a lesser degree, by DClP.

Table 4. *Action of some anticholinesterases on amino acids*

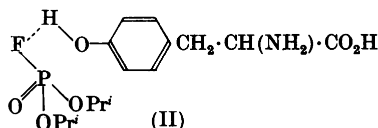
Abbreviations are as for Table 2. + indicates reaction product detected and – not detected on chromatogram. Figures in parentheses are approximate percentage reaction at 37–38°, pH 7·8 after 24 hr.

Amino acid	Anticholinesterase				
	DFP	DClP	TEPP	E600	TFPA
Tyrosine (α-amino)	(5)	(5–10)	(20)		
(hydroxyl)	+ (50)	– (0)	+ (20)	– (0)	– (0)
Phenylalanine (α-amino)	(5)	(0)	(30)	(0)	(0)
Serine (α-amino)	(0)		(10)		
(hydroxyl)	– (0)	– (0)	tr. (5)	– (0)	– (0)
Lysine (ε-amino)	–	–	+	–	–
Cysteine	–	–	–	–	–
Hydroxyproline	–	–	–	–	–
Arginine	–	–	–	–	–
Tryptophan	–	–	–	–	–
Asparagine	–	–	–	–	–

Of the other anticholinesterases we have studied only TEPP has been found to react with amino acids under the conditions of our experiments. Tetraethyl pyrophosphate (TEPP) differs from DFP in being much less specific in its reactivity towards amino acids, reacting, not only with the phenolic hydroxyl group of tyrosine, but also, and with comparable ease, with the α-amino groups of tyrosine and phenylalanine and with the ϵ-amino group of lysine; TEPP is slightly reactive towards both the α-amino and β-hydroxy groups of serine and is the only one of the anticholinesterases we have found to react at all with serine under the conditions of our experiments.

DISCUSSION

The marked difference in the reactivities of DFP and DClP towards the phenolic hydroxyl group of tyrosine suggests some connexion between the similar difference in the capacities of fluorine and chlorine to form hydrogen bonds, and it seems likely that the reaction of DFP with tyrosine involves a hydrogen-bonded intermediate, such as (II);



hydrogen-bonded intermediates, of somewhat different type, have been postulated by Epstein, Rosenblatt & Demek (1956) in the reaction of phosphorofluoridates with catechol.

The relevance of our findings for the important question of the biochemical mode of action of DFP is not clear. When our results were obtained it was tempting to conclude that the inhibition of esterases by DFP was due to di*iso*propylphosphorylation of one of the tyrosine side chains in the enzyme molecule (cf. Ashbolt & Rydon, 1952), especially since such side chains have been indicated as being involved in the active centres of both cholinesterase (Wilson & Bergmann, 1950) and chymotrypsin (Sizer, 1945); there are, however, several major pieces of evidence which stand in the way of this obvious conclusion. First, *O*-phosphorylserine and peptides of *O*-phosphorylserine have been isolated from hydrolysates of DFP-inhibited enzymes (Schaffer, May & Summerson, 1953, 1954; Cohen, Oosterbaan & Warringa, 1954, 1955; Schaffer, Harshmann & Engle, 1955; Schaffer, Harshmann, Engle & Drisko, 1955; Schaffer, Engle, Simet, Drisko & Harshmann, 1956; Turba & Grundlach, 1955; Oosterbaan, Kunst & Cohen, 1955; Cohen, Oosterbaan, Warringa & Jansz, 1955), although it is recognized that this is not proof of primary attack at a serine side chain (cf. Jandorf *et al.* 1955). Secondly, the reaction of DFP with tyrosine is very slow compared with its inhibiting action on esterases. Thirdly, there is evidence which suggests that histidine may be involved in the primary attack (Wilson & Bergmann, 1950; Doherty & Vaslow, 1952; Wagner-Jauregg & Hackley, 1953; Weil, James & Buchert, 1953). Fourthly, Jandorf *et al.* (1955) have shown that tyrosine is involved in a slow further reaction of DFP with enzymes, subsequent to the initial fast inactivation, and other proteins. None of the current views on the mode of action of DFP are, however, capable of explaining the fact that one, and only one, amino acid side chain is involved in the inactivation process, whereas the enzyme molecules all contain more than one residue of the implicated amino acids. It seems clear that the inhibition process involves some exceptionally reactive side chain, which presumably owes its enhanced reactivity to some form of interaction with neighbouring side chains; a constellation of side chains, rather than a single side chain, is involved, and the question whether this contains one or more of the amino acids serine, histidine and tyrosine must remain open for the present.

It is clear from Table 4 that TEPP is more reactive, but less selective, towards amino acids than DFP; this may be of significance in view of the notable differences between DFP- and TEPP-inhibited enzymes towards reactivation (cf. Wilson, 1955).

SUMMARY

1. The action of di*iso*propyl phosphorofluoridate, di*iso*propyl phosphorochloridate and some other phosphorus-containing anticholinesterases on a number of amino acids under 'physiological' conditions has been studied.

2. Di*iso*propyl phosphorofluoridate, but not di*iso*propyl phosphorochloridate, has been shown to react with the phenolic hydroxyl group of tyrosine under these conditions.

3. Tetraethyl pyrophosphate is more reactive, but less selective, than di*iso*propyl phosphorofluoridate towards amino acids.

4. The significance of these findings for the problem of the mode of action of di*iso*propyl phosphorofluoridate and other anticholinesterases is discussed briefly.

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The Catabolism of Glucose by Strains of *Trypanosoma rhodesiense*

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The African trypanosome, *Trypanosoma rhodesiense*, which in man causes sleeping sickness, has been maintained by blood passage in rats for many years. When isolated from rat blood, glucose or another carbohydrate is essential for the *in vitro* survival of the parasites and under these conditions the catabolism of glucose results in the formation of a number of extracellular end products (Fulton & Stevens, 1945), although the amounts of pyruvate and glycerol account for almost all the glucose utilized (Ryley, 1956). We have now used ^{14}C-labelled glucose to study the metabolic pathways involved in the formation of pyruvate and glycerol and in the further catabolism of pyruvate by the same strain of *T. rhodesiense*, its drug-resistant variants and a new strain freshly isolated from a human patient. In addition, experiments are described which indicate that pyruvate is also a major end product of the growing trypanosome *in vivo*.

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Some of these results have already been reported in a preliminary communication (Grant & Fulton, 1956).

MATERIALS AND METHODS

Strains of trypanosomes. Two distinct strains of *T. rhodesiense* were maintained in the rat as host.

The Liverpool strain ('L' strain) was isolated in 1923 from a human patient and has subsequently been maintained in either mice or rats by blood inoculation. The atoxyl-resistant variant of this strain was obtained from the Liverpool School of Tropical Medicine (Yorke & Murgatroyd, 1930). The stilbamidine-resistant variant was prepared as described by Fulton & Grant (1955).

A new strain, termed 'Maun' ('M' strain), which had been isolated from a human patient only a few weeks before the present experiments, was obtained through the courtesy of Dr W. E. Ormerod, London School of Hygiene and Tropical Medicine.

In the experiments which are to be described, the 'L' strain was used exclusively except where indicated.

Direct estimation of trypanosome numbers. Trypanosomes in infected blood or in a saline suspension were determined