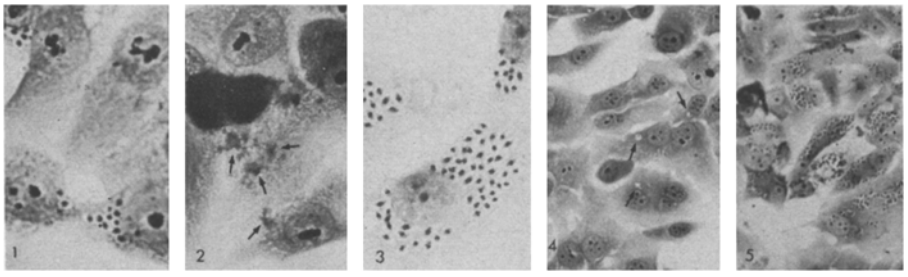

Fig. 1. HeLa cell culture infected with Trypanosoma cruzi, Y strain, incubated with Ro 7-1051, at a concentration of 100 µg/ml, for 24 h, at 37 °C. Note the rounding of the amastigote forms. Giemsa stain. × 500. Fig. 2. HeLa cell culture infected with Trypanosoma cruzi, Y strain. 48 h incubation at 37 °C with Ro 7-1051, at a concentration of 100 µg/ml. Note the karyorrhexis and lysis of the parasites within the host cell (arrows). Giemsa stain. × 500. Fig. 3. Control culture of HeLa cells infected with Trypanosoma cruzi, Y strain. Note the numerous, highly infected cells. Giemsa stain. × 500. Fig. 4. HeLa cell culture infected with Trypanosoma cruzi, Y strain (metacyclic form, incubated with Ro 7-1051 at a concentration of 100 µg/ml for 24 h at 37 °C). Incubation at 37 °C for 4 days. Note the scarcity and degeneration of parasites (arrows). Giemsa stain. × 190. Fig. 5. Control culture of HeLa cells infected with Trypanosoma cruzi, Y strain. Incubated at 37 °C for 4 days. Note the large number of infected cells, each cell containing numerous parasites. Giemsa stain. × 190.

then centrifuged at 2,500 rpm for 15–20 min and the pellets both resuspended and washed in Hanks-Wallace solution and recentrifuged. The pellets were added to HeLa cells, cultured in flasks containing floating coverslip slides, 1.5 ml of nutrient medium without the drug was added to each flask and the flasks incubated for 1, 2, 3 or 4 days.

After incubation, the slides were washed with balanced saline, fixed with Bouin's solution for 15 min and stained with Giemsa stain. Control cultures, without the drug, were run concurrently and at least 10 slide preparations of each experimental group were set up.

Results. Within 24 h of incubation (figure 1), the preparations from the first group showed an early tendency to rounding of the amastigote forms, and after 48 h incubation (figure 2), showed pyknosis, karyorrhexis and lysis of the parasites. Intensity of effect varied according to incubation time and dosage of Ro 7-1051.

Slide cultures previously treated with a dose of 100 µg/ml, and incubated for 4 days, showed very few parasites, those present being highly degenerate.

The control cultures showed plenty of highly infected cells with no signs of degeneration of cells or parasites (figure 3).

The infected cells from the second group preparations (figure 4) contained fewer parasites compared to the controls (figure 5) and most of these parasites showed degenerative signs. The intensity of degeneration varied according to the contact time between the parasite and the drug.

Discussion. Richle[12] demonstrated, in infected mice, that Ro 7-1051 acts on Trypanosoma cruzi by promoting complete destruction of the pseudocyst form. Similarly, our results show that this 2-nitroimidazole derivative caused deleterious effects on the intracellular forms of the parasite, even at a dose as low as 10 µg/ml. The degenerative signs were similar to those described by Brener[16] in Trypanosoma cruzi infected chick embryo tissue cultures, in which the amastigote forms showed nuclear pyknosis and fragmentation and eventual lysis, when submitted to the action of nitrofuran compounds, and phenanthridine derivatives.

In addition, the results of the pre-incubation of the metacyclic forms with the drug, suggests an action on the extracellular forms of the parasite, by reducing the ability of the parasite to infect the cell.

Conclusion. The drug Ro 7-1051 showed deleterious effects on the intracellular forms of Trypanosoma cruzi, represented by nuclear pyknosis, fragmentation and lysis of the parasites, at doses as low as 10 µg/ml. At higher doses, up to 100 µg/ml, the drug appeared to have no harmful effect on the cells, only on the parasites. Results also suggest an action of the drug on the extracellular forms of the parasite, as demonstrated by the reduced ability of the parasite to infect the cell.

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Desmosomal abnormalities in the liver of methotrexate-treated psoriatics

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Summary. Electron microscopy of the liver of methotrexate-treated psoriatic patients revealed junctional abnormalities consisting of detachment of desmosomal plaques between hepatocytes. Mitochondria anchoring desmosomal microfilaments were frequently noted.

While studying the fine structural changes in the liver of a psoriatic patient treated with the folic acid antagonist methotrexate (MTX), a marked abnormality of desmosomes was noted. The lesion consisted of the separation of junctional surfaces and accumulation of microfilaments at the cytoplasmic aspect of desmosomal plaques. Often, adjacent mitochondria appeared to anchor the microfilaments of desmosomes, being in direct contact

with them. In order to shed some light on the incidence and morphogenesis of this phenomenon, unreported so far to our knowledge, liver biopsy specimens of MTX-treated psoriatic patients were reviewed by electron microscopy and compared with those obtained from patients with other conditions.

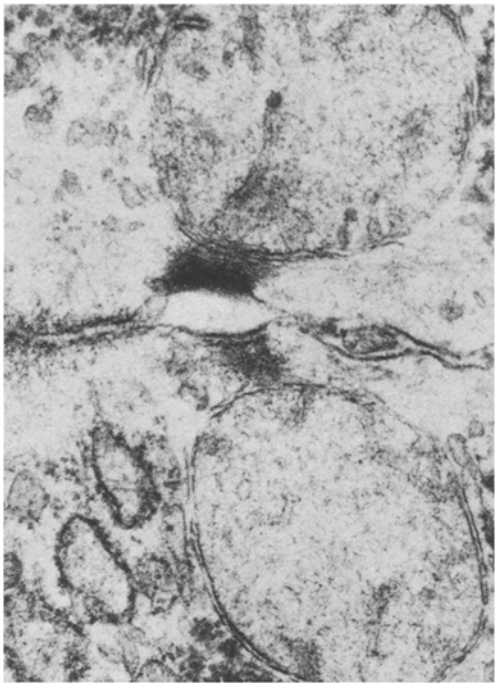

Fig. 1. Detached desmosome with mitochondria anchoring the desmosomal filaments. The density of the filamentous layer appears to be higher than that normal desmosomes. Liver biopsy of a patient treated with MTX. ×49,700.

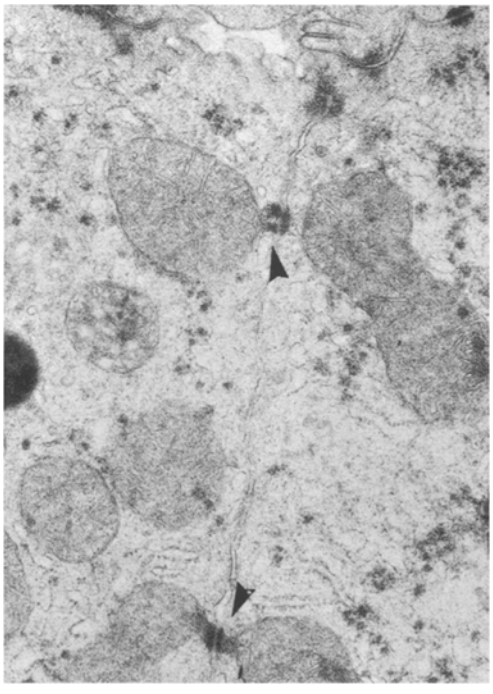

Fig. 2. Association of desmosomal filaments and mitochondria (arrowheads) in normal maculae adhaerentes of a psoriatic patient treated with MTX. ×22,500.

50 liver biopsies of psoriatic patients treated with MTX for a period from a few weeks up to 15 years, 25 biopsies of subjects with hyperlipoproteinemia or alcoholic liver disease and 10 additional biopsies showing minimal or no pathology have been studied. Liver biopsies have been obtained with the Menghini needle. Specimens were prepared for transmission electron microscopy by using conventional techniques.

In addition to Ito-cell hyperplasia[2,3] nuclear changes, prominence of Golgi apparatus and a wide variety of mitochondrial alterations[4], the most conspicuous finding was the detachment of desmosomes in 25 of the 50 biopsies obtained from MTX-treated psoriatic subjects. In these, many or practically all membrane sites with desmosomal specialization appeared to be pulled apart. The gap between the junctional surfaces increased from 250–275 Å found in unaltered desmosomes, up to 1200 Å (figure 1). The dense microfilaments attached to the desmosomal plaques frequently were in contact with and anchored by mitochondria. At the point of contact the outer mitochondrial membrane commonly became indistinct or unrecognizable. A few microfilaments appeared to be continuous with the inner mitochondrial compartment. The junctional abnormalities were regularly accompanied by the widening of pericanalicular ectoplasm

1 Acknowledgment. This work was supported in part by a grant from the Physicians' Services Incorporated Foundation, Toronto. The excellent technical assistance of Mrs Gezina Ilse and the invaluable secretarial help of Mrs Wanda Wlodarski are gratefully acknowledged.

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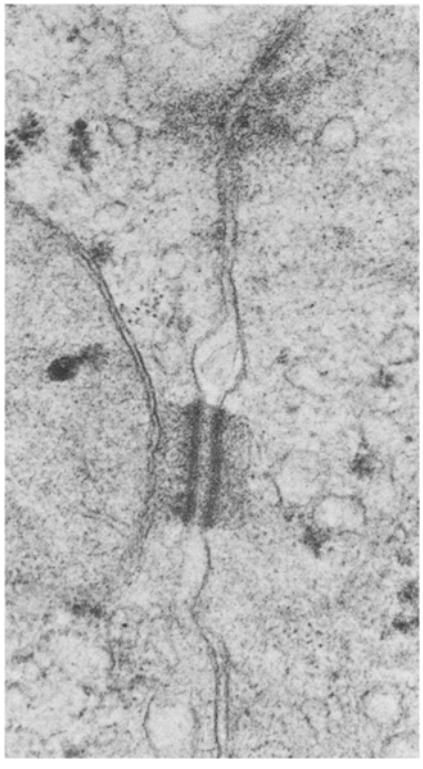

Fig. 3. High power view of a desmosome-mitochondrion contact in the liver of a MTX-treated patient. ×58,300.

and abundance of microfilaments in these zones. The cell membranes were, however, closely apposed and no alterations were seen in junctions other than desmosomes. In the remaining material among the livers of 35 patients receiving no MTX, detached desmosomes were noted only in one (hyperlipoproteinemia). However, the association between mitochondria and microfilaments of structurally unaltered desmosomes has been readily observed in the MTX-treated (figures 2 and 3) as well as untreated groups. The findings indicate that detachment of desmosomal plaques is a rare junctional abnormality; that, however, is rather prevalent in the liver tissue of MTX-treated psoriatic subjects. Direct contact between mitochondria and filaments of separated desmosomes was considerably more frequent than in that of normal maculae adhaerentes of the human liver. The association of mitochondria with desmosomes is commonly noticeable in certain normal[5] and abnormal[6] tissues, suggesting that mitochondria actively participate in the formation and/or function of adhaerent junctions. Since fine structural evidence of mitochondrial injury is commonly found in the liver cells of MTX-treated subjects, it is reasonable to suppose that mitochondrial function is also impaired. MTX is known to interfere with nucleic acid metabolism[7]. In addition to nuclear DNA and RNA, the mitochondrial genetic material may also be a target of this toxic effect possibly resulting in quantitative and qualitative changes of the synthesis of mitochondrial nucleic acids and proteins. The relation of mitochondrial injury to the structural and possibly functional alterations of desmosomal attachments remains to be elucidated.

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A cannabinoid with cardiovascular activity but no overt behavioral effects[1]

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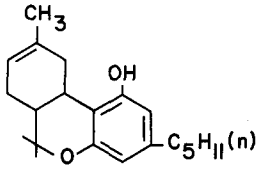
Summary. Abnormal-Δ^8-tetrahydrocannabinol (ABN-Δ^8-THC) failed to elicit central nervous system and cardiovascular effects in laboratory animals. Abnormal-cannabidiol (ABN-CBD) was also devoid of overt behavioral effects but produced marked hypotension with only slight bradycardia in anesthesized dogs.

A number of naturally-occurring constituents of marihuana, including cannabidiol (CBD), Δ^8-tetrahydrocannabinol (Δ^8-THC) and Δ^9-tetrahydrocannabinol (Δ^9-THC) have been tested for their ability to produce cannabis-like pharmacological effects. The latter 2 compounds have been found to be the primary psychoactive compounds while CBD is much less active as a behavioral agent. Synthesis and testing of analogues of these naturally-occurring compounds has provided information regarding the structural requirements for cannabinoid activity. In a recent review of the literature, Mechoulam et al.[4] presented a summary of structure-activity relationships which showed the importance of a free phenolic group and an alkyl side chain for behavioral activity (figure). To determine whether the positions of these groups were relevant for pharmacological activity, ABN-Δ^8-THC and ABN-CBD were synthesized according to a recently reported procedure[5,6]. These novel analogs differed from the natural cannabinoids in that the phenolic hydroxyl group was transposed with the pentyl sidechain (figure). The purpose of the present study was to evaluate these abnormal compounds for cannabinoid activity.



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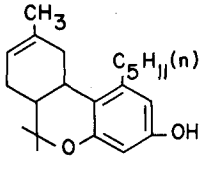
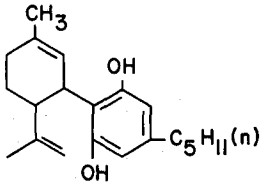
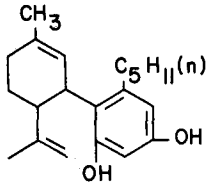


ABNORMAL - Δ^8 - THC



CANNABIDIOL



ABNORMAL CANNABIDIOL

Methods. The ability of these compounds to produce static ataxia (an effect unique to psychoactive cannabinoids) and other characteristic behavioral effects was examined in mongrel dogs of either sex (8–12 kg). The effects of cannabinoids on behavior, semiquantitated as previously described by Martin et al.[7], were observed for 30 min following i.v. administration. 3 observers independently rated the behavior of each dog, and the means of the maximum scores were recorded.

The effects of cannabinoids on spontaneous motor activity were assessed in Swiss-Webster mice (Dublin Farms, 20–25 g) after an i.p. injection of either vehicle or drug. 90 min after the injection mice were placed in photocell activity chambers (2 mice per chamber), and after a 10-min-orientation total counts were obtained for a 15-min-period. Uncorrelated sample means were compared statistically by applying Student's t-test. Cardiovascular

1 Acknowledgment. This work was supported by NIDA (grant No. DA-00574-01 and DA-00490) and Virginia Heart Society (grant No. RR-05697).
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