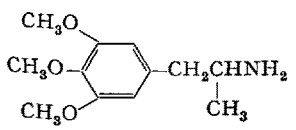
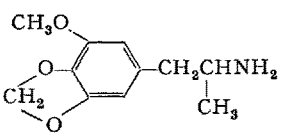
Physical and pharmacological properties

Compound	Nitrostyrene m.p.	Base·HCl m.p.	ED[a] (mice) mg/kg	LD_{50} (mice) mg/kg	ED[b] (human) µg/kg	M.U.[c] (human)
(I) TMA[d]	94	209	20	260	1700	2.2
(II) MMDA	110	191	35	150	1400	2.7
(III) TMA-2[e]	102	181	15	120	220	17.0
(IV) MMDA-2	163	187	20	130	180	21.0
(V) TMA-3	57	149	35	120	> 1900	< 2.0
(VI) MMDA-3a	106	154	25	40	210	18.0

[a] Effective dosage (as free base in saline, i.p.) at which behavioral changes were first observed.
[b] Effective dosage (as free base, *per os*) defined as the arithmetical mean between the minimum detectable dosage and the dosage above which there is a prolongation rather than an intensification of the psychotomimetic syndrome.
[c] Mescaline Units. The quotient of the effective dosage of mescaline (assumed to be 3750 µg/kg as the base) divided by the effective dosage of the substance in question. It permits a direct comparison of relative potencies, based on mescaline = 1.
[d] Physical data from [4].
[e] Literature values for the nitrostyrene m.p. 101° and for the base·HCl, 187°, see V. BRUCKNER, J. prakt. Chem. *138*, 268 (1933).

more toxic than the 3,4,5-substituted analogs. Compound VI, MMDA 3a, was the most toxic of the group and it alone produced clonic convulsions and vocalization prior to death. The other compounds led to easy deaths, apparently due to respiratory paralysis. All compounds displayed initial behavioral changes at the levels listed. The responses observed were light tremors accompanied by rapid scratching and a huddling tendency. These effects disappeared within 3 h, and there were no noticeable after-effects.


I (TMA)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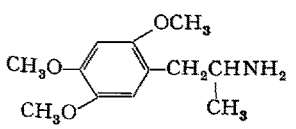
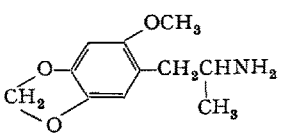
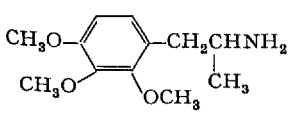
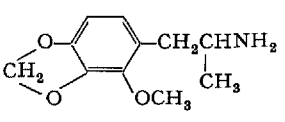

III (TMA-2)


IV (MMDA-2)


V (TMA-3)


VI (MMDA-3a)

The intoxication syndrome in human subjects, resulting from the quantities shown in the Table, is qualitatively similar to that which results from mescaline, except that the color effects, and to a large extent the nausea, are absent. As a generalization, the MMDA series leads to the more empathic and pleasant responses, whereas personal anxiety and restlessness were common with TMA-2. The vicinal analog, TMA-3, demonstrated neither physical nor psychotropic effects, even in dosages in excess of those shown to be adequate for TMA and MMDA. With the other three *ortho*-methoxy derivatives, however, hypnogogic hallucinatory synthesis and total recall are present and are similar to mescaline.

Résumé. Quatre amphétamines analogues aux substances chimiques psychomimétiques connues, la 3,4,5-triméthoxyamphétamine et la 3-méthoxy-4,5-méthylènedioxyamphétamine, ont été synthétisées. Le déplacement d'un méthoxyl à une position *ortho*- de la chaîne aliphatique a montré, dans trois cas une amplification multiple quant à l'efficacité humaine.

A. T. SHULGIN

The Dow Chemical Company, Walnut Creek (California, USA), February 10, 1964.

Adsorption of Hageman Factor (Factor XII) on Collagen

It is generally accepted that collagen fibres play an important role in haemostasis. Several authors[1-3] have described the aggregating effect of suspensions of connective tissues and collagen fibres on blood platelets *in vitro*. The platelets adhering to the traumatized vessel wall form a haemostatic plug. The purpose of our experiments was to study the adsorption of the blood clotting factors on collagen.

Collagen was prepared from bovine tendons according to the method of EINBINDER and SCHUBERT[4]. Two kinds of human citrated plasma were used: (a) platelet-rich plasma (about 400,000 platelets per mm³), and (b) platelet-poor plasma (about 30,000 platelets per mm³) obtained by two-stage centrifugation of 2000 RPM for 10 min and 10,000 RPM for 10 min. Plasma was prepared in siliconized glassware.

The following determinations were made: platelet count (Rees-Ecker method), clotting time of recalcified

[1] J. HUGUES, C.R. Soc. Biol. (Paris) *154*, 866 (1960).
[2] M. B. ZUCKER and J. BORELLI, Proc. Soc. exp. Biol. Med. (New York) *109*, 779 (1962).
[3] T. HOVIG, Thromb. Diath. Haem. *9*, 248 (1963).
[4] J. EINBINDER and M. SCHUBERT, J. biol. Chem. *188*, 335 (1950).

plasma, prothrombin time (QUICK), fibrinogen[5], 'true prothrombin', factor V, factors VII and X[6], plasminogen and antiplasmin[7]. Factors VIII, IX, XI and XII were determined by the one-stage slightly modified methods of SOULIER and LARRIEU[8]. Plasmas of patients with congenital deficiency of the above factors were used as substrates for these determinations. The so-called 'exhausted plasma' (plasma artificially deprived of factors XI and XII, prepared according to WAALER[9]) has also been used to determine the level of both total factor XI and total factor XII together. The level of blood clotting factors in the tested blood plasma was calculated by means of interpolation from standard dilution curves.

Samples of human blood plasma were shaken with various amounts of collagen for 10 min at room temperature. Platelet count and clotting factors were determined in supernatant after centrifugation of collagen fibres.

Experimental results are presented in the Table. It can be seen that collagen, shaken with platelet-rich plasma, adsorbs about 30–45% of Hageman factor. The adsorption of platelet-poor plasma on collagen causes a decrease of Hageman factor to 20% and of factor IX to 72% of their initial values. This procedure did not significantly affect other clotting factors, including PTA (factor XI).

It has been noticed that a sample of human plasma, adsorbed six times with collagen (20 mg/ml), contains about 5–7% of Hageman factor.

The following experiment was performed in order to elute Hageman factor from collagen. 10 cm^3 of plasma were shaken with 200 mg of collagen. After centrifugation, the precipitate was twice washed with distilled water and with 0.9% NaCl. Then 0.9% NaCl was added and the pH was adjusted to 10.5. The eluate contained about 0.7% of protein; the yield of Hageman factor being 25%. Its specific activity increased about 10 times as compared with human plasma.

The conclusion may be drawn that Hageman factor is almost selectively adsorbed from human plasma by collagen and that it is possible to elute this factor from collagen fibres in alkaline medium.

The adsorption of Hageman factor is greater in platelet-poor plasma. It is suggested that platelets, adhering to the collagen fibres, interfere with the adsorption of Hageman factor.

It is possible that the adsorption of Hageman factor by collagen also occurs *in vivo* and that this phenomenon is of some significance in haemostasis. It seems that certain preparative procedures, e.g. separation of PTA and Hageman factor, purification of Hageman factor, and preparation of Hageman-deficient plasma, could be elaborated on the basis of the experimental facts presented above[10].

The influence of collagen on platelet count and blood clotting factors

Clotting test	Platelet-rich plasma		Platelet-poor plasma	
	Before adsorption	After adsorption	Before adsorption	After adsorption
Platelet count	410000	158500	30000	22500
Protrombin time	15.0 sec	15.0 sec	15.6 sec	15.6 sec
Clotting time of recalcified plasma	125.0 sec	132.5 sec	139.0 sec	159.0 sec
Fibrinogen	280 mg%	250 mg%	380 mg%	390 mg%
Prothrombin-Factor II	100%	87.9 %	100%	93.7 %
Factor V	100%	100 %	100%	100 %
Factor VII + X	100%	100 %	100%	100 %
Factor VIII	100%	87.6 %	100%	91.6 %
Factor IX	100%	81.25%	100%	72.11%
Factor XI	100%	100 %	100%	100 %
Factor XI + XII[a]	100%	54.6 %	100%	21 %
Factor XII	100%	73 %	100%	20 %
Plasminogen	100%	100 %	100%	100 %
Antiplasmin	100%	100 %	100%	100 %

[a] Tested on exhausted plasma.

Résumé. Nous avons trouvé que le facteur Hageman (factor XII), contenu dans le plasma humain citraté, pauvre en plaquettes, est adsorbé presque sélectivement par le collagène. Il est également possible d'éluer ce facteur au pH alcalin.

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[6] J. P. SOULIER and M. J. LARRIEU, Le Sang *23*, 549 (1959).
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[8] J. P. SOULIER and M. J. LARRIEU, Le Sang *24*, 3 (1953).
[9] B. A. WAALER, *Contact Activation in the Intrinsic Blood Clotting System* (Oslo University Press, Oslo 1959).
[10] *Acknowledgment.* The authors are grateful to Dr. LOELIGER (Leyden) for the supply of plasma from patients with congenital Hageman factor deficiency.

Photodynamic Effect of Dye on Frog Muscle Fibre Using Microelectrodes

ROSENBLUM[1] reported that frog sartorii, when stained by Rose Bengal and illuminated by an artificial source of light from tungsten lamp, developed twitch contractions. We were interested in studying the effect of light on the resting potentials of stained muscle fibres and examining whether the photodynamic effect of the dye is exerted on the fibre membrane.

Frog sartorii from *Rana pipiens* were stained by Rose Bengal (1:25,000) for 2 h in the dark; then light from a 300 watt tungsten lamp at a distance of 18 inches was focused on the muscle for varying periods of time. An IR-glass filter was interposed to exclude the effect of heat. The membrane resting potentials were recorded by the method of NASTUK and HODGKIN[2]. The resting membrane potentials of stained fibres in frog Ringer did not differ significantly from unstained fibres. There was no

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