somal fraction and mainly in plasma membrane[12]. Corresponding with these observations, a surface mucous coat or glycocalyx and numerous lysosomes have been observed in this epithelium. Since it has been postulated that the Tamm-Horsfall urinary sialoglycoprotein is related to urinary tract cell surface[13], the notably high content of sialic acid and sialidase in sheep transitional epithelium might be coincidental findings of some biological significance. In fact, studies in course indicate that rat transitional epithelium contains low levels of sialic acid and negligeable sialidase activity[14].

Resumen. El epitelio de transición de cordero contiene actividad de sialidasa. Se demuestra que esta enzima se encuentra en lisosomas de este tejido y posee el pH óptimo ácido característico de enzimas lisosomales.

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Aggressive Behaviour Induced by Marihuana Compounds and Amphetamine in Rats Previously Made Dependent on Morphine

It has been described recently that rats, previously made physically dependent on opioids, show a traumatic aggressive behaviour when treated with amphetamine during the withdrawal phase[1,2]. The description of the fighting behaviour showed by these rats was similar to that of starved rats treated chronically with marihuana[3,4]. The present work was carried out to observe whether cannabis would induce aggressive behaviour in non-starved rats during withdrawal from previous morphine administration, and to compare the aggressive behaviour induced by these drugs.

Material and methods. Animals and Drugs. 124 male 3-month-old Wistar rats were maintained at 23 ± 1°C in air conditioned laboratories. A standard food pellet diet and tap water were provided ad libitum. Morphine hydrochloride (E. Merck, Darmstadt), D-amphetamine sulfate (Sigma Chemical Company) and (−) Δ^9-*trans*-tetrahydrocannabinol (THC) obtained from United Nations were employed. Morphine and amphetamine were dissolved in 0.9% NaCl solution; THC was suspended as recommended by others[5]. The amounts used are expressed in terms of the salts. Three experiments were carried out (see Table).

Experiment 1. 30 rats were housed in pairs in wire cages measuring $16 \times 30 \times 18$ cm. They received daily 3 i.p. injections of morphine, at 5 h intervals; the initial dosage was 12 mg/kg/day (3 injections of 4 mg/kg) which was doubled every 2nd day up to 96 mg/kg/day. 24, 48 and 72 h after the last morphine injection, therefore during withdrawal phase, 5 pairs of rats received 1.0 ml/kg of saline (group I), 5 pairs received 5.0 mg/kg of THC (group II) and the remaining 5 pairs 2.0 mg/kg of D-amphetamine (group III). The injections were i.p. and the pairs of rats were observed following the next 12 h for signs of depression, aggressiveness and stereotypy. 24 h later the animals were observed again. Depression was defined by the lack of motion and/or laying down of the rats; aggressiveness, scored in seconds, was the time the animals remained in a standing position trying to bite each other[3], and stereotypy was defined by sniffing, licking or biting the cage wire netting.

Experiment 2. In a first phase, 30 pairs of rats were treated with morphine as in experiment 1; however, the treatment was prolonged up to the dosage of 768 mg/kg/day. 8 other pairs of control rats were similarly injected with saline. At 24 and 48 h of withdrawal, 6 pairs received saline (group I), 8 pairs received 5.0 mg/kg of THC, 8 pairs received 10 mg/kg of a marihuana extract and the last 8 pairs were injected with 2.0 mg/kg of D-amphetamine (group III). As the results with THC and marihuana were similar, these 16 pairs of animals were considered together as group II. All injections were i.p. and the rats were observed as in experiment 1. Each 2 pairs of the control group received the same treatment given to groups I to III. As these animals showed only the typical effects of the drugs, they will not be included in the Table. In a second phase, starting 24 h after the last treatment with THC and amphetamine, morphine administration (and saline for control group) was reinitiated for all animals; now, 2 doses of 200 mg/kg of morphine were given daily, for 6 more days; at 48 and 72 h of a second withdrawal period the rats were submitted to same treatment as above.

Experiment 3. 18 rats were housed individually in the wire cages. Morphine addiction was induced by initial i.p. injections followed later by oral route according to a modification of the procedure by NICHOLS[6]. Injections of morphine were given twice daily at 12 h intervals; the initial dose was 10 mg/kg/day (2 daily injections of 5 mg/kg) and was doubled every 3rd day up to 160 mg/kg/day; the animals remained 3 more days on this dosage. Next, the animals were put for 6 weeks in a 3-day schedule of oral administration of liquids as follows: 1st day with water ad libitum, 2nd day without any liquid, 3rd day with morphine 0.8 mg/ml; the average intake of morphine in this phase was 141 ± 51 mg/kg/day (average $\pm$SD). 2 choice-tests between water and 0.8 mg/ml of morphine[6], at days 21 and 42, revealed that the animals preferred to drink morphine. After the second choice-test, animals were allowed to drink only the 0.8 mg/ml mor-

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Effects of 5.0 mg/kg of THC, 2.0 mg/kg of amphetamine and 1.0 ml/kg of saline on the behavior of paired rats in abstinence from morphine

Experiment	Groups and treatment	No. of pairs	Average weight (±SD)		Sec of fighting (average ± SE) and other effects observed during 12 h after drug treatment at		
			Initial	Final	24 h withdrawal	48 h withdrawal	72 h withdrawal
1	I Saline	5	188 ± 10	210 ± 8	(5)[a] none	(5) none	(5) none
	II THC	5	200 ± 7	208 ± 15	(5) depression	(5) depression	(5) depression
	III Amphetamine	5	201 ± 11	203 ± 7	(5) stereotypy	(5) stereotypy	(5) stereotypy
2	I Saline	6	182 ± 12	196 ± 15	(6) none	(6) none	–
	II THC	16	174 ± 7	186 ± 10	(16) depression	(6) 2754 ± 294	–
(phase 1)						(10) depression	
	III Amphetamine	8	183 ± 4	198 ± 17	(8) stereotypy	(8) stereotypy	–
2	I Saline	6	196 ± 15	180 ± 17	–	(6) none	(6) none
	II THC	16	186 ± 10	178 ± 12	–	(6) 830 ± 129	(8) 5064 ± 439
(phase 2)						(10) depression	(8) depression
	III Amphetamine	8	198 ± 17	188 ± 13	–	(8) stereotypy	(8) stereotypy
3	I Saline	3	192 ± 7	252 ± 13	–	(2) 352 ± 101	(3) none
						(1) none	
	II THC	3	198 ± 12	237 ± 11	–	(3) 7892 ± 260	(3) 4455 ± 1730
	III Amphetamine	3	201 ± 9	247 ± 15	–	(2) 7608 ± 154	(2) 5084 ± 440
						(1) stereotypy	(1) stereotypy

[a] Within parenthesis: number of pairs showing the effect; the number in front of parenthesis indicates sec of fighting.

phine solution for 3 more days and then the drug was withheld. 48 and 72 h later, each 6 animals were i.p. injected, respectively, with saline (group I), 5.0 mg/kg THC (group II) and 2.0 mg/kg of D-amphetamine (group III). Immediately after the 1st injection of these drugs (48 h after the last morphine intake) the animals were housed in pairs (therefore, 3 pairs for each group) and observed as before.

Results. The Table summarizes all the results. It is seen that in rats treated with morphine exclusively through i.p. route and paired by pairs from the beginning (experiments 1 and 2), amphetamine induced only stereotypy, regardless of the previous dosage of the opioid and the length of the abstinence period; on the other hand, THC induced aggressiveness in 40 and 50% of these rats at, respectively, 48 h (phase 1 of experiment 2) and 72 h (phase 2 of experiment 2) of abstinence. The saline-treated animals behaved normally.

In rats addicted by i.p. and oral routes and paired later on, the injections of both THC and amphetamine induced strong aggressive behavior either at 48 or 72 h of withdrawal (experiment 3); addicted animals injected with saline also showed some aggressiveness at 48 h withdrawal which disappeared, however, at 72 h. Amphetamine-aggressiveness appeared within 5 min after the injection, subsided within 4 h, to be replaced by stereotyped behavior. However, the animals did not seem to be really aggressive; they remained in the standing position, moving their forelegs, but they neither really tried to bite each other nor reacted to stimuli, such as a wooden stick put between them. This contrasted to the behaviour observed in THC-treated animals which were vicious and reacted violently to the wooden stick. Another difference was that in THC-treated rats, aggressive behaviour took 30 min to appear.

The Table shows also that with exception of phase 2 of experiment 2, all animals gained weight during the experiments.

Discussion. THC and a cannabis extract induced aggressiveness in rats which were in abstinence from morphine (experiments 2 and 3). However, to obtain such an effect morphine administration must be long enough; thus, when it extended only for 8 days or 96 mg/kg/day (experiment 1), THC failed to induce aggressiveness; on the other hand, after 60 days of treatment (experiment 3) aggressiveness appeared in all THC-treated rats. The influence of time and prewithdrawal dosage of morphine for the appearance of postwithdrawal spontaneous (without drugs) fighting was reported before[7]. On the other hand, THC was more efficient than amphetamine to provoke aggression in the rats. Thus, after 14 to 20 days of morphine administration, THC induced aggressive behaviour, whereas amphetamine did not (experiment 2). This does not agree with the findings of FLOREA and THOR[1] and THOR and HOATS[2], who observed fighting in rats receiving amphetamine during withdrawal from etonitazene or morphine. However, these authors used different procedures. They isolated their rats during all treatment with opioids, to aggregate them in groups of 6 per cage after amphetamine injection; amphetamine was given 90 h after the beginning of withdrawal phase[2] or repeatedly as 4 injections daily[1] of 2.0 mg/kg. We injected one dose of 2.0 mg/kg amphetamine, at most at 72 h of withdrawal, and caged the rats by pairs from the beginning of morphine treatment. The stronger aggressive-inducing property of Δ^9-THC was also demonstrated by the viciousness of these animals; thus, their fightings were fiercer and they attacked inanimate objects such as a wooden stick.

Recently, CARLINI and MASUR[4], PALERMO and CARLINI[8] and CARLINI et al.[9] have hypothesized that the stress induced by starvation is the important factor associated with the capacity of cannabis to induce aggressiveness in starved rats. The present work further supports this hypothesis; thus, aggressiveness during abstinence from morphine was obtained with THC-injected rats that had food ad libitum and did not lose weight, which indicates

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that they were not undernourished as the animals studied by CARLINI and MASUR[3,4]. Therefore, we believe that the strong stress suffered by the rats undergoing morphine withdrawal is the factor associated with the THC capacity to induce aggressiveness in these animals.

Résumé. Les rats soumis à la morphine ont été traités pendant la phase d'abstinence avec des injections i.p. d'amphétamine et (−) Δ^9-*trans*-tétrahydrocannabinol. Ces deux drogues provoquèrent une conduite agressive chez ces animaux. La durée du traitement préalable avec la morphine et le temps d'abstinence précédant l'injection des drogues furent des facteurs importants dans l'apparition de l'agressivité. Le Δ^9-THC fut à cet effet plus puissant que l'amphétamine.

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The L-4946 Leukemia in the CF[1] Mouse

The viral nature of some murine leukemias has been well established[1,2]. Yet the viral passage of some mouse leukemias has not been accomplished. This paper will present data to suggest that another murine leukemia (L-4946) may be passed by subcellular material. An extract containing RNA and protein which will induce an abdominal lymphosarcoma in the adult CF1 mouse has been isolated from L-4946 ascitic fluid.

Material. The ascitic form of this leukemia[3] in the CF1 mouse was maintained by intraperitoneal injection of 0.5 ml of leukemia ascitic fluid, each mouse receiving approximately 10^6 cells. Leukemogenic activity was defined as death from leukemia within 40 days of inoculation in 2-month-old adult female CF1 mice, mortality as the ratio of the number of mice who died of leukemia within 40 days to the total number of mice inoculated. Time of 50% mortality is defined as the day by which half of all injected mice in a specific group had died.

Methods and Results. A $12{,}000 \times g$ supernatant of the ascites fluid was found to be leukemogenic. The ascitic fluid of leukemic mice was centrigufed twice at $12{,}000 \times g$ for 10 min and the second supernatant used for experiments or further purification.

Seven strains of mice were inoculated with either ascitic cell or the $12{,}000 \times g$ supernatant. Inoculation of 1×10^5 ascitic cells caused death in all 7 strains of mice. Four strains (CF1, AKr, C3H and A) were susceptible to the $12{,}000 \times g$ supernatant. The CBA/2 and C5F Black did not die or accumulate ascitic fluid during the period of experimental observation (60 days).

Five successive 40-min centrifugations were performed on the ascitic fluid and the resulting supernatants; each pellet was obtained after increasing the centrifugal force until the last pellet and the final supernatant were obtained at $125{,}000 \times g$. Leukemogenic activity was found in all pellets and the final supernatant. The apparent heterogeneity of the leukemogenic agent, implied by the presence of activity in all fractions, could be explained by adsorption of the agent to various constituents of the ascites fluid, entrapment of activity in the sediments, or presence of an inhibitor.

The leukemogenic activity of the $12{,}000 \times g$ supernatant was precipitated by either ethanol or ammonium sulfate. Both ethanol and ammonium sulfate precipitated the leukemogenic activity in an insoluble form which was assumed to contain fibrin or fibrinogen. Plasmin was found to remove large quantities of an inert protein material from the precipitate; however, the final leukemogenic precipitate remained insoluble in saline.

Four enzymes (trypsin, DNase, RNase and lysozyme) were used to study the chemical properties of the $12.000 \times g$ supernatant and subcellular extract. The leukemogenic activity of 1.0 ml of the $12{,}000 \times g$ supernatant was not decreased after incubation with 0.1 mg of trypsin, DNase, RNase or lysozyme at 37°C for 1 h (Table I). However, when the plasmin-insoluble precipitate was incubated under these same conditions, the leukemogenic agent was sensitive to trypsin, and possibly to RNase. The resistance of the $12.000 \times g$ supernatant activity to trypsin may have been due to substrate competition between the oncogenic agent and the high concentration of plasma proteins in the supernatant.

Chemical characterization. The plasmin-insoluble precipitate was the most highly purified preparation of the leukemogenic agent. Table II presents data comparing this preparation to the $12{,}000 \times g$ supernatant from which it was isolated. Protein determination of the $12{,}000 \times g$ supernatant indicated that each mouse received 3.2 mg of protein. The composition of the plasmin-insoluble precipitate was 94% protein and 6% nucleic acid. This nucleic acid was RNA.

The saline supernatants obtained after washing L-4946 ascitic cells with saline in preparation for cell dilution experiments were found to be leukemogenic. The origin of this leukemogenic activity was thought to be the elution of adsorbed subcellular material from cell surfaces and/or the rupture of fragile cells.

The leukemogenic activity of the saline supernatant was found to be similar to that of the $12{,}000 \times g$ supernatant. Centrifugation of the saline supernatant at $125{,}000 \times g$ for 1 h yielded a leukemogenic supernatant.

The saline supernatant was also subjected to ethanol precipitation by the same technique that was described for the $12{,}000 \times g$ supernatant, except that the ethanol concentration was increased from 7 to 15% in order to obtain precipitatable material. The first precipitate at 15% ethanol contained 2 components – one saline-soluble, the other saline-insoluble. Both precipitated components were leukemogenic, killing all recipient mice.

Discussion. As previously reported[4], the leukemogenic activity of the $12{,}000 \times g$ supernatant is inactivated by

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