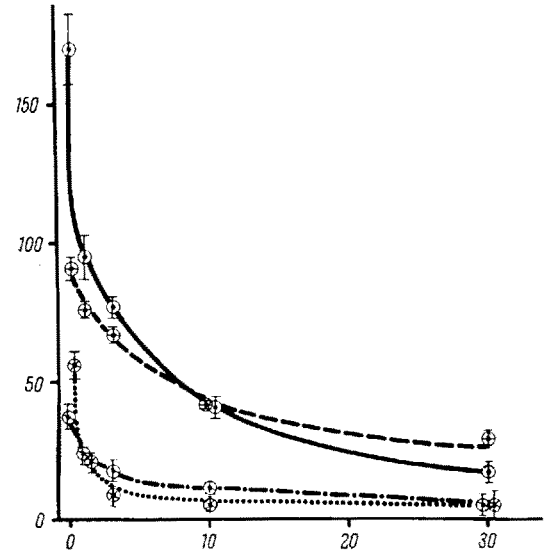
brains of animals after the combined hydrazide-harmaline treatment were not different from those of animals treated with the hydrazides alone.



Inhibition of the hydrazide-induced increase of 5-hydroxytryptamine (5HT) in the brain of rats and mice.

Ordinate: Percentage increase of 5HT in brain, as compared with untreated controls.

Abscissa: Harmaline dose in mg/kg.

The hydrazides were injected intraperitoneally in doses equimolecular to 100 mg/kg iproniazid 1 h prior to administration (intraperitoneal) of harmaline. Measurement of 5HT 16 h after application of the hydrazides. Animals treated with the hydrazides only (0 mg/kg harmaline) served as controls.

——— Hydrazide III, rats; – – – – – Iproniazid, rats;
····· Hydrazide III, mice; –·–·–· Iproniazid, mice.

Each point represents the average of 4–8 measurements. Vertical lines: standard deviation.

Discussion. The inhibition of the hydrazide-induced 5HT and NE rise in the brain after harmaline pretreatment is probably due to the fact that harmaline and the hydrazides compete for the same receptor site on the enzyme MAO. Thus, the short-acting harmaline probably blocks the receptor which then cannot be attacked by the long-acting hydrazide any more. Harmaline, however, does not reverse the action of hydrazides once the latter compounds have caused a monoamine rise in the brain. Thus, harmaline, given after the hydrazides, has no effect on the hydrazide-induced increase of the monoamines. This may be explained by the fact that hydrazides cause an irreversible damage to MAO, or that the hydrazide-enzyme bond is very strong and cannot be reversed by harmaline once it has taken place.

The above described antagonism between harmaline and hydrazides may serve as a tool to separate the hydrazide effects due to MAO inhibition from those caused by other mechanisms. Thus, the pharmacological effects of hydrazides which are counteracted by harmaline are probably due to MAO inhibition, whereas those effects which cannot be antagonized by harmaline must be caused by different mechanisms. Preliminary results show that some hydrazide-induced alterations of the reserpine and benzoquinolizine effects on brain are probably due to interference with monoamine metabolism. Iproniazid and compound III counteract some central nervous actions of reserpine and benzoquinolizines (e.g. potentiation of sedation and narcosis potentiation[6]). This effect of the hydrazides is abolished by pretreatment with harmaline. By further investigation along this line it may be possible to get additional evidence concerning the role of MAO inhibition in drug action.

A. PLETSCHER and H. BESENDORF

Medical Research Department of F. Hoffmann-La Roche & Co. Ltd., Basel (Switzerland), October 31, 1958.

Zusammenfassung

Vorbehandlung mit Harmalin hemmte den Anstieg von 5-Hydroxytryptamin (5HT) und Noradrenalin (NE) im Gehirn von Ratten und Mäusen, welcher durch zwei langdauernd wirkende Monoaminoxydasehemmer verursacht wurde (Isopropylhydrazide von Isonikotinsäure und α-Isopropylhydrazid von Glutaminsäure). Wurde Harmalin 6–8 h nach den Hydraziden appliziert, so konnte kein Absinken des durch das Hydrazid erhöhten Monoamin-Gehaltes beobachtet werden. Mit Hilfe dieses Antagonismus zwischen Harmalin und Hydraziden dürfte es möglich sein, mehr Kenntnis über die Rolle der Monoaminoxydase bei der Wirkung von Pharmaka zu gewinnen.

[6] M. CHESSIN, B. DUBNICK, E. R. KRAMER, and C. C. SCOTT, Fed. Proc. *15*, 409 (1956). – M. CHESIN, E. KRAMER, and C. C. SCOTT, J. Pharmacol. exp. Ther. *119*, 453 (1957). – H. BESENDORF and A. PLETSCHER, Helv. physiol. pharmacol. Acta *14*, 383 (1956). – P. A. SHORE and B. B. BRODIE, Proc. Soc. exp. Biol. Med., N. Y. *94*, 433 (1957). – A. PLETSCHER, Schweiz. med. Wschr. *87*, 1532 (1957). – A. PLETSCHER, H. BESENDORF, and H. P. BÄCHTOLD, Arch. exp. Path. Pharmak. *232*, 499 (1958).

The Effect of Colchicine on Plasma Unesterified Fatty Acids[1]

Although the influence of colchicine on mitotic activity has been studied extensively, comparatively little attention has been paid to other pharmacological effects of this alcaloid[2,3]. STERNBERGER and FERGUSON[4] found that a single lethal intravenous dose of colchicine causes 'fat nephrosis', which is probably a manifestation of some derangement of lipid transport. Since in CCl_4 poisoning a similar derangement occurs in association with increased plasma unesterified fatty acid (UFA) concentrations[5,6], it was decided to investigate the effect of colchicine on UFA.

Materials and Methods. 4 mg of colchicine/kg of body weight were given intravenously to male, white rats, weighing 150–300 g. The control animals received equal amounts of physiological saline. All animals were exsanguinated under Nembutal anesthesia through the inferior vena cava 17–20 h after the injection. The blood was chilled immediately and the specific gravity determined[7].

[1] Supported by the National Heart Institute, Bethesda, Md.

[2] P. F. ROBINSON and R. R. RUNGE, Fed. Proc. *15*, 154 (1956).

[3] R. W. BALEK, J. J. KOCSIS, and E. M. K. GEILING, Fed. Proc. *15*, 397 (1956).

[4] S. S. STERNBERGER and F. C. FERGUSON, Cancer *7*, 607 (1954).

[5] G. L. SELDEN and U. WESTPHAL, Proc. Soc. Exp. Biol. Med., *89*, 159 (1955).

[6] J.J.SPITZER and H.I.MILLER, Proc. Soc. Exp. Biol. Med. *92*, 124 (1956).

[7] R. A. PHILLIPS, D. D. VAN SLYKE, V. P. DOLE, R. EMERSON P. B. HAMILTON, and R. M. ARCHIBALD, Bull. U.S. Army Med. Dept. *41*, 66 (1943).

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.

J. J. Spitzer and Judy A. Spitzer

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

Zusammenfassung

Die Veränderung 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).

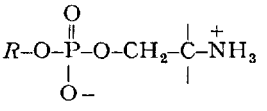
[10] H. H. Brown, A. Zlatkis, B. Zak, and A. I. Boyle, Analyt. Chem. *26*, 397 (1954).

[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.