nounced during the 1st 120 min of the session. The profiles of the other fractions (figure) are similar to that reported for nicotine by Bovet and Gatti[16], i.e. they show an amelioration in performance and a fall in reaction time (table).

Discussion. In our experiment, the Bovet-Gatti profile obtained for III_s, i.e. the smoke inhaled by the cannabis consumer, conforms with the one previously reported for hallucinogens[1]. Similar results obtained for Δ^9-THC were interpreted as indicating a hallucinogenic effect of the drug[4]. We do not interpret our results in the same way. The animal displays a highly complex behavior associating painful and visual stimuli while distributing its responses in time in the most profitable way. Cannabis products or extracts might interfer at any one of these levels, since besides psychogenic effects they induce analgesia in mice and alter the perception of time in man[14,15].

It is interesting to note that the profile of II_s conforms with that reported for nicotine[16]. Taking into consideration the concentration of Δ^9-THC in II_s, we have calculated that the dose of Δ^9-THC injected with it was 1.5 mg/kg. A similar dose of Δ^9-THC given by Webster et al. in the rat caused a significant decrease in the animal's efficient responses[4,5]. This confirms the hypothesis of Karniol and Carlini according to which the effects of several samples of cannabis sativa are not related only with their Δ^9-THC content. These investigators explained their results as due to the existence of various quantities of other cannabinoids in the marijuana extracts they used. In our experiment the relative concentration of Δ^9-THC:CBD:CBN was 2.2:1:7.7 for III_s and 2.7:1:43.5 for II_s.

On the other hand, the existence of some unknown substances, possibly pyrolysis products of the known cannabinoids, may be responsible for this modification of the action of Δ^9-THC in II_s. The hashish smoke (III_s) may be devoid of such substances. We are trying at present to evaluate the validity of this hypothesis. Similar research involving marijuana smoke might provide us with certain very interesting data in the view of the different filters used in the 2 modes of cannabis consumption.

Our results do not agree with those of H. Savaki et al. where both II_s and III_s were equally effective[17]. The specificity of their tests is questioned since fractions of tobacco were shown to be quite active as well. On the other hand, the usefulness of their results are undeniable in view of the difficulty of using the Sidman schedule as a routine method in screening drugs obtained in a serial fractionation of the highly complex cannabis pyrolysates. Consecutive testing of certain key drugs among the active ones, in the Sidman avoidance, may permit us a more reliable characterization of the drugs as active or nonactive.

1 U.R. Smythies, V.S. Johnston and R.J. Bradley, Br. J. Psychiat. *115*, 55 (1969).
2 H. Isbell, G.W. Gorodetzsky, D. Jasinski, K. Claussen, F.V. Spulak and F. Korte, Psychopharmacologia *2*, 184 (1967).
3 C.L. Scheckel, E. Boff, P. Dahlen and I. Smart, Science *169*, 1467 (1968).
4 U.C. Webster, M.C. Willinsky, B.S. Herring and G.C. Walters, Nature *232*, 498 (1971).
5 C.D. Webster, A.E. Lebanc, I.A. Marshman and I.M. Beaton, Psychopharmacologia *30*, 217 (1973).
6 A. Moschovakis, D. Liakopoulos, A. Armaganidis, B. Kapsambelis, G. Papanikolaou and G. Petroulakis, Psychopharmacology *58*, 181 (1978).
7 I.G. Karniol and E.A. Carlini, J. Pharm. Pharmac. *24*, 833 (1972).
8 I.G. Karniol, I. Shirakawa, N. Kasinski, A. Pfeferman and E.A. Karlini, Eur. J. Pharmac. *28*, 172 (1974).
9 I. Volarka, P. Grown, R. Dornbush and M. Fink, J. Psychiat. Res. *10*, 159 (1969).
10 G. Karniol and E.A. Karlini, Psychopharmacologia, Berl. *33*, 53 (1973).
11 L.E. Hollister and H. Gillespie, Clin. Pharmac. Ther. *18*, 80 (1975).
12 R.N. Takanashi and I.G. Karniol, Psychopharmacologia, Berl. *41*, 277 (1975).
13 T.A. Kephalas, I. Kibouris, I. Michael, C.M. Miras and D.P. Papadakis, Satellite Symp. 6th int. Congr. Pharmac. Helsinki, Finland, July 1975. Springer, Berlin, in press.
14 W.D.M. Paton and R.G. Pertwee, in: Marijuana, p.287. Ed. R. Mechoulam. Academic Press, London 1973.
15 W.D.M. Paton and R.G. Pertwee, in: Marijuana, p.191. Ed. R. Mechoulam. Academic Press, London 1973.
16 U. Bovet and G.L. Gatti, Proc. 2nd int. Pharm. Meeting, Prague 1963.
17 H.E. Savaki, Jomar Cunha, E.A. Karlini and T.A. Kephalas, Bull. Stupéfiants *28*, 51 (1976).

Possible stimulatory effect of retinoic acid on pulmonary macrophages

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Summary. Retinoic acid was administered to hamsters suffering from N-nitroso-N-methylurethane-induced fibrosing alveolitis. A significant increase in macrophage numbers was seen in the lungs of retinoid-treated animals as compared to the unsupplemented group.

Retinoic acid and its synthetic analogues (retinoids) have been shown to possess a number of biological activities. Among these are maintenance of normal epithelial differentiation[1], reversal of epithelial metaplasia[2,3], and inhibition of chemical carcinogenesis[4-6]. Recently, the role of retinoids in immunological reactions has come under investigation. A number of studies suggest an enhancement of humoral and cell-mediated immunity following retinoid administration[7-10]. In particular, Leutskaya and Fais[11] report stimulation of antibody synthesis and Dennert and Lotan[12] observe increased activity of killer T cells with retinoic acid. This study suggests an additional effect of retinoic acid on the immune system, that of increasing macrophage activity. Hamsters suffering from fibrosing alveolitis, a lung disease thought to be immunologically mediated[13], which were fed retinoic acid, showed significantly increased numbers of pulmonary macrophages as compared to animals on unsupplemented diets.

Fibrosing alveolitis represents a class of diseases better known as 'pulmonary fibrosis'. The disease is often asso-

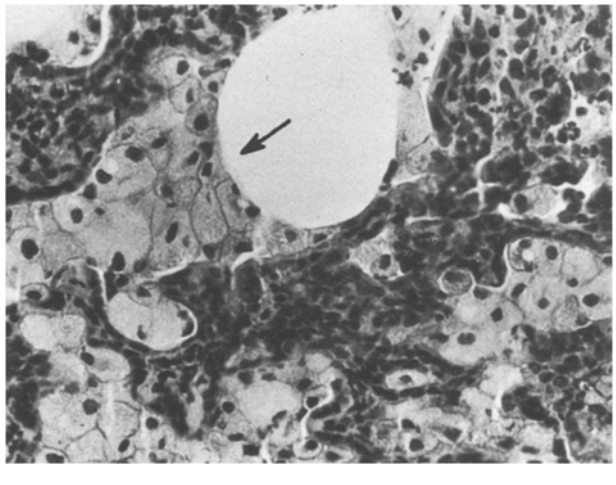
ciated with systemic immunological abnormalities such as sarcoidosis, rheumatoid arthritis, and scleroderma. It is believed to have an immunological pathogenesis. In our study, the disease was induced in Syrian Golden Hamsters by administration of the carcinogen, N-nitroso-N-methylurethane (NNNMU)[14], which in this model does not produce lung neoplasias, but rather initiates intense pulmonary inflammation with eventual scarring and distortion of lung architecture. NNNMU was given to the hamsters by s.c. interscapular injections on a weekly basis for 9 weeks. The weekly dosage was either 0.3 mg or 0.2 mg per animal. Varying the dose in this way affected the rate at which the disease developed, but not necessarily the final extent of the lung lesions. The disease continued to progress after curtailment of NNNMU injections.

The hamsters were placed either on a standard diet, to which was added retinoic acid in the form of gel beads (4.425 g/10 kg diet) or on feed containing placebo beadlets. The diets were instituted at the start of the NNNMU injection schedule. Animals were sacrificed at monthly intervals. The lungs were removed, inflated under negative pressure, and fixed in formalin. They were then cut up into random bits and processed for histological examination.

Alveolar macrophages

Month	Score* ± SE (no. animals)		Percent
	Retinoic acid	Control	increase
4	6.0±1.3 (3)	4.7±0.79 (3)	27.7
5	5.6±1.3 (4)	4.5±0.98 (4)	24.4
6	5.6±1.3 (4)	4.7±0.91 (4)	17.0
7	7.5±0.84 (3)	4.8±1.3 (3)	56.3
8	7.3±1.0 (4)	3.3±0.87 (2)	121.3

*Lungs were removed, inflated under negative pressure, and fixed in formalin. The entire tissue was processed for histological examination. Slides were coded and evaluated by a pathologist (J.O.C.), using a grading system of 0–10 for the presence of macrophages. The results were corroborated by a second pathologist (P.dSA.). Combining the results of the different intramonth comparisons, the overall significance is 0.0006 for a one-way analysis of variance.



Photomicrograph of the lung of a retinoid-treated hamster with NNNMU-induced fibrosing alveolitis. Macrophages (arrow) appear clustered within alveolar lumens and have large, vacuolated cytoplasms. These cells remain a prominent feature of the disease even after resolution of interstitial inflammation. Hamsters fed retinoids (4.425 g/10 kg diet) had greater numbers of alveolar macrophages than did untreated animals. Cellular proliferation in the interstitium is composed mostly of ballooned-out alveolar lining epithelium and type II cells.

Comparison of the lungs of animals in both diet groups during the first 3 months did not reveal significant differences in morphology. There was an intense interstitial inflammatory reaction consisting of lymphocytes, plasma cells, neutrophiles and macrophages. Ballooning of alveolar epithelial lining cells also occurred and many alveoli were denuded of these cells. Type II pneumocytes underwent abundant proliferation and showed increased numbers of lamellar inclusions which were greatly enlarged. Numerous alveoli appeared completely collapsed while adjacent areas showed dilation and distortion of air spaces.

By the 4th month, the inflammatory infiltrates began to recede and the disease entered a predominantly healing phase. Lung cell populations tended to stabilize at this point, facilitating more meaningful histological evaluation. It was noted, during this time and in succeeding months, that the retinoic acid supplemented animals had greater numbers of alveolar macrophages than did the untreated group (table). These cells appear, under the light microscope, to have pale, vacuolated cytoplasms (figure). They tend to cluster within alveolar lumens and, in general, are seen in greatest numbers in the more damaged areas of the lung.

Though NNNMU does not generally cause neoplastic changes in the lung, proliferation and metaplasia of pulmonary epithelium are a prominent feature of the disease. Retinoic acid treated animals did not show noticeable arrest of this epithelial proliferation and the overall extent of the disease was similar for both groups. At lower doses of NNNMU, however, such an effect might be seen.

How retinoic acid might produce this increase in alveolar macrophages is not clear. It may represent some general action of retinoids on the immune system which is, in part, expressed by increased macrophage activity. Alternately, retinoids may specifically act on the macrophage. Macrophages have been shown to be hormone responsive and appear to have receptors for insulin[15] and certain of the glucocorticoids[16]. The role of the macrophage in fibrosing alveolitis is similarly uncertain. Such cells are known to clear the lung of debris, and macrophages in other systems have been implicated in cell-mediated immunity[17]. Their potential for recognizing and destroying neoplastic cells is also being investigated. Retinoids appear to possess antineoplastic properties and their ability to act on macrophages may play a role in this phenomenon.

1 S.B. Woeback and P.R. Howe, J. exp. Med. *42*, 753 (1925).
2 D.P. Chopra and L.J. Wilkoff, J. natl Cancer Inst. *58*, 923 (1977).
3 A.C. Marchok, M.V. Cone and P. Nettesheim, Lab. Invest. *33*, 451 (1975).
4 P. Nettesheim, M.V. Cone and C. Snyder, Cancer Res. *36*, 996 (1976).
5 W. Bollag, Eur. J. Cancer *8*, 689 (1972).
6 R.C. Moon, C.J. Grubbs, M.B. Sporn and D.G. Goodman, Nature *267*, 620 (1977).
7 G.L. Floersheim and W. Bollag, Transplantation *14*, 564 (1972).
8 M. Jurin and I.F. Tannock, Immunology *23*, 283 (1972).
9 D.W. Dresser, Nature *217*, 527 (1968).
10 E. Seifter, G. Rettura and J. Padawer, J. natl Cancer Inst. *57*, 355 (1976).
11 Z.K. Leutskaya and D. Fais, Biochim. biophys. Acta *475*, 207 (1968).
12 G. Dennert and R. Lotan, Eur. J. Immun. *8*, 23 (1978).
13 R.P. McCombs, N. Engl. J. Med. *286*, 1186, 1245 (1972).
14 K. McD. Herrold, Am. J. Path. *50*, 639 (1967).
15 R.S. Bar, C.R. Kahn and H.S. Koren, Nature *265*, 632 (1977).
16 H. Schorn, C. Walter and J. Lafuma, J. Steroid Biochem. *8*, 25 (1977).
17 M.N. Pearson and S. Raffel, J. exp. Med. *133*, 494 (1971).