Changes of certain blood constituents after the administration of colchicine

	Colchicine administered		Controls	
	No. of animals	Mean ± S. E.	No. of animals	Mean ± S. E.
Unesterified Fatty acids mEq/l	30	0·58 ± 0·033*	41	0·26 ± 0·015*
Esterified Fatty acids mEq/l	35	7·6 ± 1·55	40	8·9 ± 0·32
Total cholesterol mg%	15	63 ± 5·1	23	66 ± 1·04
Free cholesterol mg%	5	23 ± 2·7	5	22 ± 2·7
Hematocrit %	4	57 ± 3·5	5	51 ± 1·1
Hemoglobin g%	4	19·5 ± 1·2	5	17·3 ± 0·36
Plasma proteins g%	10	7·7 ± 0·3	15	7·5 ± 0·32
Δ body weight g	32	− 14·7 ± 0·9*	35	− 3·7 ± 1·6*

* Difference highly significant.

Specific gravity[7], UFA[8], esterified fatty acid[9], and total and free cholesterol[10] concentrations were determined in each plasma. All chemical analyses were performed in duplicate.

Results. The Table shows that the administration of colchicine caused an increase in UFA levels together with a loss in body weight. Both of these changes were statistically highly significant. Esterified fatty acids, total and free cholesterol, hematocrit, hemoglobin, and plasma protein contents of the blood did not change.

Discussion. Under normal conditions lipids can be mobilized from the adipose tissue in the form of UFA[11,12]. Unesterified fatty acids are considered the most active lipid metabolites and are taken up constantly by the liver[12]. The increase of UFA in CCl_4[5,6], as well as in colchicine poisoned animals indicates either a pathological derangement of the liberation of UFA by the adipose tissue, or a change in the uptake and utilization of this metabolite by the liver and possibly by other organs. The importance of the kidneys in this respect remains to be investigated.

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Department of Physiology, Hahnemann Medical College, Philadelphia (Pa.), October 1, 1958.

Zusammenfassung

Die Verabreichung einer letalen Dosis Colchicin verursacht u. a. eine Zunahme der unveresterten Fettsäuren und eine Abnahme des Körpergewichts. Der Gehalt an veresterten Fettsäuren, freiem und verestertem Cholesterin und Plasmaeiweissen bleibt unverändert, ebenso Hämatokrit- und Hämoglobinwerte.

[8] M. I. Grossman, M. Palm, G. H. Becker, and H. C. Moeller, Proc. Soc. Exp. Biol. Med. *87*, 312 (1954).

[9] I. Stern and B. Shapiro, J. Clin. Path. *6*, 158 (1954).

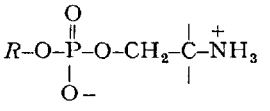
[10] H. H. Brown, A. Zlatkis, B. Zak, and A. I. Boyle, Analyt. Chem. *26*, 397 (1954).

[11] R. S. Gordon and A. Cherkes, Proc. Soc. Exp. Biol. Med., *97*, 150 (1958).

[12] P. S. Roheim and J. J. Spitzer, Amer. J. Physiol., *195*, 288 (1958).

Transesterification Reactions of Ethylene Glycol Cyclic Phosphate with 2-Aminoalcohols

Studies on the enzymatic hydrolysis of ribonucleoside-2′,3′-cyclic phosphates using tris (2-amino 2-hydroxymethyl 1,3-propanediol) buffer revealed a facile transesterification reaction occurring non-enzymatically to give nucleoside tris phosphates[1]. Periodate titration showed that the tris was linked to the P through an O atom, indicating compounds of the general structure,



(R = nucleoside residue).

The present communication describes a more detailed investigation of the reaction of several 2-aminoalcohols with the simplest five-membered ring cyclic phosphate, ethylene glycol cyclic phosphate whose preparation has recently been reported[2,3].

The aminoalcohols (ethanolamine, N,N-dimethylethanolamine, and tris) were partially neutralized with concentrated hydrochloric acid and diluted with water to a final concentration of either 0·5 M or 6·75 M. The final pH (9·1–9·4) was then measured on a pH meter. Ethylene glycol cyclic phosphate (calcium or sodium salt) was added to aliquots of the above solutions to give a final concentration of 0·025 to 0·15 M. The resulting solutions were incubated in sealed glass vials at 37°C for one week. After removal of unreacted aminoalcohol by successive passage through IRC-50(H^+) and Dowex-50(Na^+), the extent of reaction was determined directly on the eluate by one of the following methods:

(a) For ethanolamine: The difference in the periodate uptake before and after acid hydrolysis was used to measure esterified aminoalcohol.

(b) For dimethylethanolamine: Total nitrogen determination (Kjeldahl).

(c) For tris: Direct periodate titration (two moles required per mole of product). All values were corrected by substracting the figure obtained for a control (no cyclic phosphate) carried through the same procedure[4]. The recovery of phosphate was determined by a total phosphorus analysis on the eluate, thus permitting calculation of the yield of transesterification product.

As evidence supporting structures of the general type proposed above, we can cite:

(1) For the derivatives of ethanolamine and dimethylethanolamine:

Paper chromatography in isopropanol-ammonia-water (70-3-27) demonstrated, besides some glycol phosphate, a new P containing component.

[1] C. Dekker, unpublished studies.

[2] J. Lecocq, C. R. Acad. Sci. *242*, 1902 (1956).

[3] J. Kumamoto, J. R. Cox, Jr., and F. H. Westheimer, J. Amer. Chem. Soc. *78*, 4858 (1956).

[4] This correction was generally small.

Experiment No.	Aminoalcohol	pH of reaction*	Concentration (M)** Aminoalcohol	Concentration (M)** Cyclic P	Yield*** (%)	Recovery of P (%)
1	$HOCH_2CH_2NH_2$	9·35	6·75 (2·0)	0·05 (Ca^{++})	62	93
2	$HOCH_2CH_2NH_2$	9·35	6·75 (2·0)	0·15 (Ca^{++})	61	91
3	$HOCH_2CH_2N(CH_3)_2$	9·4	6·75 (2·5)	0·05 (Ca^{++})	83·5	86·6
4	$HOCH_2C(CH_2OH)_2NH_2$	9·12	0·5 (0·45)	0·025 (Ca^{++})	13 (50)	95–100
5	$HOCH_2C(CH_2OH)_2NH_2$	9·12	0·5 (0·45)	0·025 (Na^{+})	5 (10)	95–100
6	$HOCH_2C(CH_2OH)_2NH_2$	8·3	0·5 (0·31)	0·025 (Ca^{++})	5 (18)	95–100

* Measured with a pH meter. ** The values in parentheses represent the concentration of free amine calculated from the amount of concentrated HCl added. ***Values in parentheses are the % of cyclic phosphate hydrolyzed to glycol phosphate (determined by weak acidity titration). In experiments 1, 2, and 3, paper chromatography showed only glycol P and the transesterification product. No cyclic P appeared to remain.

R_P[5] = 0·88 for the derivative of ethanolamine, giving a ninhydrin reaction;

R_P = 1·23 for the derivative of dimethylethanolamine.

Passage through the anion-exchange resin Dowex-1(Cl^-) at neutral pH resulted in the disappearance of the glycol phosphate but the new P containing component was quantitatively recovered, confirming its amphoteric nature.

(2) For the derivative of ethanolamine it was shown by paper chromatography that acid hydrolysis gave glycol phosphate and ethanolamine. 1·085 M of the aminoalcohol (determined by periodate) was formed per mole of glycol phosphate (determined by the increase in weak acidity due to secondary phosphate[6]).

(3) For the derivative of dimethylethanolamine, titration after removal of accompanying glycol phosphate by passage through Dowex-1(Cl^-) revealed little weak acidity[6].

The results of the main experiments are summarized in the Table.

The catalytic effect of Ca^{++} is seen when one compares the results of Experiments 4 and 5. Such effect of polyvalent cations on the alkaline hydrolysis of phosphodiesters has been observed by several investigators.

Experiments 4 and 6 show the influence of pH.

Although further studies will be necessary to ascertain the mechanism of the reaction, our present results indicate a direct attack of the oxygen of the aminoalcohol on the phosphorus of the cyclic phosphate rather than an attack of the amino-N followed by a N to O migration.

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Department of Biochemistry, University of California, Berkeley, October 3, 1958.

Résumé

Il a été trouvé que l'éthylène glycol phosphate cyclique réagit en solution aqueuse avec plusieurs amino-2 alcools pour donner les phosphodiesters résultants d'une transesterification.

[5] $R_P = R_f$ of new compound/R_f of ethylene glycol cyclic phosphate.

[6] Quantitative data are difficult to obtain because of the partial titration of the amino group in the region pH 4–8.

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Il guanosintrifosfato quale attivatore dei sistemi enzimatici ossidasici dei grassi Nota III

Nelle note precedenti[1,2] si è formulata l'ipotesi che l'α-chetoglutarato attivi i sistemi enzimatici ossidasici dei grassi tramite il guanosintrifosfato (G.T.P.) che viene sintetizzato durante l'ossidazione dell'α-chetoglutarato stesso secondo le seguenti reazioni:

α-chetoglutarato + D.P.N.$^+$ + CoA ⟶
Succinil-CoA + D.P.N.H + H^+ + CO_2 (1)

Succinil-CoA + G.D.P + Pi ⟷
Acido Succinico + G.T.P. + CoA. (2)

Con le ricerche qui riferite si è tentato di convalidare o meno tale ipotesi determinando l'eventuale potere attivatore del G.T.P. in confronto di quello dell'α-chetoglutarato, dell'A.T.P. o dell'A.T.P. più succinato.

Metodi. Sono stati impiegati due lotti di cavie: uno in condizioni di alimentazione, l'altro in condizioni di digiuno da 120 ore. L'intensità dell'azione del sistema enzimatico ossidasico dei grassi, preparato dal fegato secondo il metodo di LEHNINGER[3], è stata dedotta, mediante la tecnica descritta nelle note precedenti, dalla quantità di O_2 consumata per ossidare l'acido caprilico (vedi Tabella, colonna A). Si è determinato anche l'acido acetacetico formatosi (metodo di GREENBERG e LESTER[4]; modificato da BARKULIS e LEHNINGER[5]), e dai valori trovati si è calcolata la quantità di O_2 teoricamente occorsa per la sua formazione (vedi Tabella, colonna B). Il G.T.P. è stato impiegato nella quantità di 10 μM/100 mg di preparato enzimatico secco, quantità corrispondente a quella che si forma teoricamente durante il processo di ossidazione dell'α-chetoglutarato calcolando P/O = 1. L'A.T.P. è stato impiegato nella quantità di 10 e di 65 μM.

Risultati e discussione. Impiegando preparati enzimatici estratti da fegati di cavie in condizioni di alimentazione, risulta che il G.T.P. alla concentrazione di 10 μM/100 mg di preparato enzimatico secco (quantità corrispon-

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[3] A. L. LEHNINGER, J. biol. Chem. *161*, 437 (1945).
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