CELLULAR EFFECTS OF CANNABIS - SUPPORTING INTERFERENCE OF THE DRUG WITH ARGININE UTILIZATION AND/OR METABOLISM: A NEW HYPOTHESIS ON MECHANISM OF ACTION

Marietta R. Issidorides

Department of Psychiatry
University of Athens
Athens, Greece

I. INTRODUCTION

A single unifying mechanism by which cannabis exerts its many actions in a variety of tissues may not be a biological prerequisite. Data, however, obtained from cellular studies of chronic hashish users and matched controls, consistently indicated repercussions of the drug on arginine metabolic pathways in several functionally diverse cell populations. Analysis and interpretation of these data led to the formulation of the hypothesis that cannabis may act through a common metabolic denominator, namely arginine "depletion" (1). In the first studies (2), electron cytochemical methods (3,4) demonstrated a reduction of arginine-rich histones in the nuclei of lymphocytes and of arginine-rich protamines in the nuclei of spermatozoa. This reduction seemed counterbalanced by increased amounts of lysine-rich proteins in both cell types. Such altered histone ratios in the lymphocyte denotes over-repression of the genome (5), i.e., a reduced potential to respond to antigenic stimuli (6). In the sperm nucleus, however, the altered protein ratio denotes arrested maturation since during the late stages of spermiogenesis lysine-rich histones are shed and substituted by newly synthesized arginine-rich protamines (7) which endow: a) the sperm tail with motility, and b) the sperm chromatin with stability and resistance to noxious agents during its trajectory from testis to ovum (8). Clearly, the similarly altered histone ratio in lymphocytes and spermatozoa of chronic hashish users had entirely different implications for the functional capacity of

each cell type. Functional repercussions, predictable from the low levels of arginine-rich proteins in the lymphocytes and sperm, have been substantiated by the findings of inhibited cellular-mediated immunity (9) and of decreased motility and total sperm count (10) in heavy marijuana users. The causal relation of low protamine content to sperm functional impairment and oligospermia was established by Silvestroni and his collaborators in their studies of congenital fertility problems (11). Their findings would suggest that long-term cannabis use mimics the specific metabolic aberration, i.e. inhibition of protamine synthesis, that characterizes infertility or "idiopathic spermatidic arrest".

The dependence of normal spermatogenesis on the amino acid arginine was first reported by Holt and Albanese (12), who also demonstrated the experimental modulation of fertility solely by deprivation or supplementation of arginine in the diet, both in humans and rats. Subsequent studies established the therapeutic effectiveness of arginine administration in oligospermia (13) as well as its stimulatory action on sperm motility (14). Effects on sperm motility exactly opposite to those of arginine supplementation have been observed with the exposure of spermatozoa to THC (15). These data support our hypothesis that THC and arginine actions overlap at the same sites with opposite biochemical and functional effects.

In view of the mounting evidence that cannabis inhibits transcription in a variety of cellular systems (16, 17) we sought in cells of chronic hashish users morphological correlates of macromolecular synthesis inhibition, as those are defined in cell biology. Chromatin which is highly condensed, either as heterochromatin or as compact chromosomes of cell division, is transcriptionally inert (18). A wide spectrum of drugs and metabolic inhibitors induce structural changes in nuclei and these changes were found to have functional significance (19, 20). In previous studies with the electron microscope (EM) we had observed that structural changes, i.e., chromatin condensation, in leukocytes of patients after neuroleptic treatment paralleled clinical changes (21,22). These findings prompted the initiation of similar investigations on cells of chronic cannabis users and controls for the study of chromatin with a special EM method (23). The results showed significantly that in users the somatic cells (leukocytes) displayed highly condensed nuclear chromatin, while the germ cells (ejaculated spermatozoa) showed the opposite state: decondensed chromatin and undifferentiated nuclei (1). In a recent report (24) we demonstrated with the EM four types of sperm head abnormalities, in 50% of the spermatozoa of chronic hashish users, that exemplify the abnormalities classified by Holstein and Schirren (25) as indicative of infertility. It is well documented that sperm nuclear condensation during

maturation is a process requiring the coordinated synthesis of arginine-rich proteins (26, 27). The different abnormalities in sperm nuclear condensation observed in the EM were, therefore, the natural consequence of the low protamine content, which distinguishes the sperm of chronic hashish users from that of controls (2). Similarly, the inappropriate excessive condensation of nuclear chromatin in somatic cells, observed in the leukocytes of the users, can rapidly be induced by deficiency of arginine in the medium (28). Arginine-poor media will also cause an arrest of DNA, RNA and protein synthesis (29), which also are well documented actions of cannabinoids (30). Other reports have shown that in the absence of arginine morphological disorganization of nuclei and fragmentation of chromosomes take place in cultured cells (31). Similar findings were reported by Leuchtenberger et al. (32) and Morishima et al. (33) in cultures exposed to cannabinoids, as well as in chronic hashish users (24), the neutrophils of which displayed in the EM protrusions of nucleus (drumsticks) and attached fragments of chromosomes in the cytoplasm (micronuclei). A synthesis of all these data reveals that a putative "depletion" of arginine by cannabis would suffice to explain both the chemical and the ultrastructural aberrations that we and others have observed in the cells of chronic hashish users and in cultures exposed to cannabinoids, respectively.

II. NEW FINDINGS

We pursued the concept of cannabis/arginine interaction further by examining in chronic hashish users and controls other cell types - known to be affected by cannabis -, where arginyl proteins have a major functional role.

The clinical group consisted of 16 chronic users and 10 matched controls. All were paid volunteers recruited from a larger population that was extensively used for a long-term controlled investigation of the effects of chronic cannabis use in man (34). Inclusion criteria in the users' group were 1) age below 58 years, 2) regular hashish use for more than 10 years continuing up to the day of testing, 3) no use of other addictive substances except for tobacco smoking and irregular social alcohol drinking, and 4) absence of gross neurological disorders or incapacitating physical illness. Controls had to match the users with respect to age, place of upbringing and residence, education and socio-economic level. They had also to be regular tobacco smokers and to never have used any addictive substances except alcohol irregularly and moderately. Data regarding hashish use by the group have appeared

in detail elsewhere, (2,24). The material smoked by the subjects contained 4-5% delta-9-tetrahydrocannabinol (THC).

A. Chronic Effects of Hashish Smoking in Blood Platelets

In human platelets alpha-granules store basic proteins (Factor 4) with anti-herparin activity (35,36), which are released following stimulation by ADP or epinephrine (37,38). The precursor of these proteins is an immumologically identical more basic protein with essential arginines (39). Pellets of platelets from users and controls were prepared by the ammoniacal silver reaction for arginine (4) in order to localize this precursor at the ultrastructural level (24). The results showed that in control subjects all platelets contained numerous granular deposits of arginine-rich proteins, but in chronic users all platelets had suffered a 90% reduction of their granular arginine-rich deposits. This finding is consistent with the impaired ability of platelets from marijuana smokers to aggregate when stimulated by ADP in vitro (40). The greatly reduced level of the arginine-rich protein in the platelets of chronic users justifies cannabis-induced impairment of aggregation potential since this protein promotes clotting (38). We point out that polyamines, which are present in platelets (41) and modulate clotting by inhibiting platelet aggregation induced by ADP (42), are also derivatives of arginine through the ornithine pathway. Given limited substrate levels, a diversion of arginine in the latter pathway would be adequate to explain both cannabis' inhibitory effect on ADP-stimulated aggregation (through polyamines?) and the observed depletion of arginyl proteins from their granulated stores.

B. Acute Effects of Marijuana Smoking in Erythrocytes

Five subjects were selected from the group of 16 on the basis of their continuous daily smoking during the previous 30-day period. They served as their own controls in a pilot study of the acute effects of cannabinoids on various ultrastructural and chemical parameters in blood (43). The subjects were instructed to abstain from cannabis smoking for 12 hours before testing. On the day of testing, each subject was allowed to smoke ad libitum within a period of 15 minutes home-made cigarettes containing 1 g active marijuana supplied by NIDA with a 2.6% THC content. Blood samples were obtained before smoking and again 30 minutes after smoking, i.e., at a time that coincides with the peak effect on heartbeat, with a

subjective "high" and with the highest concentration of the drug in the blood (44).

We report the preliminary results obtained from blood smears (24), which were used to examine direct cannabis/arginine interations with the following rationale. The erythrocyte, which represents a well known target of cannabis actions (45-49), contains at least two membrane proteins rich in arginine residues. The first, enzyme acetylcholinesterase, is a major outer surface protein (50) with 31 arginine residues (51). The second, the structural band 3, is the preponderant transmembrane polypeptide of the human erythrocyte with 44 arginine residues (52), a certain of which is essential for cholride transport (53). Erythrocyte function relies heavily on lipoprotein associations in the membrane structure, especially on surface phospholipids, which are required also for the catalytic activity of ($Na^+ + K^+$) ATPase (54). Recent studies have provided clear and convincing evidence that arginine residues may play an important role in phospholipid-protein interactions by acting as binding sites for negatively charged phosphate groups in phospholipid molecules (55). This was demonstrated by the finding that modification of the phosphatidycholine-transfer (exchange) protein with butanedione and phenylglyoxal, which are arginine-specific dicarbonyl reagents, completely inactivated the enzyme. It has also been shown that the reactive arginines, essential for binding, are embedded in hydrophobic sequences of the protein (56), a property of the active site which favors strong anion binding (57) and for which THC would have special affinity (58).

In an earlier experimental trial, of a partly similar design, with a group of subjects drawn from the same population of users, it was shown that 30 minutes after heavy hashish smoking the concentration of phosphatidylcholine and of other membrane phospholipids decreases significantly in the erythrocytes (48). In light of Akeroyd's data (55) this acute effect of cannabis suggested a loosening by the drug of the phospholipid-protein association in surface molecules, presumably leaving the protein-bound arginyl residues unmasked. To test this particular eventuality we employed an arginine-specific ligand on formaldehyde-fixed blood smears collected from the five subjects before and after marijuana smoking. This reagent, phenanthrenequinone (PQ), is a non-fluorescent compound reacting specifically with arginine to form a condensation product, which exhibits strong fluorescence when viewed under ultraviolet light (59). The PQ reaction is an extremely sensitive and specific test for polypeptide-bound arginine in fixed smears (60) and in molecules with low arginine content such as hemoglobin (59). Because of these properties PQ is uniquely suitable for determining any acute changes in arginine availability in the erythrocytes following marijuana

smoking. Staining and fluorescence microscopy were applied according to Magun and Kelly (60).

As expected, PQ/arginine condensation product was formed in the leukocytes (with histones) and the platelets (with basic proteins) and showed no changes at 30 minutes post-smoking. In contrast, the uniform diffuse fluorescence of the erythrocytes was blocked following marijuana smoking while intense fluorescence emerged along their membrane. These changes reveal cannabis/arginine interactions, an interpretation inherent in the PQ method employed. These results support first the presence of reactive arginyl residues at the erythrocyte surface following the presence of cannabis in the blood. Such a finding would be consistent with a perturbation in the lipoprotein structures and with the observed loss of phospholipids from the membrane (48). It is not clear from this experiment which of the membrane proteins is responsible for the cannabis-induced PQ-arginine fluorescence. Of the three proteins which have essential arginines at their active site, i.e. acetylcholinesterase (51), ($Na^+ + K^+$) ATPase and band 3 at the chloride channel (53), the activity of the first two has been found to be inhibited by THC (62-64). Since phospholipids are required for the active ATPase configuration and for catalytic activity (54) it is reasonable to suggest that at least this protein would provide unmasked arginyl residues free to react with PQ after cannabis-induced phospholipid loss from the membrane.

The diffuse PQ fluorescence of the erythrocytes evident before smoking, but blocked after smoking, may tentatively be attributed to cannabis/hemoglobin interactions, without excluding alternative interpretations (24). Erythrocytes exhibit a lowered capacity to engage oxygen after cannabis use (46). Hemoglobin, which represents the major intracellular red cell protein shows decreased affinity for oxygen following modification of its arginyl residues by specific reagents (65). Furthermore, PQ generates intense fluorescence with the arginines in hemoglobin molecules (59). These data suggest that in our experiment the blocking of the diffuse PQ/arginine fluorescence in the erythrocytes 30 minutes post-smoking may be due to masking of the arginine residues in hemoglobin by a cannabis/arginine interaction, which produces hindrance to PQ attachment, and which would be in accord with both the chemical and physiological data.

C. Comment

The postulate that cannabis may produce its varied biological effects through interactions with arginine, in many cellular systems and pathways, does not appear to conflict

with the data obtained from chronic or acute exposures to the drug. To test the validity of this hypothesis, however, we need to survey a wider spectrum of known biological effects of cannabis, as well as of known biological effects of arginine deficiency, deprivation, depletion or inactivation and, then, determine the degree of overlap by taking into account the proposed mechanism of action for each case. The review that follows is designed to serve this goal with no intent of being exhaustive.

III. REVIEW OF EXPERIMENTAL EVIDENCE SUPPORTING CANNABIS/ARGININE INTERACTIONS

Although limited to the discussion of our own findings, the previous sections have introduced some of the data defining the principles according to which THC, a highly lipophilic molecule, could modulate biological processes which are dependent upon the basic amino acid arginine. A comparatively recent realization in the field of protein chemistry is, that arginine, because of its unique molecular properties (66), has secured during evolution an important position in a broad spectrum of metabolic functions (67-69). The co-planarity of the three nitrogens and the central carbon atom enables the guanidium side chain of arginine to enter into extended patterns of hydrogen bonding that are unique, also a unique aspect of the side chain is its very high pKa; of all the common amino acids it is probably the only one whose charged nature can never be suppressed under physiological conditions (69). Present investigations emphasize important generality, that arginyl residues can serve as positively charged recognition sites for negatively charged substrates and anionic cofactors in enzyme active sites, and in all proteins that interact with anions (70). Arginine-specific reagents were unknown prior to benzil (71). The application of these alpha-dicarbonyl arginine-specific reagents (72), i.e. diacetyl or 2,3-butanedione (73), 1,2-cyclohexanedione (74) and phenylglyoxal (75) for the modification of arginyl residues opened up a rapidly progressing field and established the functional importance of essential arginines. Thirteen of the fourteen enzymes in the glycolytic pathway, in which all the intermediates are phosphoric acid esters, were shown to contain essential arginines at the active site (66). Arginyl residues are essential in carboxyl and nucleotide binding sites, as well as in dehydrogenases for binding of NADH and in kinases for binding of coenzymes (70). The functional importance of essential arginines also in strategic sites of other non-enzyme proteins is illustrated by hypophyseal pro-opiocortin, where arginine

is essential for the processing of this molecule into its hormonally active subunits (76,77).

A. Macromolecular Synthesis

Nahas et al. (78, and refs. therein) were the first to introduce the notion of inhibition of macromolecular synthesis by cannabinoids *in vitro*. Jakubovic and McGeer (79) were the first to document extensively that under a variety of conditions THC brings about marked changes in the biosynthesis of key cerebral macromolecules. Experiments with isolated rat brain slices showed inhibition by THC of the incorporation of labeled leucine into protein fraction, while active transport of the amino acid into the cells was not affected. Similarly, THC inhibited incorporation of labeled uridine into nucleic acids, but also decreased uridine nucleotides in the soluble fraction, while the relative amount of labeled uridine was high. These results would imply that the decreased incorporation into nucleic acids was related to the reduced phosphorylation of the uridine. Another important finding was that a large amount of THC and/or metabolites was retained by brain slices during incubation which suggested the possibility of prolonged action in inhibiting the synthesis of cerebral macromolecules (79). Comparable results obtained with THC on rat testis (80) confirmed further that a general inhibition of protein synthesis occurs. It was found that *de novo* synthesis of nucleic acids was inhibited as much as synthesis through the salvage pathway. The decreased biosynthesis of labeled RNA appears to be directly related to the inhibition of phosphorylation of the precursor of RNA. A severe reduction of TDP and TTP indicated the same mechanism for the inhibition of DNA synthesis. The authors suggested that "the reduced ATP levels in the cell and/or the phosphorylation or perhaps even direct inhibition or nucleic acid polymerases may be the principle steps at which cannabis derivatives act on nucleic acid synthesis".

Arginine or its metabolite(s) have been implicated in the regulation of DNA synthesis in cultured liver cells (81). These authors presented evidence that quiescent primary liver cells in culture can be induced by a) serum, b) ornithine or c) arginine to synthesize DNA and to divide, suggesting that low-molecular-weight nutrients might be involved in the control of DNA synthesis. In these experiments arginine seemed to be limiting for biochemical events which led to the initiation of DNA synthesis. When the pool size of arginine dropped below a certain level, the cells became arrested in the G_1 phase of the cell cycle. It was concluded that ornithine and/or arginine and/or their proximal metabolites have regula-

tory functions in the control of DNA synthesis, the latter being regulated by intracellular levels of these compounds. Alterations in liver nucleic acids and nucleotides in arginine-deficient rats were demonstrated by Hassan and Milner (82). A depression of approximately 30% in RNA and DNA synthesis in the livers of arginine-deficient rats were closely paralleled with growth depression, increased liver lipids and a 52% decrease in the concentration of ATP (82). In all these experiments the arginine deficiency increased total pyrimidine nucleotides while decreasing total purine nucleotides. Such altered ration of nucleotides leads to altered base ratios and concomitantly to altered nucleic acid synthesis.

Purine nucleoside phosphorylase has an important role in purine metabolism, probably in purine salvage. The enzyme from both human erythrocytes and calf spleen is inactivated by butanedione and phenylglyoxal through the covalent modification solely of arginine residues (83). In addition, each of the various classes of nucleotide polymerases has been reported to be inactivated by arginine modification (84-86). As in all other cases of enzyme inactivation by arginine-specific reagents, these inhibitions are reversible, and can be prevented by the simultaneous presence of the natural substrate of the enzyme in the test system; the substrate protects the active site arginine from reacting with the modifying agent. It is of interest that the results obtained by Jakubovic and McGeer (79, 80) from the exposure of brain and testis slices to cannabinoids were interpreted as being due to inhibition of phosphorylation, low ATP and, probably, to interference with the activity of nucleic acid polymerases, all of which could occur if cannabis interacted solely with arginine in these systems as described above.

Similar inhibition of macromolecular synthesis by THC has been observed not only in isolated mammalian cells such as normal human fibroblasts, human and mouse neuroblastoma cells (87), but also in the unicellular Tetrahymena pyriformis, where it resulted in depressed exponential growth (88). The reduction in DNA, RNA and protein synthesis in this unicellular organism was accompanied by effects also on the ribosomes. A reduction in the amount of polysomes was observed in cellular fractions obtained from THC-treated cells, while the amount of monosomes was the same in THC-treated and control preparations. Dissociation of polyribosomes in vivo has been reported in electron microscopic studies in brain, both after chronic THC administration to adult monkeys (89) and after acute administration to infant rats (90). It is common knowledge that RNA synthesis from chromosomal DNA templates occurs in the nucleus, whereas translation occurs on cytoplasmic polyribosomes, Polyribosomes either in the cytoplasm or on the nuclear membrane are an index of attached mRNA emerging

from the nucleus respectively. The dissociation of the polyribosomes entails interruption of these processes (91).

The reduction in nuclear membrane-attached ribosomes, following acute exposure to pharmacologically active cannabinoids, is dose-related and rapid, peaking at 30 min (90). This observation is important and relevant to our main thesis. A parallel is to be found in the modification of ribosomes with butanedione, which was accompanied by loss of mRNA-binding capacity and arrest of protein synthesis upon an incubation of 30 min (92). These experiments showed that arginine residues in ribosomal proteins are essential for the binding of mRNA through interaction of their positively charged guanidinium groups with the negatively charged phosphate moieties of the messenger nucleic acid. As in all other proteins discussed so far, these essential arginines are embedded in apolar sequences of the protein (57) and constitute sites of affinity for hydrophobic molecules (93). An essential condition for a putative interference by hydrophobic THC effects could have a documented common denominator, a cannabis/arginine interaction across a wide spectrum of tissues, species and phyla.

Although the data reviewed here are all consistent with such an interaction, it should be emphasized that it is far from clear how these interactions take place, especially since the necessary experiments have not been conducted. In the pursuit of the stated goal of this paper, i.e., to review the effects of THC and arginine on, as far as feasible, identical experimental systems, the HeLa S_3 human cell line constitutes a good model for analysis. In recent experiments (94, 95), it was demonstrated that cannabinoids depress in a dose-dependent way proliferative activity as well as incorporation of radiolabeled precursors into nucleic acids and proteins of HeLa S_3 cells growing in exponential cultures. These studies, as well as previous ones from the same group (30), demonstrated that the effects of cannabinoids on the intact cells could not be observed in *in vitro* nuclear and chromatin transcription experiments. No alteration in the template activity of chromatin isolated from cannabinoid-treated cells was found when compared to that of chromatin from untreated cells. It was concluded that "the findings taken together could be explained by cannabinoid-induced modifications at the level of the cell membrane". Experiments with phenylglyoxal which reacts with arginine moieties in proteins, but does not enter intact cells, have shown that this reagent causes a dose-dependent inhibition of HeLa S_3 cell proliferation by chemically masking arginine moieties at the cell surface (96,97). With the use of labeled phenylglyoxal these authors demonstrated that there is a 24-fold increase in arginine at entry into G_1 from M when they propagate in mono-layer culture. Growth was inhibited by

the chemical masking of arginine by phenylglyoxal only during the phases of the cycle when arginine-rich protein is exposed at the surface (96). Interaction of cannabinoids with the arginine-rich membrane protein in HeLa S_3 cells would be consistent with the findings, interpretations and conclusions of Stein et al. (30) and Mon et al. (94,95) based on their experiments with HeLa S_3 cells and cannabinoids. It is of further interest that this arginine enrichment at the cell surface has been implicated in cell surface-mediated growth control and/or transformed or tumor cell surface changes (98).

B. Steroids, Lipoproteins and Cholesterol

Diethyl stilbestrol bisphosphate, which has similar properties to estradiol and is commonly employed to control prostatic carcinoma, was recently found to interact directly with arginyl residues in protein substrates through binding to their guanidino group (99). Harris et al. (100) have emphasized that the binding of cannabinoids resembles that of steroids, on the basis of their strong spatial structural resemblance, their high lipid solubility and the absence of specific antagonists. Evidence was also presented that the cannabinoids may be interacting at steroid-specific sites (101). Carchman et al. (102) have shown that the cannabinoids can inhibit steroidogenesis both in normal adrenal and adrenal tumor cells. This inhibition was demonstrated to be very specific in that the cannabinoids had no significant effects on cell growth, O_2 consumption, etc. They proposed that this specific inhibition of steroid production involved early steps in the pathway, possibly cholesterol transport or cholesterol esterase, and attributed it to the strong structural and biological relationships between steroids and cannabinoids. A possible explanation may reside in the numerous lipid droplets, rich in cholesterol esters, present in the THC-inhibited cells (102). In the light of the section that follows, it is conceivable that cholesterol binding sites may be occupied by the cannabinoid.

Cholesterol in the adrenal gland is either converted into steroid hormones or esterified and stored in the intracellular lipid droplets, the hydrolysis of the esterified cholesterol being catalyzed by the cytoplasmic enzyme cholesterol esterase (103). A stepwise examination of the steroidogenic pathway in isolated Leydig cells for the purpose of locating the site of action of THC demonstrated that "virtually the only step inhibited" was the liberation by the esterase of "precursor" cholesterol from its lipid droplet storage (104). These studies demonstrated a direct effect of THC on steroid secreting cells. It has been suggested that the "inability to

demonstrate reduced corticosteroid levels in animals treated with THC may reside in the ability of the animals to compensate physiologically, or pharmacologically by a secondary drug action, for reduced steroid synthesis with enhanced ACTH release" (101).

THC has a structural similarity to cholesterol (105). In the blood it is strongly bound to, and migrates in association with, lipoproteins (106). In electrophoretic experiments these authors showed that labeled THC in human plasma in vitro is located in a peak in the albumin area and in a broad peak around the transferring; these two peaks which must be attributed to protein-bound THC amount to about 87% of the total radioactivity. Other experiments showed that THC and its 11-OH metabolite, which is also pharmacologically and psychologically active, are bound not only to albumin but also to alpha$_1$-lipoproteins and beta-lipoproteins (107). Arginine is known to be a constituent of beta- and prebeta-lipoproteins (108). Furthermore, there are arginine residues at or close to the strong hydrophobic anion-binding sites of bovine serum albumin (93). Chemical modification of the arginines in transferring with phenylglyoxal, 1,2-cyclohexanedione and 2,3-butanedione decreased iron binding activity by 50% (109) by modifying the guanidino group of arginine at the bicarbonate binding site. More importantly, very low density lipoproteins (VLDL) contain the arginine-rich apolipoprotein E, which is also found in HDL (110). Apoprotein E (apo-E) is a glycoprotein which occurs in several classes of lipoproteins of both humans and animals (111). Metabolically this apo-E is implicated in cholesterol transport in the plasma and mediates lipoprotein recognition and uptake by the liver. As a matter of fact, cyclohexanedione modification of arginine residues of LDL results in a retardation in plasma clearance, showing that the hepatocytes have a receptor for the arginines in apo-E (112). It has been shown that the guanidino groups of arginine (10%) in apo-E participate in cholesterol binding (113). This has been established by in vitro experiments of cholesterol interaction with compounds containing guanidino groups (114). The unique importance of hydrophilic arginine residues interrupting an otherwise hydrophobic portion of a peptide, for effective cholesterol-protein interaction, has been established by recent experiments (115). It is evident that also in this general area of lipoproteins a unifying mechanism of cannabis effects may be envisaged, through interactions with arginine in many varied systems.

C. Chromosome Aberrations and Immune Function

Despite consistent evidence that THC and marijuana admin-

istrations induce immunological defects in experimental animals (116), the findings so far available in humans are conflicting (117). Two early papers dealing with the in vitro blastogenic responses of lymphocytes from long-term marijuana smokers exemplify the conflicting findings (9,118). Subsequent experiments have established the strong dependence of such results upon culture media and culture conditions (119). It was shown that with serum concentrations of 0.5% in the culture medium, thymidine incorporation was nearly completely inhibited by THC, while with the same dose of THC, but in the presence of 10% serum concentration, thymidine incorporation was 90% of control. Analysis of the data indicated a direct interaction between THC and serum as well as the reversibility of the inhibition of thymidine incorporation by THC through washing the cells in RPMI medium and replacing them in culture. Cytogenetic analyses of lymphocyte cultures from chronic marijuana smokers have also yielded contradictory results (120) despite the fact that heavy doses of marijuana in humans (33), natural cannabinoids on normal human lymphocytes (121) and marijuana smoke in cultures (122) consistently induce hypoploidy and chromosomal aberrations. The questions arises whether the contradictory findings may not be attributed to the varied experimental designs. Aside from cannabis studies, probably in few other areas of cellular research have the methodological procedures played so important a role for the outcome of experiments as in cytogenetics (123) and in studies of the immune process using cell lines of diverse origins (124, and refs. therein).

Numerous studies initiated for the elucidation of the role of media and of culture conditions led to the unanimous conclusion that the main factor involved that determines the results obtained - and, under certain conditions, the artifacts produced - is the level of arginine in the experimental system. The many reasons why, and ways in which, this level may vary have been reviewed in the classic study of Freed and Schatz (31). Specifically, the chromosome damage resulting from arginine deprivation was initially observed in cultures recovering from inhibition of multiplication induced by liver extracts that inadvertantly contained arginase. The enzyme acted by degrading arginine in the culture medium thereby producing nutritional deficiency in the cells. Chromosome aberrations induced in cultured cells by infection of Mycoplasma appear also to stem from an arginine deficiency (29) since these organisms liberate arginine deaminase in the medium and consume the amino acid. Extracts prepared from mycoplasmas are inhibitory to the secondary production of antibody to rabbit lymph node fragments in vitro (125), to blastogenesis and to mixed lymphocyte culture reactions (126-128). These effects are due to the arginine depleting enzyme

since they can be overcome by either adding excess arginine or eliminating the enzyme activity by heat or washing the cultures. Mitotic inhibition and increase in chromosome breakage have both been experimentally produced in arginine-deficient culture media and reversed again by addition of the amino acid (29). Cells cultured in limiting concentrations of arginine just adequate to produce multiplication, incur a significantly elevated level of chromosome abnormalities. The experiments showed that lack of any one of the essential amino acids was capable of inducing chromosome aberrations when the cells were "rescued" after a 24- or 48 h exposure. These findings suggested that "karyotype instability might stem from disparity between the amino acid requirement of the cells and the nutritional availability of these compounds" (31). Barile and Leventhal showed that only the species of mycoplasma which derive their energy from arginine utilization inhibit photohemagglutinin (PHA) - stimulated transformation of lymphocytes in cultures by their ability to deplete available arginine (129). Thus, PHA-induced lymphocyte transformation is inextricably linked to a normal level of arginine available in the culture medium, the lack of which inhibits the incorporation of thymidine into DNA (29). Lastly, it has been proposed that chromosome changes could be expected from the introduction into the culture of any drug tending to make an amino acid unavailable to the cell by whatever mechanism (31, and refs therein).

When the experiments and findings described above are taken into account, it becomes clear that an interaction of cannabis with arginine would suffice to explain a large number of the drug's effects in culture systems. Assuming that cannabis derivatives "deplete" available arginine, a given dose of the drug would cause a more effective inhibition in a 0.5% concentration of serum (which supplies the amino acid) than in a 10% concentration, which supplies a proportionally higher level of arginine to the same number of cells. Furthermore, the reversibility of THC inhibition of thymidine uptake by washing the cells in RPMI medium and resetting in fresh culture (78) would be equivalent to overcoming the metabolic block since the RPMI medium contains 200 mcg/ml arginine, i.e., much more than the 100 mcg/ml found required to abrogate inhibition caused by arginine deprivation (130). It is obvious, then, that media containing normally less arginine (Fisher's: 15 mcg/ml), if used, would lead to entirely different results and, probably, opposite conclusions. Cytogenetic experiments seem also to support a cannabis/arginine interaction at many levels. Stenchever et al. showed an increase in the incidence of chromosome breakage in marijuana smokers when their cells were placed in culture with nutritive media and PHA (131). No increase, however, in chromosome

breakage was determined in normal lymphocytes when THC was added *in vitro* after PHA stimulation (132). Far from being contradictory these results support the experiments of Freed and Schatz (31), in which chromosomal breaks will appear *after* rescue from the deprivation of arginine (presumably occuring *in vivo* under marijuana smoking) when the cells are stimulated to enter mitosis, as in the Stenchever et al. experiment (131). On the other hand the addition of THC after PHA in a culture of lympocytes which have not been subjected to arginine depletion (those of non-smokers) will inhibit growth and block mitosis by acute arginine depletion (29) without evidence of chromosome aberrations, as in the Stenchever and Allen experiment (132). These results are consistent with the finding (31) that the frequency of chromosome aberrations increases with increasing periods of exposure of the resting cells to limiting levels of arginine, which may occur, as we proposed (1), in long-term cannabis users.

The impaired development of the F_2 generation of cannabis-treated pregnant rats (133) appears to support a mutagenic potential of THC. Limitation of available arginine is known to sensitize cells to the clastogenic activity of mutagens (134-136). Furthermore, death of a cell due to loss of genetic material may not be evident until later generations when the cell no longer possesses essential gene products, having depleted its intracellular pool (137). THC is capable of inducing segregational errors (121), which are studied in anaphase preparations of chromosomes obtained by omission of colchicine (138). These THC-induced anomalies were attributed to disruption of the spindle and of microtubule formation, a conclusion supported also by the findings of Leuchtenberger et al. (139). No other mechanism need be invoked for these effects than interference of cannabis with arginine. Modification of pig brain tubulin with the arginine specific reagent 2,3-butanedione results in a decrease of its microtubule formation capacity (140). These data suggested that at least one arginyl residue plays an essential role in tubulin polymerization through its interaction with the negatively charged moiety of GTP, which is required for tubulin polymerization. The preceeding analysis and critique of the experimental data does not exhaust the subject, but highlights fundamental mechanisms that may be operant in a wider spectrum of cannabis associated effects in development and in the modulation of central nervous system activity dependent upon microtubule function.

The health-related problem of whether cannabis consumption produces immune dysfunction in humans can be approached only indirectly. Conclusive evidence may prove elusive since cells *in situ* in a healthy subject may be protected by homeostatic mechanisms absent in cultures. One such approach is suggested by the observation of the interactive effects of nutrition and

cannabis in experimental animals (141). This study showed that an enriched protein diet counteracted the effects of cannabis exposure. Although a relationship between nutritional status and cannabis induced immunosuppression in man is often mentioned no controlled studies have been carried out. However, modern concepts of the role of nutrition in the treatment of patients with tumors suggest that food intake plays a role in preventing or correcting the immune depression consequent to the tumor (142, and refs. therein). In healthy human volunteers eating their usual diets, supplemental arginine greatly enhances the in vitro blastogenesis of peripheral lymphocyte response to T-cell mitogens (143). In rodents with depressed immune function secondary to tumors or stress, dietary arginine supplementation can restore thymic weight, the number of thymic lymphocytes and in vitro thymocyte mitogenic response (142). Although the basal diet in the latter study contained 1.8% arginine and was therefore not arginine-deficient, the described restoration of function was observed only after supplementation of the diet and the drinking water with 0.5% arginine. Further experiments clearly showed for the first time that supplemental arginine improves thymic function also in animals with genetically decreased thymic function (144). Dietary arginine was also found to prevent thymic involution and adrenal hypertrophy due to stress or injury (142, 145). In experimental animals long-term and short term exposure to pure cannabinoids and crude marijuana extracts lead to adrenal weight increase and thymus weight decrease (146), while in humans several studies have failed to show any changes in adrenocortical function (120).

Taken together the foregoing studies emphasizes the opposite effects of cannabis and arginine in the intact organism and demonstrate the modulating role of nutritional state, i.e., dietary arginine, on immune function in man. Despite the lack of similar studies in long-term and short-term cannabis users, it seems valid to consider that plasma levels of arginine by some approximation could be a modifying factor in cannabis-induced immunosuppression in man, assuming that the in vitro effects of cannabis have functional significance for health. The reported reactivation of dormant genital herpes shortly after smoking of cannabis (147) could be explained by an arginine-depleting effect of cannabis. Actually, it has been shown that arginine deficiency can increase Herpes Simplex virus yields by inhibiting the induction of interferon (148). On the other hand Herpes Simplex virus fails to replicate and to produce extensive c.p.e. in human cell monolayer cultures exposed to THC (149); this effect of THC was observed only in the absence of serum from the culture since addition of 10% serum overcomes the inhibition in the presence of THC. This antiviral effect of THC in vitro is also consistent with

a putative cannabis/arginine interaction since it is well documented that arginine is essential for the replication of Herpes Simplex virus (150). Absence of the amino acid from the culture medium prevents the formation of virions and addition of arginine immediately stimulated protein synthesis within 2 min which is followed by virus formation. Serum provides a high concentration of arginine (81) and, thus, may overcome the antiviral (arginine-depleting?) effect of THC as observed in the Blevins and Dumic experiments (149).

D. Carcinogenicity and Antineoplastic Activity

The data of the previous section on the effects of cannabis and on the role of arginine in immune function cover the main concepts that are also applicable to tumors. It is obvious, though, that the metabolic individuality of tumors, either in the transforming or in the proliferating state, will be a factor affecting the interaction of cannabis with these tissues. Available evidence indicates that cannabis has carcinogenic potential (122,151) as well as antineoplastic activity both in vivo and in vitro when added to certain cell cultures (117,153). From the data available in the literature it can be inferred that the various cell lines appear to react differently to the drug. The field is vast and complex, but some general considerations have emerged that permit us to examine cannabis/arginine interactions. The literature on the roles of arginine and arginase in cancer is voluminous and spans at least 35 years. An arbitrary sampling revealed that although the growth of some tumors is arrested by the administration of arginine (154-156), the growth of the majority of tumors or cell lines is arrested by the depletion of arginine by arginase (157-162). Far from being empirical this latter approach seems to simulate a natural defense mechanism of the organism. It has been shown that activated macrophages kill tumor cells by releasing arginase (130). The selective in vitro cytotoxic activity of zynosan or lipipolysaccharide (LPS) - activated rodent macrophages for cultured malignant cells is due to the lethal depletion of arginine mediated by arginase (163). Since arginine is present in high concentration throughout the extracellular fluid, arginase-mediated arginine deprivation of malignant cells "can be envisaged as a microenvironmental phenomenon occuring at or near the surface of the macrophage" (164). Arginine depletion of the extracellular fluid creates conditions inimical to the successful implantation and proliferation of the tumor. Effective arginine-degrading enzymes are currently being studied for use in cancer chemotherapy (165). The known inhibitory action of THC on macrophage function (47) would appear to support an in vivo

enhancement by cannabis of cancerous growth, in those instances where macrophage arginase is inhibitory to tumor growth. On the other hand, by the same mechanism (paralysis of macrophages) cannabis would arrest cancerous growth in those instances where arginine is inhibitory to the tumor (154-156). It is evident that the metabolic idiosyncrasies of tumors introduce an ambiguity in the understanding of cannabis' effects. This is compounded by the fact that arginine may enter many alternative metabolic pathways (protein synthesis, urea cycle, production of creatine, proline, polyamines, etc.) subserving both growth and function, all of which are claimed by both host tissue and tumor tissue. Malignant cells are reported to require a higher concentration of arginine in the medium than their normal counterparts (163). Tumors obtain the amino acid from their host thus causing arginine depletion to healthy tissues (157), which results in immunosuppression as already discussed. We suggest that cannabis interaction with arginine in its different pathways could account for the apparent variability of the drug's effects in tumors.

E. Muscle and Nerve

Limitations to this review are set by the fact that not all areas of cannabis research have a counterpart in arginine investigations. Some new data in the literature, however, permit to close this paper by examining cannabis/arginine interactions in muscle and nerve. It has been reported that the hypotensive and cardiac slowing actions of THC in rats are not mediated by activation of parasympathetic nerves or by beta-adrenotropic blockade (166). It has also been shown that THC has a depressant effect on the beating of dissociated myocardial cells in vitro (167). During muscle contraction myosin catalyzes, in the presence of actin, the hydrolysis of ATP. Mornet et al. have reported that phenylglyoxal reacts rapidly with isolated myosin heads and induces two successive and distinguishable effects on their enzymic properties: activation followed by inhibition (168). It was demonstrated that the sequential modification of two reactive arginyl residues is responsible for the observed activation-inhibition phenomenon. Furthermore, Johnson ad Blazyk demonstrated that modification of arginine-95 with 1,2-cyclohexanedione abolishes the ability of F-actin to interact with tropomyosin (169). Arginine-95, one out of 18 arginine residues per actin monomer, is part of the 'activated state' binding site. It is currently held (169, and refs. therein) that the interaction between one tropomyosin and seven adjacent actin molecules can control the interaction between actin and myosin for contraction. The tachycardia regularly observed as a consequence of cannabis

smoking in humans and the bradycardia observed in animals could be reconciled by a cannabis/arginine interaction with species-dependent emphasis on the activation-inhibition phenomenon and on tropomyosin binding in the mechanism of muscle contraction described above.

The recent reports that muscle spasticity, associated with spinal cord injury or multiple sclerosis, improves after administration of marijuana strongly support a cannabis/arginine interaction in the urea cycle. Narayanareddy and Swami showed that there is a 100% increase in arginine during denervation in muscle (172). The atrophy process causes in addition a shift in ammonia metabolism. Although every enzyme in the urea cycle is subject to inherited abnormality and typical symptoms of hyperammonemia include lethargy, drowsiness and recurrent vomiting attacks (173), it is well documented that patients with arginase deficiency have an extreme degree of limb spasticity. This appears to be caused by some detrimental effect ofarginine accumulation since thespasticityis relieved by a low-arginine diet, which reduces blood arginine levels (174). The proposed depletion of arginine by cannabis (1) could cause the reduction of spasticity by reducing the amount of accumulated arginine. One report has been found to date that examines the question of THC effects on the urea cycle (175). Exposure of rabbits to hashish smoke every other day for a period of one month resulted in a marked increase in blood ammonia, not related to hepatic damage. Though behavioral data are not included in this study, still the biochemical findings offer the first direct roof of a cannabis/arginine interaction in, at least, one of the arginine pathways. Only during recent decades with the discovery of congenital hyperammmonemia in man has it been fully appreciated that ammonia at high levels can cause aberrant behavior, permanently damage the brain as well as inhibit growth (176, and refs. therein). This author stressed the fact that ammonia may have subtle but significant effects on metabolism, particularly in the brain, which are not apparent from neurological signs or blood ammonia concentrations, but which are responsible for abnormal neuro-transmission. The clinical symptoms of hyperammonemia: episodes of intermittent ataxia, irritability, lethargy and coma -- also observed with high doses of cannabis (177) -- can be reversed by dietary arginine (176, and refs. therein), which lowers blood ammonia levels (178). The above data taken together suggest that the results of Ghoneium et al. (175) give an important insight into the mechanismof action of cannabinoids and offer experimentalsupport to the working hypothesis of a cannabis/arginine interaction.

One of the most intriguing effects of cannabis on the nervous system is its biphasic action not only on behavior, but also on distinct neurochemical parameters (63,179,180).

In view of the consistently inhibitory action of cannabis on macromolecular synthesis in the brain, it is far from clear how these biphasic effects are produced. There is agreement that cannabis preparations interact with the cholinergic system (181, and refs. therein). Marijuana and THC affect human memory and cognition in ways that are always detrimental to the user (182). Neurochemical research suggests that these undesirable effects are related to a decrease in the release of neuronal acetylcholine (ACh) and its turnover in certain brain regions (181). The kinds of effects that marijuana can have on the memory process (183) are strikingly similar to those found in patients experiencing amnesia due to Herpes Simplex encephalitis (184), where cholinergic limbic pathways are disrupted (185). Physiological reactions as well as behavioral effects of cannabis are similar to those found with anticholinergic drugs (186). A number of studies have suggested that THC has antiepileptic effects; these effects appear paradoxical when THC has also produced epileptiform activity in hippocampus (183). Behavioral effects of cannabinoids can be blocked by anticholinesterase-type drugs (186). It has also been reported that THC has anticholinesterase-like activity (62). All these contradictory findings could stem from a common substrate assuming a cannabis/arginine interaction. Choline acetyltransferase, the enzyme involved in ACh synthesis, can be inactivated by butanedione and phenylglyoxal, the arginine specific reagents, through the modification of the coenzyme A binding active-site arginine residue (187). Acetylcholinesterase (AChE), the ACh degrading enzyme, can, however, also be inactivated by butanedione through the modification of one essential arginine residue which alters the conformation of the protein (51). Dose-dependent inhibition of choline acetyltransferase and/or AChE would have profound effects on the metabolism and turnover of ACh. An interaction of cannabinoids with active site arginines of these enzymes could account for their biphasic effects on the cholinergic system. Inactivation by phenylglyoxal of adenyl cyclase (188) and catechol-O-methyltransferase (189) extends the role of arginine into the catecholaminergic system. It has been reported that low doses of THC stimulate while high doses depress the noradrenergic and dopaminergic systems (179). This could be mediated through the antagonistic relationships between adenyl cyclase and ATPase activities (190). Interaction of cannabis with the active-site arginines of both enzymes (61,188) could disrupt feedback regulations. Furthermore, the well documented disrupting effect of THC and cannabis on microtubules (33,139) which mediate monoamine exocytosis (191) has also to be taken into account as a factor contributing to the drug's biphasic effects on neurotransmitters.

It is of interest that, also in this process, one arginyl residue of tubulin, the binding site of GTP, is essential for microtubule formation (140) and its modification with butanedione decreases the capacity of tubulin for polymerization. Thus, a putative interaction of cannabis with arginine would permit modulation of synaptic events at multiple sites through a common molecular mechanism. Other enzymes involved in neurotransmitter metabolism that possess essential arginine residues are glutamic decarboxylase (192) and gamma-aminobutyric acid (GABA) aminotransferase (193). Experiments with phenylglyoxal, which inactivates these enzymes, have shown that the decarboxylase, responsible for the biosynthesis of GABA, has essential arginine residues at both the glutamate and the pyridoxal phosphate binding sites; the transferase, responsible for GABA degradation, has an essential arginine at the pyridoxal phosphate binding site.

GABA is the main inhibitory neurotransmitter in the mammalian brain and excitability tends to vary inversely with GABA levels, so that increased brain GABA concentrations were thoughtto lead to protection against convulsions (194). The convulsant action of hydrazines was once attributed to a decrease in GABA, but it soon developed that seizures can occur in the absence of this change (195). It was later demonstrated that hydrazine interferes with the chloride channel "pumps" (196) thus causing postsynaptic disinhibition. THC enhances focal epileptic potentials and seizure activity, while cannabidiol has unique anticonvulsant properties (197) and marijuana has paradoxical anticonvulsant and convulsant effects (198). A differential interaction of cannabinoids with essential arginine in the GABA metabolizing enzymes as well as with the essential arginine in the chloride-channel protein (53) could well underlie their complex effects in convulsions. Roberts et al. have proposed the possibility that hydrazine might displace guanidino groups of arginyl residuesin proteins of nerve cell membranes from interacation with adjacently-located negatively-charged groups (199). This they assume to be the mechanism of hydrazine toxicity in convulsions, since arginine tested as a possible protective agent in hydrazine toxicity was found to be effective. That arginine may have a complex homeostatic role in the mechanisms of excitation/inhibition in the neuronal microenvironment is suggested by the finding that gamma-guanidinobutyric acid, a normal component of the brain, which is synthesized from GABA and arginine (200), inactivates inhibitory synapses (201) and may be important in the excitation of the central nervous system which leads to seizures (202). Chronic injections of THC increase the content of GABA in rat brain (203). Since the biphasic effects of cannabis suggest the interaction ofthe drug with homeostatic mechanisms of the brain, it would be

consistent with these data to view the interaction as occuring via arginine involvement at the multiple sites described, where arginine appears to be essential to neuronal function.

Last, but not least, and relevant to THC's lipophilic nature, is the emerging role of arginine in the biochemistry of receptor binding. This role was anticipated by the work of Curtis and Watkins (204), predicted by Roberts et al. (199), elaborated by the hypothesis of Watkins (205), and is now supported by specific experiments (55, 206). Briefly, Watkins proposed that the pharmacological actions of ACh, GABA and glutamic acid result from dissociation of phospholipid-protein complexes contained in the membrane, and the ensuing permeability changes. He reached this conclusion after noting the similarity in the structure and charge distributions of ACh, GABA and glutamic acid with phosphatidyl-chlorine, phosphatidylethanolamine and phosphatidylserine respectively. The theory requires that receptor sites for GABA and glutamicacid should be ascribed to basic amino acid side chains in the protein. Confirmation of Watkins' hypothesis with respect to GABA was obtained when a preparation enriched in junctional complexes from rat cerebellum was incubated with labeled GABA, and binding was determined in the absence and presence of the enzyme that specifically hydrolyzes phosphatidylethanolamine (206). It was demonstrated that phospholipase C, in fact, enhanced the specific binding of GABA by 260% and, furthermore, that there was competition between GABA and the phospholipid for binding to the receptor. Hence, removal of the phospholipid unmasked a high affinity site for transmitter binding. Recent studies have now provided evidence that arginylresidues in proteins are the high affinity sites for negatively charged phosphate groups of phospholipid molecules (55). Other studies have established that the microenvironment of the binding-site arginines is always a hydrophobic sequence of the protein molecule (56), i.e., a locus for which THC has special affinity. Since the GABA is similarly a hydrophobic protein (206) a cannabis/arginine interaction at the receptor would be consistent with the data and may explain the observed increase in GABA content of rat cerebellum after chronic THC administration (203).

F. Comment

In the light of the data surveyed and analyzed from the published reports available in the literature it appears that the hypothesis of the cannabis/arginine interaction, as the common denominator of the drug's biological effects, remains valid. However, a hypothesis hovers around truth, it is not

truth itself. It is hoped that the evidence offered in the present review in favor of the "common denominator" may stimulate the pertinent analytical disciplines into designing the crucial experiments that suggest themselves, and through which the hypothesis may claim reality status. The references cited may also generate a cascade of further contributory data, here witheld due to the limitation of space.

ACKNOWLEDGMENTS

The author thanks Mrs. Judy Ashmore of the Marine Biological Laboratory, Library, Woods Hole, Mass. for her invaluable help in collecting the literature. This study was supported by a grant of the National Research Foundation of Greece.

REFERENCES

1. Issidorides, M. R., Observations in chronic hashish users: nuclear aberrations in blood and sperm and abnormal acrosomes in spermatozoa, Adv. Biosci., 22 & 23: 377-388 (1979).
2. Stefanis, C. N., and Issidorides, M. R., Cellular effects of chronic cannabis use in man, in "Marihuana: Chemistry, Biochemistry and Cellular Effects" (G. G. Nahas, ed.) pp. 533-550, Springer-Verlag, New York, 1976.
3. Black, M. M., and Ansley, H. R., Histone specificity revealed by ammoniacal silver staining, J. Histochem. Cytochem. 14:177-181, 1966.
4. MacRae, E. K., and Meetz, G. D., Electron microscopy of the ammoniacal silver reaction for histones in theerythropoietic cells of the chick, J. Cell Biol., 45:235-345 (1970).
5. Littau, V. C., Burdick, C., Allfrey, V. G., and Mirsky, A. E., The role of histones in the maintenance of chromatin structure, Proc. Nat. Acad. Sci. USA 54:1204-1212 (1965).
6. Black, M. M., and Ansley, H. R., Antigen-induced changes in lymphoid cell histones. I. Thymus, J. Cell Biol., 26:201-208 (1965).
7. Vaughn, J.C., The relationship of the "sperm chromatophile: to the fate of displaced histones following histone transition in rat spermiogenesis, J. Cell Biol. 31:257-278 (1966).
8. Calvin, H. I., and Bedford, J. M., Formation of disulfide bonds in the nucleus and accessory structures of mamma-

lian spermatozoa during maturation in the epididymis, J. Reprod. Fert. Suppl. 13: 65-75 (1971).
9. Nahas, G. G., Suciu-Foca, N., Armand, J. P., and Morishima, A., Inhibition of cellular mediated immunity in marijuana smokers, Science, 183:419-420 (1974).
10. Hembree III, W. C., Nahas, G. G., Zeidenberg, P., and Huang, H. F. S., Changes in human spermatozoa associated with high dose marijuana smoking, Adv. Biosci., 22 & 23: 429-439 (1979).
11. Silvestroni, L., Frajese, G., and Fabrizio, M., Histones instead of protamines in terminal germ cells of infertile oligospermic men, Fertil Steril. 27:1428-1437 (1976).
12. Holt, L. E., and Albanese, A. A., Observations with amino acid deficiencies in man, Trans. Assoc. Amer, Physicians, 58:143-156 (1944).
13. Schachter, A., Friedman, S., Goldman, J. A., and Eckerling, B., The treatment of oligospermia with the amino-acid arginine, Int. Gynecol. and Ostet., 11:206-209 (1973).
14. Radany, E. W., and Atherton, R. W., Arginine-induced stimulation of rabbit sperm motility, Arch. Androl., 7:351-355 (1981).
15. Shahar, A., and Bino, T., In vitro effects of delta-9-tetrahydrocannabinol (THC) on bull sperm, Biochem. Pharmacol., 23:1341-1342 (1974).
16. Nahas, G. G. (ed.) "Marihuana: Chemistry, Biochemistry, and Cellular Effects". Springer-Verlag, New York, 1976.
17. Nahas, G. G., and Paton, W. D. M. (eds.) "Marihuana: Biological Effects, Analysis, Metabolism, Cellular Responses, Reproduction and Brain", Advances in Biosciences, Vol. 22 & 23, Pergamon Press, Oxford, 1979.
18. Dardick, I., and Setterfield, G., Volume of condensed chromatin in developing primitive-line erthrocytes of chick, Exp. Cell Res., 100:159-171 (1976).
19. Brasch, K., and Sinclair, G. D., The organization, composition and matrix of hepatocyte nuclei exposed to a-amanitin, Virchows Arch. B Cell Pathol., 27:193-204 (1978).
20. Harris, J. R., The biochemistry and ultrastructure of the nuclear envelope, Biochem. Biophys. Acta., 515:55-104 (1978).
21. Issidorides, M. R., Stefanis, C. N., Varsou, E., and Katsorchis, T., Altered chromatin ultrastructure in neutrophils of schizophrenics. Nature, 258:612-614 (1975).
22. Issidorides, M. R., Zioudrou, C., Lykouras, E., and Stefanis, C. N., Drug-induced changes in chromatin ultrastructure and nuclear basic proteins of the neutrophils of chronic schizophrenics, Prog. Neuro-Psychopharmacol.,

2:79-85 (1978).
23. Issidorides, M. R., and Katsorchis, T., Dispersed and compact chromatin demonstrated with a new EM method: Phosphotungstic acid hematoxylin block-staining, Histochemistry, 73:21-31 (1981).
24. Issidorides, M. R., Interaction of cannabis with arginine metabolism underlies biological effects: A new hypothesis on mechanism of action, in "Adverse Health and Behavioral Consequences of Cannabis Use. Working Paper for the ARF/WHO Scientific Meeting, Toronto, 1981", K. O. Fehr and H. Kalant, eds.) Addiction Research Foundation, Toronto, 1982.
25. Holstein, A. F., and Schirren, C., Classification of abnormalities in human spermatids based on recent advances in ultra-structure research on sermatid differentiation, in "The Spermatozoon: Maturation, Motility, Surface Properties and Comparative Aspects (D. W. Fawcett and J. M. Bedford, eds.), pp. 341-354. Urban & Schwarzenberg, Baltimore, MD, 1979.
26. Coelingh, J. P., Rozijn, T. H., and Monfoort, C. H., Isolation and partial characterization of a basic protein from bovine sperm heads, Biochem. Biophys. Acta., 188:353-356 (1969).
27. Fawcett, D. W., Anderson, W. a., and Phillips, D. M., Morphogenetic factors influencing the shape of the sperm head, Dev. Biol., 26:220-251 (1971).
28. Weissfeld, A. S., and Rouse, H., Arginine deprivation in KB cells, II. Characterization of the DNA synthesized during starvation, J. Cell Biol. 75:889-898 (1977).
29. Aula, P., and Nichols, W. W., The cytogenetic effects of mycoplasma in human leukocyte cultures, J. Cell Physiol., 70:281-29 (1967).
30. Stein, G. S., Mon, M. J., Haas, A. E., and Jansing, R. L., Cannabinoids: The influence on cell proliferation and macromolecular biosynthesis, in "Marihuana: Biological Effects: (G. G. Nahas and W. D. M. Paton, eds.) pp. 171-28. Pergamon Press, Oxford and New York, 1979.
31. Freed, J. J., and Schatz, S. A., Chromosome aberrations in cultured cells deprived of single essential amino-acids, Exp. Cell Res., 393-49 (1969).
32. Leuchtenberger, C., Leuchtenberger, R., Zbinden, I., and Schleh, E., Cytological and cytochemical effects of whole smoke and the gas vapor phase from marihuana cigarettes on growth and DN metabolism of cultured mammalian cells, in "Marihuana: Chemistry, Biochemistry, and Cellular Effects" (G. G. Nahas, ed.) pp. 243-256. Springer-Verlag, New York, 1976.
33. Morishima, A., Henrich, R. T., Jayaraman, J., and Nahas, G. G., Hypoploid metaphases in cultured lymphocytes of

marihuana smokers, Adv. Biosci., 22 & 23: 371-376 (1979).
34. Stefanis, C., Dornbush, R., and Fink, M., (eds.) "Hashish: Studies of Long-Term Use". Raven Press, New York, 1977.
35. Da Prada, M., Jakabova, M., Luscher, E. F., Pletscher, A., and Richards, J. G., Subcellular localization of the heparin-neutralizing factor in blood platelets, J. Physiol., 257:495-502 (1976).
36. Fukami, M. H., Niewiarowski, S., Rucinski, B., and Salganicoff, L., Subcellular localization of human platelet antiheparin proteins, Thromb. Res., 14:433-443 (1979).
37. Niewiarowski, S., Poplawski, A., Lipinsi, B., and Farbiszewski, R., The release of platelet clotting factors during aggregation and viscous metamorphosis, Exp. Biol. Med., 3:121-128 (1968).
38. Kaplan, K. L., Dauzier, M. J., and Rose, S., ADP and epinephrine-induced release of platelet fibrinogen, Blood, 58:797-802 (1981).
39. Holt, J. C., Harris, M., Lange, E., Rucinski, B., Henschen, A., and Niewiarowski, S., Platelet basic protein (PBP) is the precursor of low affinity platelet factor 4 (LA-PF_4) and b-thromboglobulin (BTG), Fed. Proc., 41:528 (1982).
40. Scgaefer, C. F., Brackett, D. J., Gunn, C. G., and Dubowski, K. M., Decreased platelet aggregation following marihuana smoking in man, J. Okla. State Med. Assoc., 72:435-436 (1979).
41. Cooper, K. D., Shukla, J. B., and Rennert, O. M., Polyamine distribution in cellular compartments of blood and in aging erythrocytes, Clin. Chem. Acta., 73:171-88 (1976).
42. Rennert, O. M., Buehler, B., Miale, T., and Lawson, D., Polyamines and platelet aggregation, Life Sci., 19:257-263 (1976).
43. Issidorides, M. R, et al., in preparation.
44. Miras, C., and Koutselinis, A., The distribution and excretion of tetrahydrocannabinol-C^{14} in humans, Med. Sci. Law., 11:197-199 (1971).
45. Raz, A., Schurr, A., and Livine, A., The interaction of hashish components with human erythrocytes, Biochem. Biophys. Acta., 247:269-271 (1972).
46. Rubin, V., and Comitas, L., "Ganja in Jamaica". Moulton Publ., The Hague, 1975.
47. Chari-Bitron, A., Effect of delta-9-THC on red blood cell membranes and on alveolar macrophages, in "Marihuana: Chemistry, Biochemistry and Cellular Effects" (G. G. Nahas, ed.) pp. 273-281. Springer-Verlag, New York, 1976.
48. Kalofoutis, A., and Koutselinis, A., Changes induced by hashish constituents on human erythrocyte phospholipids,

Pharmacol. Biochem. Behav., 11:383-385 (1979).
49. Kalofoutis, A., Koutselinis, A., Lekakis, J., and Miras, C., Levels of erythrocyte 2,3 DPG and ATP in heavy hashish smokers, Experientia. 36:897-898 (1980).
50. Martin, K., The effect of proteolytic enzymes on acetylcholinesterase activity, the sodium pump and choline transport in human erythrocytes, Biochem. Biophys. Acta. 203:182-184 (1970).
51. Mullner H., and Sund, H., Essential arginine residues in aceltylcholinesterase from Torpedo californica, FEBS Lett., 119:283-286 (1980).
52. Steck, T. L., Koziarz, J. J., Singh, M. K., Reddy, G., and Kohler, H., Preparation and analysis of seven major topographically defined fragments of band 3, the predominant transmembrane polypeptide of human erythrocyte membranes, Biochemistry, 17:1216-1222 (1978).
53. Zaki, L., Inhibition of anion transport across red cells with 1, 2-cyclohexanedione, Biochem. Biophys. Res. Commun., 99:243-251 (1981).
54. Goldman, S. S., and Albers, R. W., Sodium-potassium-activatedadenosine triphosphatase. IX. The role of phospholipids, J. Biol. Chem., 248:867-874 (1973).
55. Akeroyd, R., Lange, L. C., Westerman, J., and Wirtz, K. W. A., Modification of phosphatidylcholine-transfer protein from bovine liver with butanedione and phenylglyoxal. Evidence for one essential arginine residue, Eur. J. Biochem., 121:77-81 (1981).
56. Norne, J. E., Hjalmarsson, S. G., Lindman, B., and Zeppezauer, M., Anion binding properties of human serum albumen from halide ion quadrupole relaxation, Biochemistry, 14:3401-3408 (1975).
57. Patthy, L., and Thesz, J., Origin of the selectivity of adicarbonyl reagents from arginyl residues ofanion-binding sites, Eur. J. Biochem. 105:367-393 (1980).
58. Paton, W. D. M., Concluding summary, in "Marihuana: Chemistry, Biochemistry, and Cellular Effects" (G.G. Nahas, ed.) pp. 551-552. Springer-Verlag, New York, 1976.
59. Yamada, S., and Itano, H. A., Phenanthrenequinone as an analytical reagent for arginine and other monosubstituted guanidines, Biochem. Biophys. Acta., 130:538-540 (1966).
60. Magun, B.E., and Kelly, J. W., A new fluorescent method with phenanthrenequinone for the histochemical demonstration of arginine residues in tissues, J. Histochem. Cytochem., 17:821-827 (1969).
61. Grisham, C. M., Characterization of essential arginyl residues in sheep kidney ($Na^+ + K^+$)-ATPase, Biochem, Biophys. Res. Commun., 88:229-236 (1979).
62. Brown, H., Possible anticholinesterase-like effects of trans (-)-delta-8 and delta-9-THC as observed in the

general motor activity of mice, Psychopharmacologia, 27:111-116 (1972).
63. Luthra, Y. K., Rosekrantz, H., Heyman, I. A., and Braude, M. C., Differential neurochemistry and temporal pattern in rats treated orally with delta-9-tetrahydrocannabinol for periods up to six months, Toxicol. Appl. Pharmacol., 32:418-431 (1975).
64. Toro-Goyco, E., Rodriguez, M. B., Preston, A., and Jering, H., Effect of (-)-delta-9-THC on ATPase systems from various sources, Adv. Biosci., 22 & 23: 229-242 (1979).
65. Kookkini, G., Stevens, V. J., Peterson, C. M., and Cerami, A., Modification of hemoglobin by ninhydrin, Blood, 56:701-705 (1980).
66. Riordan, J. F., McElvany, K. D., and Borders, Jr., C. L., Arginyl residues: anion recognition sites in enzymes, Science, 195:884-886 (1977).
67. Campbell, J. W., Comparative biochemistry of arginine and urea metabolism in invertebrates, Excerpta Med. Intnol. Congr. Series No. 195:48-68 (1968).
68. Jukes, T. H., The 'intruder' hypothesis and selection against arginine, Biochem. Biophys. Res. Commun., 58:80-84 (1974).
69. Wallis, M., On the frequency of arginine in proteins and its implication for molecular evolution, Biochem. Biophys. Res. Commun., 56:711-716 (1974).
70. Riordan, J. F., Arginyl residues and anion binding sites in proteins, Mol. Cell. Biochem., 26:71-92 (1979).
71. Itano, H. A., and Gottlieb, A. J., Blocking of tryptic cleavage of arginine bonds by the chemical modification of the guanido group with benzil, Biochem. Biophys. Res. Commun., 12:405-08 (1963).
72. Means, G. E., and Feeney, R. E., in "Chemical Modification of Proteins", pp. 194-18, Holden-Day, Inc. San Francisco, 1971.
73. Yankeelov, J., Jr., Kochert, M., Page, J., and Westphal, A., Reagent for the modification of arginine residues under mild conditions, Fed. Proc., 25:590 (1966).
74. Toi, K., Bynum, E., Norris, E., and Itano, H. A., Studies on the chemical modification of arginine I. The reaction of 1,2-cyclohexanedione with arginine and arginyl residues in proteins, J. Biol. Chem., 242:1036-1043 (1967).
75. Takahashi, K., The reaction of phenylglyoxal with arginine residues in proteins, J. Biol. Chem., 243:6171-6179 (1968).
76. Loh, Y. P., and Loriaux, L. L., Adrenocortiocotropic hormone, beta-lipotropin, and endorphin-related peptides in health and disease, J. Amer. Med. Assoc., 247:1033-1034 (1982).

77. Hook, V. Y. H., Eiden, L. E., and Brownstein, M. J., A carboxypeptidase processing enzyme for enkephalin precursors, Nature, 295:341-342 (1982).
78. Nahas, G. G., Desoize, B., Hsu, J., and Morishima, A., Inhibitory effects of delta-9-tetrahydrocannabinol on nucleic acid synthesis and proteins in cultured lymphocytes, in "Marihuana: Chemistry, Biochemistry, and Cellular Effects", (G. G. Nahas, ed.)pp. 299-312. Springer-Verlag, New York, 1976.
79. Jakubovic, A., and McGeer, P.L., Inhibition of rat protein and nucleic acid synthesis by cannabinoids in vitro, Can. J. Biochem., 50:654-662 (1972).
80. Jakubovic, A., and McGeer, P. L., In vitro inhibition of protein and nucleic acid synthesis in rat testicular tissue by cannabinoids, in "Marihuana: Chemistry, Biochemistry, and Cellular Effects", (G. G. Nahas, ed.) pp. 223-241. Springer-Verlag, New York, 1976.
81. Paul, D., and Walter, S., Initiation of DNA synthesis in primary fetal rat hepatocytes in cultures, Proc. Soc. Exp. Biol. Med., 145:456-460 (1974).
82. Hassan, A. S., and Milner J. A, Alterations in liver nucleic acids and nucleotides in arginine deficient rats, Metabolism, 30:739-744 (1981).
83. Jordan F., and Wu, A., Inactivation of purine nucleoside phosphorylase by modification of arginine residues, Arch. Biochem. Biophys. 190:699-704 (1978).
84. Borders, C. L., Jr., Riordan, J. F., and Auld, D. S., Essential arginyl residues in reverse transcriptase, Biochem. Biophys. Res. Commun. 66:490-495 (1975).
85. Armstrong, V. W., Sterbach, H., and Eckstein, F., Modification of an essential arginine in E. coli DNA-dependent RNA polymerase, FEBS Lett., 70:48-50 (1976).
86. Salvo, R. A., Serio, G. F., Evans, J. E., and Kimball, A. P., The affinity labeling of amino acids in or about the active enter of DNA-dependent polymerase I, Biochemistry, 15:493-497 (1976).
87. Blevins, R. D., and Regan, J. D., Delta-9-tetrahydrocannabinol: Effect on macromolecular synthesis in human and other mammalian cells, in "Marihuana: Chemistry, Biochemistry, and Cellular Effects", (G. G. Nahas, ed.) pp. 213-222. Springer-Verlag, New York, 1976.
88. Zimmerman, A. M., and Zimmerman, S., The influence of marihuana on eukaryote cell growth and development, in "Marihuana: Chemistry, Biochemistry, and Cellular Effects", (G. G. Nahas, ed.) pp. 195-204. Springer-Verlag, New York, 1976.
89. Myers, III, W. A., and Heath, R. G., Cannabis sativa: Ultra-structural changes in organelles of neurons in

brain septal region of monkeys, J. Neurosci. Res., 4:9-17 (1979).
90. Hattori, T., Jakubovic, A., and McGeer, P. L., The effect of cannabinoids on the number of nuclear membrane-attached ribosomes in infant rat brain, Neuropharmacology, 12:995-999 (1973).
91. Gierer, A., Function of aggregated reticulocyte ribosomes in protein synthesis, J. Mol. Biol., 6:148-157 (1963).
92. Hernandez, F., Lopez-Rivas, A., Pintor-Toro, J. A., Vazquez,D., and Palacian, E., Implication of arginyl residues in mRNA binding to ribosomes, Eur. J. Biochem. , 108:137-141 (1980).
93. Jonas, A., and Weber, G., Presence of arginine residues at the strong, hydrophobic anion binding sites of bovine serum albumin, Biochemistry, 10:1335-1339 (1971).
94. Mon, M. J., Haas, A. E., Sten J. L., and Stein, G. S., Influence of psychoactive and non psychoactive cannabinoids on cell proliferation and macromolecular biosynthesis in human cells, Biochem. Pharmacol., 30:31-43 (1981).
95. Mon, M. J., Haas, A. E., Steink, J. L., and Stein, G. S., Influence of psychoactive and nonpsychoactive cannabinoids on chromatin structure and function in human cells, Biochem. Pharmacol., 30:45-58 (1981).
96. Stein, S. M., and Berestecky, J. M., Inhibition of growth by masking of arginine moieties in protein at the cell surface, Cancer Res., 34:3112-3116 (1974).
97. Stein, S. M., and Berestecky, J. M., Exposure of an arginine-rich protein at surface of cells in S, G_2 and M phases of the cell cycle, J. Cell. Physiol., 85:243-250 (1975).
98. Mehrishi, J. N., Positively charged amino groups on the surface of normal and cancer cells, Eur. J. Cancer., 6:12-137 (1980).
99. Steven, F. S., Griffin, M. M., Hulley, T. P., and Brooman,P., Interaction of alpha,beta-stilbestrol-4,4'-bisphosphate with arginyl substrates resulting inapparent inhibition of typsin and thrombin, Eur. J. Biochem. 125: 305-309 (1982).
100. Harris, L. S., Carchman, R. A., and Martin, B. R., Evidence for the existence of specific cannabinoid binding sites, Life Sci., 22:1131-1138 (1978).
101. Warner, W., Harris, L. S., and Carchman, R. A., Inhibition of corticosteroidogenesis by delta-9-tetrahydrocannabinol, Endocrinology, 101:182101827 (1977).
102. Carchman, R. A., End, D. W., and Parker, M. R., Marihuana cell function, Adv. Biosci., 22-23:219-229 (1979).
103. Beins, D. M., Vining, R., and Balasubramaniam, S., The regulation of neutral cholesterol esterase and acetyl-Co

A:cholesterol acetyltransferase in the rat adrenal gland, Biochem. J., 202:631-637 (1982).
104. Burstein, S., Hunter, S. A., and Shoupe, T. S., Site of inhibition of Leydig cell testosterone synthesis by delta-1-tetrahydrocannabinol, Mol. Pharmacol., 15:633-640 (1979).
105. Gill, E. W., Jones, G., and Laurence, D. K., Chemical mechanisms of action of THC in "Cannabis and Its Derivatives: Pharmacology and Experimental Psychology" (W. D. M. Paton and J. Crown, eds.) pp. 76-87. London:Oxford University Press, 1972.
106. Wahlqvist, M., Nilsson, I. M., Sandberg, F., and Agurell, S., Binding of delta-1-tetrahydrocannabinol to human plasma proteins, Biochem. Pharmacol., 19:2579-2584 (1970).
107. Widman, M., Nilson, I. M., Nilson, J. L. G., Agurell, S., Borg, H., and Granstrand, B., Plasma protein binding of 7-hydroxy-delta-1-tetrahydrocannabinol: An active delta-1-tetrahydrocannabinol metabolite, J. Pharm Pharmacol., 25:453-457 (1973).
108. Fredrickson, D. S., Lux, S. E., and Herbert, P. N., Apolipoproteins, Adv. Exp. Med. & Biol., 26:25-56 (1972).
109. Rogers, T. B., Borresen, T., and Feeney, R. E., Chemical modification of argininein transferrins, Biochemistry, 17:1105-1109 (1978).
110. Havel, R. J., Lipoprotein biosynthesis and metabolism, Ann. N. Y. Acad. Sci., 348:16-29 (1980).
111. Weisgraber, K. H., Rall, S. C., Jr., and Mahley, R. W., Human E apoprotein heterogeneity, J. Biol. Chem., 256:9077-9083 (1981).
112. Mahley, R. W., Innerarity, T. L., and Weisgraber, K. H., Alterations in metabolic activity plasma lipoproteins following selective chemical modification of the apoproteins, Ann. N. Y. Acad. Sci., 348:265-280 (1980).
113. Titova, G. V., Kliueva, N. N., Kozhevnikova, K. A., and Klimov, A. N., Interaction of cholesterol with apoprotein E-An arginine-rich protein of very low density lipoproteins, Biokhimia, 45:51-55 (1980).
114. Klimov, A. N., Titova, G. V., Kozhenvnikova, K. A., Kliueva, N. M., and Smirnova, I. V., Interaction of cholesterol with polypeptides and amino acids, Biokhimia, 47:226-232 (1982).
115. Fukushima, D., Yokoyama, S., Kezdy, F. J., and Kaiser, E. T., Bindingof amphiphilic peptides to phospholipid/cholesterol unilamellar vesicles: A model for protein-cholesterol interaction, Proc. Natl. Acad. Sci. USA, 78: 2732-2736 (1981).
116. Levy, J. A., Munson, A. E., Harris, L. H., and Dewey, W.

L., Effects of delta-9-THC on the immune response of mice, Fed. Proc., 34:728 (1975).
117. Munson, A. E., and Fehr, K. O., Immunological effects of cannabis, in "Adverse Health and Behavioral Consequences of Cannabis Use. Working Papers for the ARF/WHO Scientific Meeting, Toronto 1981" (K. O. Fehr and H. Kalant, eds.). Addiction Research Foundation, Toronto, 1982.
118. White, S. C., Brin, S. C., and Janicki, B. W., Mitogen-induced blastogenic responses of lymphocytes from marihuana smokers, Science, 188:71-72 (1975).
119. Nahas, G. G., Morishima, A., and Desoize, B., Effects of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes. FASEB Conference, "Organismal Response to the Environment", 60th Annual Meeting of FASEB, Anaheim, CA, April 15, 1976.
120. Bloch, E., Effects of marihuana and cannabinoids on reproduction, endocrine function, development and chromosomes, in "Adverse Health and Behavioral Consequences of Cannabis Use. Working Papers for ARF/WHO Scientific Meeting, Toronto, 1981". (K. O. Fehr and H. Kalant, eds.). Addiction Research Foundation, Toronto, 1982.
121. Henrich, R. T., Nogawa, T., and Morishima, A., In vitro induction of segregational errors of chromosomes by natural cannabinoids in normal human lymphocytes, Environmental Mutagenesis, 2:139-147 (1980).
122. Leuchtenberger, C., Effects of marihuana (cannabis) smoke on cellular biochemistry, utilizing in vitro test systems, in "Adverse Health and Behavioral Consequences of Cannabis Use. Working Papers for the ARF/WHO Scientific Meeting, Toronto, 1981". (K. O. Fehr and H. Kalant, eds.). Addiction Research Foundation, Toronto, 1982.
123. Schempp, W., and Krone, W., Deficiency of arginine and lysine causes increases in the frequency of sister chromatid exchanges, Human Genet., 51:315-328 (1979).
124. Birke, C., Peter, H. H., Langenberg, U., Muller-Hermes, J. P., Peters, J. H., Heitman, J., Leibold, W., Dallugge, H., Krapf, E., and Kirchner, H., Mycoplasma contamination in human tumor cell lines: Effect of interferon induction and susceptibility to natural killing, J. Immunol., 127: 94-98 (1981).
125. Simberkoff, M. S., Thorbecke, G. L., and Thomas, L., Studies of PPLO infection: V. Inhibition of lymphocyte mitosis and anti-body formation by mycoplasma extracts, J. Exp. Med., 129:1163-1181 (1969).
126. Callewaert, D. M., Kaplan, J., Peterson, Jr., W. D., and Lightbody, J. J., Suppression of lymphocyte activation by a factor produced by Mycoplasma arginini, J. Immunol., 115:1662-1664 (1975).
127. Copperman, R., and Morton, H. E., Reversible inhibition

of mitosis in lymphocyte cultures by non-viable mycoplasma, Proc. Soc. Exp. Biol. & Med., 123:790-795 (1966).
128. Kaklamanis,E., and Pavlatos, M., The immunosuppressive effect of mycoplasma infection. I. Effect on the humoral and cellular response, Immunology, 22:695-702 (1972).
129. Barile, P. M., and Leventhal, B. G., Possible mechanism of Mycoplasma inhibition of lymphocyte transformation induced by phytohemagglutinin (PHA), Nature (Lond.), 219:758-759 (1978).
130. Currie, G. A., Activated macrophages kill tumour cells by releasing arginase, Nature (Lond.), 273:758-759 (1978).
131. Stenchever, M. A., Kunysz, T. J., and Allen, M., Chromosome breakage in users of marihuana, Amer. J. Obstet. Gynecol., 118-113 (1974).
132. Stenchever, M. A., and Allan, M., The effect of delta-9-THC on the chromosomes of human lymphocytes in vitro, Amer. J. Obstet. Gynecol., 114:891-821 (1972).
133. Fried, P. A., and Charlebois, A. T., Cannabis administered during pregnancy: First- and second-generation effects in rats, Physiol. Psychol., 7:307-310 (1979).
134. Carrano, A. V., Thompson, L. H., Lindl, P. A., and Minkler, J. L., Sister chromatid exchanges as an indicator of mutagenesis, Nature, 271:551-555 (1978).
135. MacRae, W. D., MacKinnon, E. A., and Stich, H. F., Effects of arginine deprivation upon chromosome aberrations, SCEs and survival of CHO cells treated with mutagenic agents, Mutat. Res., 62:495-504 (1979).
136. MacRae, W. D., MacKinnon, E. A., and Stich, H. F., Induction of sister chromatid exchanges and chromosomes alterations in CHO cells arrested in the cell cycle by arginine deprivation, In Vitro, 15:555-564 (1979).
137. Carrano, A. V., and Heddle, J. A., The fate of chromosome aberrations, J. Theor. Biol., 38:289-304 (1973).
138. Hsu, T. C., Editorial comments, Mammal. Chromo. Newslett., 17:1-7 (1976).
139. Leuchtenberger, C., Leuchtenberger, R., and Ritter, U., Effects of marihuana and tobacco smoke on DNA and chromosomal complement in human lung explants, Nature (Lond.) 242:403-404 (1973).
140. Maccioni, R. B., Vera, J. C., and Slebe, J. C., Arginyl residues involvement in the microtubule assembly, Arch. Biochem. Biophys., 207:248-255 (1981).
141. Charlebois, A. T., and Fried, P. A., Interactive effects of nutrition and cannabis upon rat perinatal development, Dev. Psychobiol., 13:591-505 (1980).
142. Rettura, G., Padawer, J., Barbul, A., Levenson, S. M., and Seifter, E., Supplemental arginine increases thymic cellularity in normal and murine sarcoma virus-inoculated mice and increases resistance to murine sarcoma virus

tumor, JPEN, 3:409-416 (1979).
143. Barbul, A., Sisto, D. A., Wasserkrug, H. L., and Efron, G., Arginine stimulates lymphocyte immune responsein healthy human beings, Surgery, 90:244-251 (1981).
144. Barbul, A., Sisto, D. A., Wasserkrug, H. L., Levenson, S. M., Efron, G., and Seifter, E., Arginine stimulates thymic immune function and ameliorates the obesity and hyperglycemia of genetically obese mice, JPEN, 5:492-495 (1981).
145. Barbul, A., Rettura, G., Levenson, S. M., and Seifter, E., Arginine, a thymotropic and wound-healing promoting agent, Surg. Forum., 28:101-103 (1977).
146. Block, E., Thysen, B., Morrill, G. A., Gardner, E., and Fujimoto, G., Effects of cannabinoids on reproduction and development, Vit. Hor., 36:204-258 (1978).
147. Juel-Jensen, B. E., Cannabis and recurrent herpes simplex, Brit Med. J., iv:296 (1972).
148. Manischewitz, J. E., Young, B. G., and Barile, M. F., The effectof mycoplasmas on replication and plaquing ability of Herpes Simplex virus, Proc. Soc. Exp. Biol. Med., 148: 859-863 (1975).
149. Blevins, R. D., and Dumic, M. P., Delta-9-tetrahydrocannabinol and Herpes Simplex, This volume, 1982.
150. Becker, Y., Olshevsky, U., and Levitt, J., The role of arginine in the replication of Herpes Simplex virus, J. Gen. Virol., 1:471-478 (1967).
151. Tennant, F. S., Jr., Clinical toxicology of cannabis use, in "Adverse Health and Behavioral Consequences of Cannabis Use. Working Papers for the ARF/WHO Scientific Meeting, Toronto, 1981", (K. O. Fehr and H. Kalant, eds.). Addiction Research Foundation, Toronto, 1982.
152. Munson, A. E., Harris, L. S., Friedman, M. A., Dewey, W. L., and Carchman, R. A., Antineoplastic activity of cannabinoids, J. Natl. Cancer Inst., 55:597-603 (1975).
153. Jering, H., and Toro-Goyco, E., Effect of delta-9-THC in nucleoside and amino acid uptake in Reuben H-35 hepatoma cells, Adv. Biosci., 22 & 23:161-169 (1979).
154. Milner, J. A, and Stepanovich, L. V., Inhibitory effect of dietary arginine on growth of Ehrlich ascites tumor cells in mice, J. Nutr., 109:489-494 (1979).
155. Cho-Chung, Y. S., Clair, T., Bodwin, J. S., and Hill, D. M., Arrest of mammary tumor growth in vivo by L-arginine: stimulation of NAD-dependent activation of adenyl cyclase, Biochem. Biophys. Res. Commun., 95:1306-1313 (1980).
156. Cho-Chung, Y. S., Clair, T., Bodwin, J. S., and Berghoffer, B., Growth arrest and morphological changes of human breast cancer cells by dibutyryl cyclic AMP and L-arginine, Science, 214:77-79 (1981).

157. Bach, S. J., and Lasnitzki, I., Some aspects of the role of arginine and arginase in mouse carcinoma 63, Enzymologia, 12:198-205 (1947).
158. Bach, S. J., and Maw, G. A., Creatine synthesis by tumour-bearing rats, Biochem. Biophys. Acta., 11:69078 (1953).
159. Bach, S. J., and Simon-Reuss, I., Arginase, an antimitotic agent in tissue culture, Biochem, Biophys. Acta., 11:369-402 (1953).
160. Storr, J. M., and Burton, A. F., The effects of arginine deficiency on lymphoma cells, Br. J. Cancer, 30:50-59 (1974).
161. Okabe, J., Hayashi, M., Honma, Y., and Houmi, M., Induction of differentiation of cultured mouse myeloid leukemic cells by arginase, Biochem. Biophys. Res. Commun., 89:879-884 (1979).
162. Scallan, C., Clynes, M., and Joyce, P., The effect of shark liver arginase on the growth of cells in culture, Biochem. Soc. Trans., 9:317 (1981).
163. Currie, G. A., and Basham, C., Differential arginine dependence and the selective cytotoxic effects of activated macrophages for malignant cells in vitro, Br. J. Cancer, 38:653-659 (1978).
164. Currie, G. A., Gyure, L., and Cifuentes, L., Microenvironmental arginine depletion by macrophages in vitro, Br. J. Cancer, 39:613-629 (1979).
165. Holcenberg, J. S., Enzyme therapy: problems and solutions, Ann. Rev. Biochem., 51:795-812 (1982).
166. Li, D. M., The lack of beta-adrenoreceptor involvement in the cardiac action of delta-1-tetrahydrocannabinol in rats, Clin. Exp. Pharmacol. Physiol., 7:23-29 (1980).
167. Choisy, H., Choisy, G., Millart, H., and Legris, H., Influence of delta-9-tetrahydrocannabinol on contraction rate and enzymatic activity of embryonic heart cells, Adv. Biosci., 22-23:265-277 (1979).
168. Mornet, D. Pantal, P., Audemard, E., and Kassab, R., Involvement of an arginine residue in the catalytic activity of myosin heads, Eur. J. Biochem., 100:421-431, (1979).
169. Johnson, P., and Blazyk, J. M., Involvement of an arginine residue of actin in tropomyosin binding, Biochem. Biophys. Res. Commun., 82:1013-1018 (1978).
170. Petro, D. J., and Ellenberger, Jr., C., Treatment of human spasticity with delta-9-tetrahydrocannabinol, J. Clin. Pharmacol., 21:413S-416S (1981).
171. Malec, J., Harvey, R. F., and Cayner, J. J., Cannabis effect on spasticity in spinal cord injury, Arch. Phys. Med. Rehabil. 63:116-118 (1982).

172. Narayanareddy, K., and Swami, K. S., Free aminoacid composition in the denervation atrophy of gastronemius muscle of the frog Rana hexadactyla, Indian J. Exp. Biol., 13:343-345 (1975).
173. Smith, I., The treatment of inborn errors of the urea cycle, Nature (Lond.), 291:38-380 (1981).
174. Snyderman, S. E., Sansaricq, C., Norton, P. M., and Goldstein, F., Argininemia treated from birth, J. Pediatr., 95:61-63 (1979).
175. Ghoneim, M. Th., Mikhal, M. M., Mahfouz, M., and Makar, A. B., Effect of hashish smoke on some blood and serum parameters in rabbits, Pharmazie, 35:226-228 (1980).
176. Visek, W. J., Ammonia metabolism, urea cycle capacity and their biochemical assessment, Nutr. Rev., 37:273-282 (1979).
177. Rosenkrantz, H., Cannabis marihuana and cannabinoid toxicological manifestations in man and animals, in "Adverse Health and Behavioral Consequences of Cannabis Use. Working Papers for the ARF/WHO Scientific Meeting, Toronto, 1981", (K. O. Fehr and H. Kalant, eds.), Addiction Research Foundation, Toronto, 1982.
178. Wergedal, J. E., and Harper, A. E., Metabolic adaptations in higher animals IX. Effect of high protein intake on amino acid nitrogen catabolism in vivo, J. Biol. Chem., 239:1156-1163 (1964).
179. Poddar, M. K., and Dewey, W. L., Effects of cannabinoids on catecholamine uptake and release in hypothalamic and striatal synaptosomes, J. Pharmacol. Exp. Ther., 214:63-67 (1980).
180. Fehr, K. O., and Kalant, H., Long-term effects of cannabis on cerebral function: A review of the clinical and experimental literature, in "Adverse Health and Behavioral Consequences of Cannabis Use. Working Papers for ARF/WHO Scientific Meeting, Toronto, 1981" (K. O. Fehr and H. Kalant, eds.), Addiction Research Foundation, Toronto, 1982.
181. Domino, E. F., Cannabinoids and the cholinergic system, J. Clin. Pharmacol., 21:2496-2556 (1981).
182. Ferraro, D. P., Acute effects of marihuana on human memory and cognition, in "Marihuana Research Findngs: 1980" (R.C. Peterson, ed.) pp. 98-119. NIDA Research Monograph 31, Department of Health and Human Services, NIDA, Rockville, MD, 1980.
183. Miller, L. L., Cannabis and the brain with special reference to the limbic system, Adv. Biosci., 22 & 23:539-566 (1979).
184. Rose, F. C., and Symonds, C. P., Persistant memory defect following encephalitis, Brain, 83:195-211.

185. Drachman, D. A., and Adams, R. D., Herpes Simplex and acute inclusion body encephalitis, Arch. Neurol., 7:61-79 (1962).
186. Brown, H., Some anticholinergic-like behavioral effects of trans (-)-delta 8-THC, Psychopharmacologia, 21:294-301 (1971).
187. Mautner, H. G., Pakula, A. A., and Merrill, R. E., Evidence for presence of an arginine residue in the coenzyme A binding site of choline acetyltransferase, Proc. Natl. Acad. Sci. USA 78:7449-7452 (1981).
188. Franks, D. J., Tunnicliff, G., and Ngo, T. T., Inactivation of adenylate cyclase by phenylglyoxal and other dicarbonyls. Evidence for the existence of essential arginyl residues, Biochem. Biophys. Acta., 611:358-362 (1980).
189. Tunnicliff, G., and Ngo, T. T., Involvement of arginyl residues in catalytic activity of catechol-O-methyltransferase, Gen. Pharmacol. 10:373-376 (1979).
190. Moszik, G., Some feedback mechanisms by drugs in the inter-relationship between the active transport system andadenyl cyclase system localized in the cell membrane, Eur. J. Pharmacol., 7:319-327 (1969).
191. Thoa, N. B., Wooten, G. R., Axelrod, J., and Kopin I. J., Inhibition of release of dopamine-beta-hydroxylase and norepinephrine from sympathetic nerves by colchicine, vinblastine, or cytochalasin-B, Proc. Natl. Acad. Sci. USA, 69:520-522 (1972).
192. Tunnicliff, G., and Ngo, T. T., Functional role of arginine residues in glutamic acid decarboxylase from brain and bacteria, Experientia, 34:989-900 (1978).
193. Tunnicliff, G., Essential arginine residue at the pyridoxal phosphate binding site of brain gamma-aminobutyrate aminotransferase, Biochem. Biophys. Res. Commun., 97:160-165 (1980).
194. Roberts, E., Prospectus. Epilepsy and antiepileptic drugs: A speculative synthesis, in "Antiepileptic Drugs: Mechanisms of Action" (G. H. Glaser, J. K. Penry and D. M. Woodbury, eds.) pp. 667-713. Raven Press, New York, 1980.
195. Stone, W. E., Action of convulsants: Neurochemical aspects, in "Basic Mechanisms of the Epilepsies" (H. H. Jasper, A. A. Ward, and A. Pope, eds.) pp. 184-193, Little Brown and Co., Boston, 1969.
196. Liebl, L., Convulsant action of hydraine:impairment of chloride-dependent inhibition in cat spinal motor neurons, Exp. Brain Res., (Suppl.) 23:125 (1975).
197. Karler, R., and Turkanis, S. A., Cannabis and epilepsy, Adv. Biosci., 22 & 23:619-641 (1979).

198. Feeney, D. M., Marihuana and epilepsy: paradoxical anticonvulsant and convulsant effects, Adv. Biosci., 22 & 23:643-657 (1979).
199. Roberts, E., Simonsen, D. G., and Roberts, E., Structural requirements of nitrogenous substances which have protective effects against acute hydrazine toxicity in mice, Proc. Soc. Exp. Biol. Med., 119:683-687 (1965).
200. Pisano, J. J., and Udenfriend, S., Formation of gamma-guanidino-butyric acid and guanidinoacetic acids in brain, Fed. Proc., 17:403 (1958).
201. Purpura, D., Girado, M., and Grundrest, H., Central synaptic effects of omega-guanidino acids and amino acid derivatives, Science, 127:1179-1181 (1958).
202. Jinnai, D., Sawai, A., and Mori, A., gamma-guanidinobutyric acid as a convulsive substance, Nature (Lond.), 212:617 (1966).
203. Edery, H., and Gottesfeld, Z., The gamma-aminobutyric acid system in rat cerebellum during cannabinoid-induced cataleptoid state, Br. J. Pharmacol., 194:74-81 (1975).
204. Curtis, D. R., and Watkins, J. C., The excitation and depression of spinal neurons by structurally related amino acids, J. Neurochem., 6:117-141 (1960).
205. Watkins, J. C., Pharmacological receptors and general permeability phenomena of cell membranes, J. Theoret. Biol., 9:37059 (1965).
206. Giambalvo, C. T., and Rosenberg, P., The effect of phospholipases and proteases on the binding of gamma-aminobutyric acid to junctional complexes of rat cerebellum, Biochem. Biophys. Acta., 436:741-756 (1976).