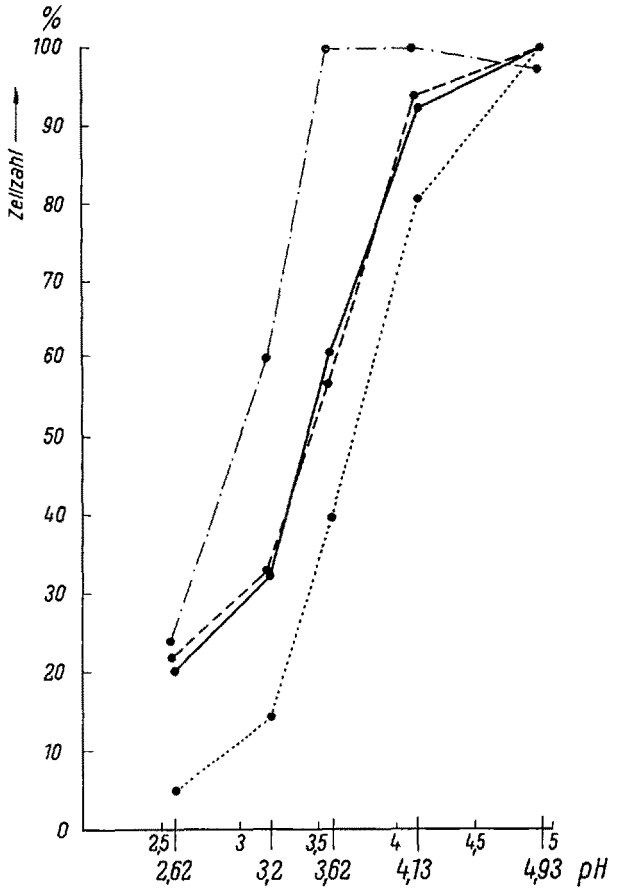
offenbar durch das Ferment herausgelöst sind. In Kurve IV, die von der Mastzellenretikulose gewonnen wurde, ist die histochemische Abartigkeit der maligne entarteten Mastzellen deutlich erkennbar.



—— I: Nicht vorbehandelte Gewebsmastzellen formolfixierten Femurmarkes von M. WALDENSTRÖM.

– – – II Mit *Aqua dest.* vorbehandelte Gewebsmastzellen bleiazetatfixierten Femurmarkes von M. WALDENSTRÖM.

·–·–· III Mit Luronase vorbehandelte Gewebsmastzellen bleiazetatfixierten Femurmarkes von M. WALDENSTRÖM.

····· IV: Gewebsmastzellen formolfixierten Femurmarkes von Mastzellenretikulose.

(Nähere Beschreibung im Text)

Es steht zu erwarten, dass mit dieser Methode eine Reihe von Fragen, die sich auf die Natur und Funktion der Mastzellengranula richten, zu lösen ist.

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Pathologisches Institut der Johann-Wolfgang-Goethe-Universität, Frankfurt am Main, den 9. Mai 1956.

Summary

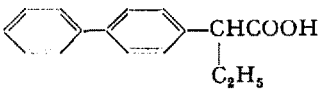
A method is described for histochemical definition of the metachromatic substance of mast cells. A slide of tissue containing mast cells is treated with substances dissolving metachromasia, such as hyaluronidase and water, and later on stained in aqueous solutions of gradually heightened pH. A marked spot of the slide is counted at each pH. Concentrations of hydrogen and numbers of mast cells result in a characteristic curve.

Diphenylilethylacetic Acid Inhibits Hypercholesteremia Induced by Triton

Aware of the great importance of Coenzyme A for *in vitro*[1] and *in vivo*[2] synthesis of cholesterol, we thought that by testing several substances showing inhibitory activity on the Co A, we could obtain drugs able to interfere with cholesterol metabolism.

For this purpose we undertook some researches in connection with the work of COTTET *et al.* on the hypocholesteremic activity of the phenylethylacetic acid[3], and with our previous results demonstrating that acetylation activity of Co A, studied according to the procedure of KAPLAN and LIPMANN[4], is inhibited *in vitro* by phenylethylacetic acid.

On the other hand, also *in vitro*, we have proved, on a series of molecules, that suitable substitutions of 1 or 2 atoms of hydrogen in the methyl group of acetic acid, compounds are obtained which are much more active than phenylethylacetic acid itself[5]. One of the compounds showing the highest inhibiting activity on the acetylation process, also *in vivo*[6], is diphenylilethylacetic acid[7].



As a test for evaluating the action of diphenylilethylacetic acid, hypercholesteremia and hyperlipemia have been obtained by means of an administration of Triton[8]. According to recent results, the action of Triton appears to be connected with an improvement of endogenous synthesis, since an increased incorporation of labelled acetate in cholesterol has been obtained[9].

We used male albino rats of mean weight of 250 g; cholesterol was determined according to GRIGAUT[10], serum lipids with the micromethod of SWAHN[11], and lipoproteins pattern with paper electrophoresis according the classical method modifies as elsewhere reported[12].

The results are summarized in the following 2 Tables.

We want to point out that diphenylilethylacetate not only has fmarked anti-hypercholesteremic and anti-hyperlipemic activity, but is also able to modify intensively the lipoproteic pattern. In fact, while treatment

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[6] S. GARATTINI *et al.*, Arch. Int. Pharm. (1956) (in press).

[7] Synthetized by G. CAVALLINI and E. MASSARANI of Maggioni Lbs. of Milan, Farmaco (1956) (in press).

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[11] B. SWAHN, Scand. J. clin. Lab. Invest. *5* (1953), suppl. 9.

[12] S. GARATTINI and B. MURELLI, G. Biochim. *5*, 98 (1956).

with Triton clearly displaces the β/α-ratio towards the high molecular weight lipoproteins (those same lipoproteins which increase in human and experimental atherosclerosis, diphenylilethylacetic acid makes the lipoproteic pattern return to normal, inhibiting the formation of β-lipoproteins.

Number of rats	Treatment: Triton mg/kg intravenous	Diphenylilethylacetate* mg/kg intraperitoneal**	Serum cholesterol mg/100 ml (and standard deviation)
50	–	–	77 ± 3·70
50	200	–	269 ± 8·54
15	200	100	152 ± 16·53

* Triton and Sodium diphenylilethylacetate were given at the same time to animals fasted for 12 h. The animals were killed 18 h after administering Triton.

** It has been demonstrated in other experiments that diphenylilethylacetic acid can inhibit the effects of Triton also when given *per os* (250 mg/kg). Diphenylilethylacetic acid does not cause variations of normal cholesteremia when used in the doses reported by us.

Number of rats	Triton mg/kg	Treatment: Diphenylilethylacetate mg/kg intraperitoneal *	Serum total lipids mg/100 ml	Lipoproteins**			
				α	β	X	β/α
30	–	–	200 ± 15·93	34·8	48·4	14·8	1·3
15	200	–	1008 ± 56·24	11·52	71·7	16·2	6·2
10	200	100	481 ± 49·37	23·15	53·8	22·6	2·2

* We demonstrated in other experiments that diphenylilethylacetic acid can inhibit the effects of Triton also when given *per os* (250 mg/kg).

** In the present elaboration of data, lipoproteins have been divided on the grounds of their localization on the protein electrophoretic pattern.

Diphenylilethylacetic acid, which is very well tolerated by man in doses of 300 mg daily, has been used recently also in clinical trials and gave good results in cases of hypercholesteremia[13].

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Institute of Pharmacology, University of Milan, Italy, April 20, 1956.

Résumé

On prouve que l'acide diphénylilléthylacétique, qui exerce un effet d'inhibition sur l'activité acétylante du coenzyme A, peut aussi causer une diminution de l'hypercholestérolémie et de l'hyperlipémie provoquées par une administration de Triton.

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Isolation of a Cytochrome of the Antimycin A Sensitive Pathway for DPNH Oxidation

A cytochrome has been isolated from pig, guinea pig or rat liver, having the following absorption peaks: 404 and 500 mμ in oxidized state and 428 and 556 mμ in reduced state.

This cytochrome was prepared from liver using essentially the method of EDELHOCH *et al.*[1] for DPNH-cytochrome c reductase. With rat and guinea pig liver the cytochrome is not extracted by 10% ethanol at 40° C, but can be obtained by a subsequent extraction with *M*/20 orthophosphate buffer pH 7·4. With pig liver this cytochrome is obtained in the ethanol extract, together with DPNH-cytochrome c reductase, but can be separated from it by ammonium sulfate fractionation.

This cytochrome is reduced by hydrosulfite, and leucomethylene blue, but not by reduced glutathione or ascorbic acid. It is not reduced by DPNH-cytochrome c reductase, unless SLATER[2] factor is present.

To obtain the Slater factor, rat liver homogenate is taken to pH 5·4, and the precipitate extracted with a digitonin solution. Reduction of the present cytochrome by DPNH-cytochrome c reductase is a specific test for this factor, the reaction being inhibited by antimycin A. In this reaction Slater factor can be replaced by methylene blue, which is reduced by the reductase and reoxidized by the present cytochrome, but not inhibited by antimycin A.

It is remarkable that DPNH-cytochrome c reductase is able to reduce cytochrome c directly, but the present cytochrome, which has a lower redox potential, only through Slater factor.

0·01 *M* orthophosphate inhibits the reduction of cytochrome c, by reductase, probably by combining with its iron[3], and inhibits also the reduction of the present cytochrome. Removal of the bound iron of the reductase by dialysis against 8-hydroxyquinoline transforms this enzyme to a diaphorase, which reduces the new cytochrome even in the presence of orthophosphate.

Although the present cytochrome has a different absorption spectrum from the other cytochromes recently described[4], it may turn out to be identical with one of them.

The present cytochrome transports electrons from leucomethylene blue to hydroxylamine. This fact has been erroneously interpreted[5], as hydroxylamine inhibiting the reduction of methylene blue by reductase. This "inhibition" required a factor which had been called hydroxylamine inhibition factor. The factor is now identified with the present cytochrome, which reoxidizes the methylene blue, reduced by the reductase. It was shown[6] that this cytochrome is necessary for the oxydative phosphorylation coupled to DPNH oxidation by cytochrome c, in a soluble system.

I. RAW

Departamento de Química Fisiológica, Faculdade de Medicina da Universidade de Sao Paulo, Brasil, April 17, 1956.

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[5] I. RAW, Science *118*, 159 (1953).

[6] I. RAW, J. Amer. chem. Soc. *77*, 503 (1955).