Using the Wittig reaction[7], after hydrolysis of the intermediate acrylic ester, *acid* I is obtained in 82% yield from formylferrocene and carbethoxymethylenetriphenylphosphorane (in benzene). We have not been able to carry out a similar reaction on acetylferrocene under the various experimental conditions.

Catalytic hydrogenation of I and II on palladinized charcoal in methyl alcohol or acetic ether leads smoothly to the corresponding saturated acids, that is *β-ferrocenylpropionic*[4,8] and *β-ferrocenyl-β-methylpropionic acid*; the latter not yet described (orange crystals from ligroine, m.p. 95–96°; calcd. for $C_{14}H_{16}FeO_2$: C, 61.79; H, 5.93; found: C, 61.97; H, 5.77) and also characterized as *ethyl ester* (red-orange oil, b.p. 125–130°/0.1 Torr; calcd. for $C_{16}H_{20}FeO_2$: C, 64.01; H, 6.72; Fe, 18.60; found: C, 64.25; H, 6.48; Fe, 18.59) which is obtained on catalytic reduction of the corresponding acrylic ester.

Aldol condensation between formylferrocene and nitromethane, carried out according to the general method of BOILEAU[9], furnishes in 30% yield a well crystallized product (bronze crystals from ligroine, m.p. 114–115°) to which, on the basis of elemental analysis and IR-spectrum, the structure of *1-ferrocenyl-2-nitroethyl alcohol* (III) is just assigned (calcd. for $C_{12}H_{13}FeNO_3$: C, 52.39; H, 4.76; N, 5.09; found: C, 52.48; H, 5.10; N, 5.10. $\nu_{NO_2}$ 1550, $\nu_{OH}$ (broad) 3400–3500 $cm^{-1}$, in Nujol). Moreover, by passing a benzene solution of III through alumina or by warming III in acetic acid, its dehydration product, the *ferrocenyl-2-nitroethylene* (IV), is obtained in 32–37% yield (brown needles from *n*-hexane, m.p. 139–140°); structure of the latter being also proved by elemental analysis and IR-spectrum (calcd. for $C_{12}H_{11}FeNO_2$: C, 56.06; H, 4.31; N, 5.45; found: C, 56.36; H, 4.04; N, 5.29. $\nu_{C=C}$ 1625, $\nu_{NO_2}$ 1500 $cm^{-1}$, in Nujol).

*Riassunto.* Si riferisce sulla applicazione delle reazioni di Reformatskii e di Wittig al formil- e all'acetil-ferrocene per preparare i corrispondenti acidi ferrocenilacrilici. Viene anche descritta la condensazione del formilferrocene col nitrometano.

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*Research Department of Recordati s.a.s., Milano (Italy), May 21, 1965.*

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## Physiological Cell Permeability and Pharmacological Action of DMSO

Among the hypotheses that have been put forward to explain the cell membrane permeability, are some which relate it to its lipids and the corresponding peroxide derivatives.

USSING[1] explains the facts observed by considering that the cell membrane is a *lipoid pore-membrane*, provided with specialized 'carriers' for certain substances.

ALLISON[2] said: 'We suppose that the hyperoxia increases the permeability of lipoprotein membrane of cells and cell organelles, probably as a consequence of lipid peroxidation'. TAPPEL et al.[3,4] have already demonstrated release of lysosomal enzymes from rabbit liver homogenates by lipid peroxidation damage. But no explanation was given for the stereochemical aspects of the formation of pores. Years ago, one of us (P.P.M.) proposed a hypothetical geometric mechanism of the micropore formation[5].

This concept of the mechanism of peroxide permeability is as follows: When an unsaturated fatty acid is autoxidated or peroxidated, a shift of double bonds takes place, together with a modification of the geometrical isomerism. In linoleic acid, for instance, the *cis-cis* structure of the hydrocarbon chain is changed by peroxidation to the *cis-trans* form. This stereoisomeric change produces a specific alteration of the IR-spectra. Some published papers deal with this modification and with its application for the study of lipid peroxide formation[6–8].

Such a stearic change may be important, especially in the case where a lipidic layer contains saturated fatty acid together with a variety of the non-saturated type. For instance, in the simpler case of the copresence of stearic and linoleic acids, peroxidation induces structural change in the linoleic acid, whilst stearic acid remains unaffected. This is shown in Figure 1.

When we studied the pharmacological actions of dimethyl sulphoxide (DMSO) on tissues and upon the increasing cellular permeability, we confirmed for the first time some of the surprising experimental results previously published by JACOB et al.[9] and STOUGHTON and FRISCH[10].

With regard to the stereometric structure of DMSO, and for the known special bond between S and O in the sulphoxides, we studied the possibility that DMSO might produce some change of the isomeric conformation of linoleic acid and oleic acids.

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*Methods.* The IR-spectra of the stated solutions were measured under the following conditions, using the Parking-Elmer spectrophotometer[11]. Linoleic, oleic and stearic acids were dissolved separately in DMSO, and the IR-absorption spectra were determined between 0.85 and 2.5 $\mu$; the spectra of the pure substance were also recorded. Linoleic and oleic acids were dissolved in their equivalent weights of DMSO. The IR-spectra were taken by dissolving 400 mg of the substance to be assayed in 3 ml of $Cl_4C$ using 1 cm quartz cells.

*Results.* In the $CCl_4$ control solutions of oleic and linoleic acids, two maxima were observed at 2.15 and 2.19 $\mu$ which show the presence of the double bond in position *cis*.

In the spectrum corresponding to mixtures of oleic and linoleic acid in DMSO, the maximum absorption at 2.15 $\mu$ is almost absent for linoleic acid (Figure 2) and completely absent in the case of oleic acid (Figure 3), which seems to indicate a diminution or disappearance of the isomer *cis*.

In the control solution, in no case was a maximum of absorption observed at 2.07; however, there are slight maxima at 1.46 and 1.42 which are to be attributed to the hydroxyl bonded to hydroperoxide, which is probably present in small quantity as an impurity. These small maxima disappear when the fatty acids are in solution of DMSO, which probably indicates the destruction of the hydroperoxide.

[11] The IR-spectra were registered under the supervision of Dr. J. CASTELLS, of the Laboratory of Organic Chemistry of the University of Barcelona, to whom we are grateful for his collaboration.

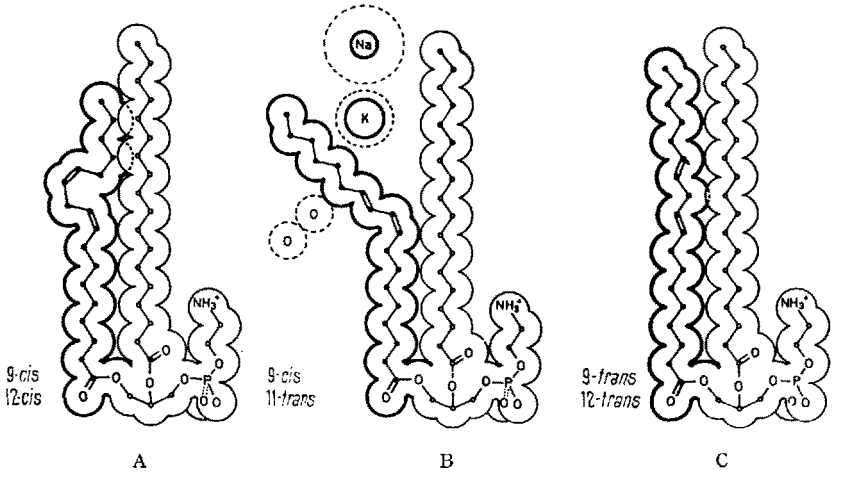


Fig. 1. A, Structure of phosphatidylethanolamine with stearic and linoleic acids. The latter appears in its normal structure. B, Change of the structure of linoleic acid by fixation of peroxide, with the appearance of a space wide enough to allow the passage of potassium and sodium ions in the hydrated forms. C, Structure of the other isomeric form; the space disappears.

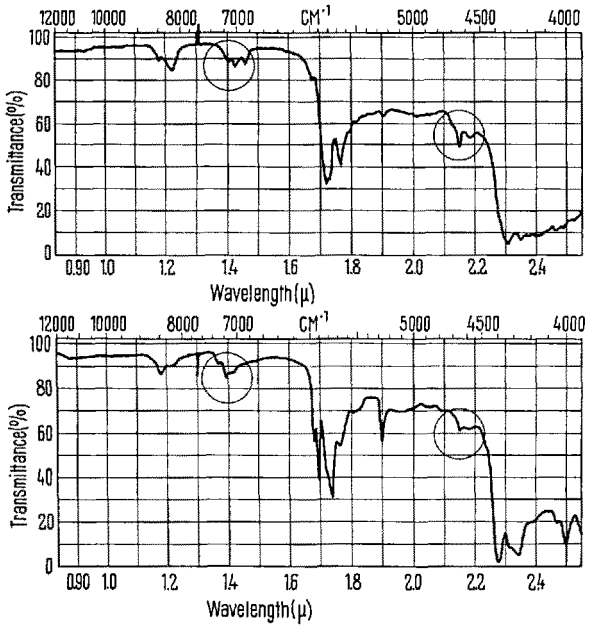


Fig. 2. IR-spectra of linoleic acid without (above) and with (below) DMSO. The maximum absorption at 2.15 $\mu$ is nearly absent in the presence of DMSO.

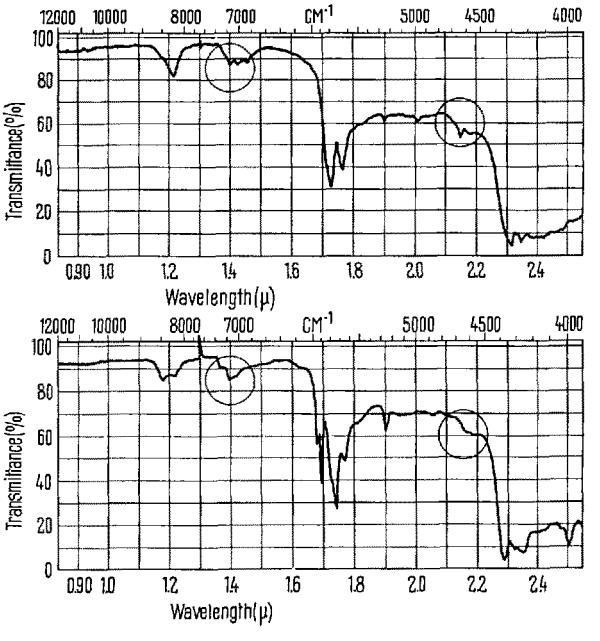


Fig. 3. IR-spectra of oleic acid without (above) and with (below) DMSO. The maximum absorption at 2.15 $\mu$ is absent in the presence of DMSO.

Taking into consideration the experimental results we have obtained with DMSO – a product that shows a capacity for tissue and cellular diffusion, as well as the capacity of modifying the stereoisomery of fatty acids – we have now reinforced our previous hypothesis of the lipoperoxide permeability of membrane.

*Zusammenfassung.* Es wird gezeigt, dass das DMSO die Möglichkeit hat, die *cis-cis*-Struktur der ungesättigten Fettsäure zu ändern. Diese strukturelle Veränderung, wenn sie in den gesättigten Fettsäuren benachbarten Molekülen stattfindet, bedingt die Bildung einer Pore. Sie hängt überdies zusammen mit der Veränderung, welche die Bildung von Lipoperoxyden bedingt und ihrer möglichen Rolle in der zellulären Permeabilität.

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## The Influence of Continuous Irradiation and Experimental Diphtherial Intoxication upon the Origin and Incidence of Non-Specific Necrotic Changes in the Cardiac Muscle

In the course of our research work, concerning the interrelations between continuous irradiation and experimental diphtherial intoxication[1-3], an interesting phenomenon in the myocardium has been observed. This phenomenon is described in this communication.

*Material and methods.* The experiments were performed on albino Wistar rats of both sexes. The mean body weight at the beginning of experiments was 160 g.

80 rats were divided into 4 groups. The first group received a relatively small dose of the diphtherial toxin – 250 MLD/150 g body weight. The second group was represented by the rats which were only continuously irradiated. In the third group the dose of the diphtherial toxin was the same as in the first group, but the animals were pretreated with continuous irradiation. The rats in the fourth group were treated with a massive dose of the diphtherial toxin: 1000 MLD/150 g body weight.

The irradiation was continuous for 23–23 1/2 h a day. The daily dose was 90 R, total dose within 25 days 2250 R. The animals were kept in special cages in the $\gamma$-field in a semicircular area around the source of $Co^{60}$-type irradiation with an activity of 45 C. Not a single rat died in the course of irradiation.

The diphtherial toxin used in this experiment was submitted by Biogena, Prague; series HPA, Tk-5-6, titer 115 Lf, 2500 MLD in 1 ml of the stock solution. At the end of the first week, after irradiation was finished, all rats, both irradiated and non-irradiated, received diphtherial toxin intravenously into the right jugular vein. From all 80 rats, 40 animals were selected (10 rats from every group) for the evaluation of the myocardial lesions. The animals which died or were sacrificed, were all dissected and the organs were examined by means of stereoscopic section lens. Organ specimens were fixed in the 10% neutral formalin, and the cardiac tissue was stained using acid fuchsin method. Other organs were stained with hematoxylin eosin and sudan III. These results are reported elsewhere[1-3]. The histological evaluation of the myocardial tissue was performed from several sections of every organ according to the arbitrary scale 0–3.

*Results.* The characteristic feature of the lesions in the cardiac muscle was the incidence of minute necroses in the myocardial fibres. These necroses were characterized by the accumulation of a strongly fuchsinophilic material in the myocardial fibres, and in the 3rd and 4th group were accompanied by the simultaneous occurrence of large foci of infarctoid necroses. The appearance of a strongly fuchsinophilic material in the sarcoplasm between the myofibrils is the earliest detectable change in the necrotizing myocardium. This change usually begins in the immediate vicinity of nuclei and then tends to spread, so that larger portions of a muscle fibre are transformed into a more or less hyaline, strongly fuchsinophilic mass. As the lesion progresses further, the nucleus becomes indistinct and eventually disappears, while an entire segment of the muscle fibre, still sharply delimited by sarcolemma and intercalated discs, is completely transformed into a fuchsinophilic tube. The fuchsinophilic material is characteristic only for the initial stages of cardiac lesion. It disappears completely from muscle fibres which have totally disintegrated and are in the process of being absorbed. Most affected was the cardiac tissue in the 4th group, where massive doses of diphtherial toxin were administered. The incidence of the lesions was slightly reduced in the 3rd group, where the animals received relatively small doses of the diphtherial toxin, but were pretreated by continuous irradiation. In the second group, where the animals were treated only with continuous irradiation, the occurrence of necrotic lesions could also be confirmed. Finally, in the first group (small dose of the diphtherial toxin) the lesions observed are practically unimportant, considering the fact that some small degree of incidence of these lesions may be found even in the normal, intact cardiac tissue. The findings are summarized in the Table and in Figure 1, and the description of the necrotic cardiac lesions in the text is illustrated by the appearance of the cardiac tissue, as shown in Figures 2 and 3.

*Discussion.* If we have to explain the pathogenesis of the large, infarctoid foci in the myocardium of our experimental rats, then it is necessary to pay attention to the simultaneous incidence of these minute, miliary necroses in the cardiac tissue. Under these conditions we assume the pathogenesis of infarctoid lesions in the myocardium as a non-specific stress process, which is initiated by the disturbance of the electrolyte metabolism in the isolated fibres of the cardiac muscle. This may be caused by the direct action of the diphtherial toxin upon the electrolyte

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