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Effects of Cocaine, Nicotine and Diet on Voluntary Alcohol Consumption in Rats

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EFFECTS OF COCAINE, NICOTINE AND DIET ON VOLUNTARY ALCOHOL CONSUMPTION IN RATS

by

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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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CHAPTER 1

INTRODUCTION

Alcoholism has become an important social and economic problem that affects 5,000,000 people in the United States (16, 12) and has been rated the number three health problem in this country (9). The term alcoholism includes all who are addicted to the use of alcohol to such a degree that it interferes with their health, personal relations, social adjustment, and economic efficiency. Alcoholism thus constitutes one of the major diseases of modern life (16).

The production and use of alcohol goes back to prehistory. Archaeological records reveal the widespread use of alcohol from different forms. Greek writers distinguished between sources of fermented beverages based respectively on honey (to give mead), cereals (to give beer) and the grape for wine. Later (about fifth century A.D.) references began to appear of other sources, notably the apple (to give cider), the pear (for perry) and other fruits.

The products described in the classical literature or archeological records of the Mediterranean world were derived products from fermentation. Distillation awaited the rise of chemistry by way of the Arabian alchemists, which led to the production of a wide variety of distilled spirits to supplement the old fermented beverages. It led also to the pro-

duction of a third group of beverages, namely wines (or similar products) fortified by the addition of alcohol.

The cultivation of the grape and the art of wine making spread from Asia Minor westward with Greek civilization and colonization. Under the Romans, the legions spread the vine to Spain, France, England, Germany, Austria and Hungary. The vine, with the olive and the wheat grain, became symbolic of the Roman way of life, and of the civilization and dietary pattern of Western Europe.

Today Italy, France and Spain are the main producers of wine. Wine production has become well established in South America, in countries like Argentina, Chile and Uruguay. In the United States, California is the major producer; other countries are Canada, Australia, South Africa. Algeria in North Africa ranks as sixth in world production (3).

Different parts of the world have different fermented beverages, like the palm wines in North Africa, the "tequila" in Mexico (from maguey), and the different chichas used by the Indians in Peru and Bolivia. These customs date clear back to the pre-colonial and colonial times. We find citations about this in different chronicles and history books like this one by Rowe, "The Inca made a number of fermented drinks or chicha from different cultivated plants, maize, quinoa, ocas and molle berries, but had no destilled liquor. Chicha was made by women who chewed the pulp of the fruit used, and spat the mash out into jars or warm water. The liquid then was allowed to ferment to desired strength" (27).

Garcilazo de la Vega in 1555, in his book The Royal Commentaries of the Incas, describes another common way used to obtain alcoholic beverages: "Some Indians who are more fond of inebrity than their fellows, steep the maize until it sprouts and then mash it in the same water, and keep it until it ferments. This produces a very strong liquor which intoxicates at once. They call it Vinapu and in another language Sora. The Incas prohibited its use, because it was so intoxicating (13).

Also Cieza de Leon (1550) takes a critical look at the drinking habits of the Incas and writes: After they have eaten (Indians) they pass the rest of the day in drinking chicha, or wine made from maize. They always continue drinking until they are very drunk. And the following words are unfit for translation, "Y como estan sin sentido, algunos toman las mujeres que quieren, y llevadas a alguna casa, usan con ellas sus luxurias, sin tenerlo por cosa fea, porque ni entienden el don que esta debaxo de la verguenza, ni miran mucho en la honra, ni tienen mucha cuenta con el mundo (8).

In 1613, Poma de Ayala describes the social implications of drinking in the Spanish colonies as follows. "Now drunkeness is condoned saying that the killer was drunk, and thus there begins to thrive so much drunkeness and harm, and they do not serve God, but rather fall into more offense and nothing is done. It's the fault of the justice, he who quarrels or fights while he is drunk, fifty lashes and then (let him) be shorn, and who ever kills, hang him. Then there is

good justice" (24). Since those times, with the discovery of distillation, the drinking patterns in alcohol consumption within the Incas and other countries have changed in some way or another.

I. REVIEW OF THE LITERATURE

ABOUT ALCOHOL

Factors Affecting Ethanol Consumption: Sex and Strain

Some studies reveal that some strains of animals would select solutions containing alcohol while others if given the choice would avoid them. Such happens with the C57BL/10J and C57BL/6J mice who prefer 10% alcohol to water. In contrast, C57BL/6A mice prefer water instead of alcohol (23). In our laboratory when three different strains (Sprague-Dawley, Wistar and Fisher) were tested for alcohol or water preference, the Sprague-Dawley rats (albino) showed the higher consumption of a 10% ethanol solution, second came the Wistar strain and third the Fisher strain which showed a very low level of ethanol intake (19). This was the reason for our preference of the Sprague-Dawley rats in this study.

Also sex has an influence upon alcohol intake, Russell and Stern showed in one of their studies that the female rats of the Wistar and Tryon Maze Bright strains consumed more alcohol than water compared to the males (28).

Availability of Fluids: The length of exposure to ethanol is another factor that influences alcohol intake. Rats exposed to ethanol for prolonged periods of time will progressively increase consumption of even concentrated solutions which were previously avoided. Also an increase intake of alcohol

is observed after periods of withdrawal. Several periods of food withdrawal will also lead to an increased consumption of alcohol (34). When different methods to induce alcohol intake in rats were used by Wise et.al. (36) they found that the forced choice schedule (that is 20% ethanol solutions as their only fluid on every other day, and water in between days) will induce high levels of ethanol preference. On the other hand a period of fluid deprivation will decrease the ethanol preference in rats when offered again a 10% ethanol solution (6). Interestingly enough, housing density has also an effect on alcohol intake, Deatherage (10) observed that rats housed alone will consume more 20% ethanol solution than do rats housed in groups.

Nutritional Factors: Experiments show that consumption of alcohol is controled by physiological incentive or discentive provided by taste receptors in the mouth. Alcohol can be first detected in human adults as a sweet taste (concentration about 4.2 $\pm$ 0.24% v/v), then as the concentration of alcohol increases (about 21.2% v/v) the sweet taste changes to a burning taste. This is defined as the human burning taste threshold to alcohol. Experiments in rats and man confirm that alcohol taste discrimination must be an innate physiological incentive in both, being the factor that determines the initial choice and continued consumption of alcohol at higher concentrations. When the aversion taste threshold is exceeded in rats, central mechanisms probably provide the determining factor for continued consumption. Similar mech-

anisms may determine the initial selection and continued consumption of alcohol in man, not only that but also the degree of dilution of alcoholic drinks. Alcohol itself raises the taste threshold. This effect is related to the blood alcohol level, but whether it is produced by direct effect on the taste receptors or through a central action it is unknown (35).

It is well known that alcohol consumption may interfere with many biochemical and nutritional functions, such is the case with thiamine. Animal studies show that when labeled thiamine S^{35} is administered, it's rate of absorption from the intestines is reduced when alcohol is administered orally or parenterally. Since the liver is an important organ for both the storage and metabolism of certain vitamins, interference by alcohol of it's functions can cause nutritional deficiencies. Chronic taking of ethanol reduces the amount of the active form of vitamin B_6. The phosphorylation of thiamine in the liver is also impaired by alcohol. This could be caused by deficient ATP in the liver. This deficiency is known to occur in alcoholism. This explains the persistence of peripheral neuropathy and low transketolase activity of erythrocytes in alcoholic cirrhosis despite therapy with thiamine (35).

Also ingestion of alcohol has been shown to increase cholesterol levels in the blood of man, chicken, rabbit and rats. Alcohol is related to cholesterol metabolism, as it is converted to acetyl CoA the precursor of cholesterol biosynthesis (25).

Among the detrimental effects of alcohol is hyperlipidemia, which can be induced by combination of dietary fat and alcohol. This is often associated with hemolytic anemia, jaundice and fatty liver a combination known as Zieve's syndrome. Additional factors may also play a part in the etiology of this syndrome.

In rats as well as in non-alcoholic human volunteers it was observed, that alcohol can profoundly affect the ultrastructure of the liver cell despite adequate nutrients, protein and choline. When alcohol (95% diluted 1 in 6) was isocalorically substituted for carbohydrate in an adequate diet so that it supplied about 44% of the energy during 12-18 days, there was enlargement and marked morphological changes in the mitochondria of the liver, and proliferation of the smooth endoplasmic reticulum. The mitochondria of chronically treated rats oxidized less fatty acids, acetate and chylomicrons; and they respired slower than normal (32).

Also gout has traditionally been associated with alcohol, and for a century its ingestion has been known to be accompanied by hyperuricemia. This condition is common in drunken persons, and infusions of alcohol cause hyperuricemia by raising blood lactate which is known to suppress the excretion of uric acid (32).

In studies done in our laboratory it was seen that rats on a poor basal diet tend to drink more alcohol than water when given a choice of tap water and a 10% solution of alcohol (26, 2, 33).

Another aspect that may be pertinent to this study are the effects of caffeine on the blood concentration, Siegers et.al. (31) has observed in rats a depression of blood alcohol with caffeine, however elimination of alcohol was not observed to be affected. This was due to the delaying of the emptying time of the stomach by caffeine, since 80% of ethanol is normally absorbed in the small intestine. Rinkel and Myerson (1941) have also shown that sympatomimetic substances or atropine lowered blood alcohol levels in man by delaying stomach emptying (31).

Rats that prefer alcohol to water may also prefer other drugs, thus Amit et.al. (1) observed that rats preferring alcohol would choose instead of water other drugs like diazepam, when alcohol was withheld.

It is interesting to find in the literature ethnic differences in alcohol sensitivity. Taiwanese, Japanese, and Korean after drinking amounts of alcohol that have no detectable effect on caucasoids, respond with a marked facial flushing and mild to moderate symtoms of intoxication (37).

ABOUT COCAINE

Before the colonization of America by the Spaniards, the chewing of coca (Erythroxylon Coca) leaves was already being practiced by the Indians in Peru and Bolivia. References about this fact is found in the literature such as this, "The Inca used coca leaves for divining and sacrifice, and chewed them with lime to absorb a small amount of cocaine.

The lime was made by burning quinoa stalks, bones, limestones, or sea shells. The Indians made a quid of leaves and lime about the size of a walnut and held it in their cheek, swallowing only the juice. Coca chewing was believed to be very good for the teeth" (27).

According to the chronicler Santillan and the Viceroy Francisco de Toledo, after the Spanish conquest coca became a lucrative business. In the mines of Potosi alone 100,000 baskets of leaves were consumed in 1538. Also several ordinances from Viceroys (1570, 1571, 1574) authorized coca cultivation. This wide use of coca can be attributed to several factors: 1. The diminished food supply, and the obligation of hard labor in the mines, 2. The feeling of artificial well being after chewing coca, which allowed them to block out feelings of inferiority due to the conquest by the Spanish, and 3. The income that derived from the trade of coca leaves (38).

It is estimated today that 6,000,000 people are coca addicts in Peru and Bolivia. This habit has been extended to some neighboring countries like Ecuador and the northern part of Argentina. The majority of users consume about 30-50 grams of coca leaves per day, divided into three treats or so. Very few consume 200 grams or more.

It has been estimated that in Peru 8,500,000 kilos of coca are consumed per anum. Taking as an average 0.5% of cocaine content in the leaves, this means 40,000 kilos of cocaine per year. This figure is 25 times larger than the

amount of cocaine for medical purposes needed in the whole world (38). During chewing, an average of 86% of the alkaloids contained in the coca leaves are ingested, the remaining 14% are put aside in the residue. The reason for adding alkali substances to coca (while chewing), is to facilitate the liberation of cocaine for absorption through the intestinal tract.

It is believed that after cocaine (methyl benzoil ecgonine) is ingested through the mouth it undergoes constant hydrolysis, first by the alkali that accompanies it, and then by the different digestive juices, giving rise to benzoil ecgonine and finally to ecgonine. So the amount of cocaine passing to the bloodstream is insignificant. This is totally converted into benzoil ecgonine and ecgonine in the liver. The blood that passes through the suprahepatic vein and into the bloodstream may contain traces of cocaine, which would be hydrolyzed by the cocainesterase in the blood, forming ecgonine, the metabolite eliminated in the urine (20). It must be kept in mind that there are differences between those who chew coca leaves and those who take cocaine directly.

The physiological effects of coca include acceleration of the pulse, a mild rise in blood pressure, blood sugar, body temperature, basal metabolism plus a mild increase in respiratory rate and muscle reflexes. Resistance to fatigue, hunger, cold and sleep are the effects that coca users seek. Chewers associate coca primarily with work (14), they state for instance that "without coca it is impossible to work" or

"we always use it because of work."

The alleviating sensation of fatigue by cocaine may be due to the fact that epinephrine and norepinephrine levels are elevated during work, and cocaine potentiates the action of both. If the fatigue alleviating action of epinephrine is potentiated by coca, endurance is extended (18). A paradox is observed in cocaine, although it is a known stimulant, researchers note a retardant or depresant effect on regular consumers (21). This may be due to the pharmacological actions of ecgonine, which causes a slight arterial hypotension and high degree of innocuity (20).

It is interesting to notice that both in formal and informal gatherings, when these people chew coca they also drink. Goddard (14) while studying the social factors associated with coca, observed that the chewers drank heavier than the non chewers.

Also the relation of poor nutrition to cocainism is very significant. Any effort to eradicate cocainism has to be parallel with an improvement in nutrition. An example of this is the Peruvian army. People tend to quit cocainism as soon as they join the army because they have better food and housing conditions. Once they are discharged from the army they return to the habit of chewing coca (38).

TABLE I

Nutrients Available from 4.4 gm Coca Leaves and 8 gm Llujta (Ash) Mixture*

	mg
Fluorine	0.18
Calcium	140
Carotene	0.8
Thiamine	0.08
Riboflavin	0.07
Niacin	0.6
Ascorbic acid	
reduced	7.0
dehidro-	7.4

* From "Bolivia. Nutrition Survey. A Report by the Inter-departmental Committee on Nutrition for National Defense." June 1964

ABOUT NICOTINE

Nicotine (1 methyl, 2 - (3 pyridyl) pirrolidine), is a well known alkaloid extracted from the dried leaves of Nicotiana Tabacum and Nicotiana rustica (Content 2% - 8%) combined with citric and malic acids. The alkaloid was identified by Possett and Reimann in 1829 and named after the French ambassador, Jean Nicot, who obtained the plant in Florida and introduced to Portugal. It was first used at this time to treat many skin lesions. However, tobacco was smoked by the American Indian at the time of the European discovery of America in the 15^{th} century (7). Nicotine is readily absorbed by all tissues. The fact that it can be absorbed through the skin makes it a very dangerous substance. In fact it is known that smugglers used to hide the leaves in contact with their skin, suffering later from deadly poisoning.

The acute lethal dose of nicotine is around 50-75 mg. Alarming symptoms may occur in individual non smokers after as little as 4 mg. One cigarette contains about 20-30 mg (70, 15). A cigar may have ten times this amount. Of the nicotine entering the body fluids 80-90% is metabolized by the liver, kidneys and lungs. Nicotine and its breakdown products, cotinine (γ- (3 pyridyl) - γ oxybutiric acid), 3-pyridyl acetic acid and isomethylnicotinium, are eliminated completely by the kidney (7).

It should be pointed out that there is a difference between the cigarette and cigar smoke. Cigarette smoke is acid, while cigar smoke is alkaline. This difference has a toxicological significance. Because of local irritation of cigar smoke it is not inhaled. Therefore, its absorbed by the mucous of the mouth, swallowed, absorbed in the gastro intestinal tract, and quickly detoxified in the liver. In contrast, in the acid smoke of cigarette, the nicotine salt is found as an aerosol, with a particle size of 0.2 - 0.3 microns. According to Keith and Derrick (22) this will not be filtered by the cigarette filter, and only a little of this aerosol lands in the mucous membranes of the mouth and nose. Only with a deep inhalation of a spread of the lung surface between 100-200 square meters will the inhaled aerosol pass from the lungs to the circulation. This will not be easily detoxified as the cigar smoke because it bypasses the liver. If the smoke stays in the lungs for seconds practically all can be absorbed, but if the smoke comes only into contact with the mucous membranes of the nose and mouth by "puffing," only a small fraction of nicotine is absorbed. The World Health Organization's Expert Committee on Addiction-Producing Drugs, labeled nicotine (cigarette smoking) as habituating, and not as addicting, although reports in the literature describe the use of tobacco as addicting (11).

Apparently the determinant factor for the desirability of smoking is nicotine Gue (17) observed an accurate discrimination in the preference for cigarettes. People that smoke are very often addicted to other drugs. When L. Schmidt (30)

studied 500 alcoholics, he found out that the more cigarettes they smoke the higher the tendency of alcohol consumption.

It is well known that smoking produces undesirable effects in the organism. A suggested mechanism for the tumorigenic effect of cigarette smoke in animals is enzymatic inhibition, the inhibition of sulfhydryl containing enzymes being the mode of action (other mechanisms may operate too). Peroxide, acetaldehyde, free radicals and hydrogen cyanide have been implicated as the inhibitors in smoke. Yeast alcohol dehydrogenase was found to be inhibited by the vapor phase(in smoke), whole smoke and much more by particulate matter present in the smoke of cigarettes (5).

Cigarette smoke has been shown to precipitate at bends of points of swelling in the capillaries. Smoke from dry cigarettes precipitates more effectively on these parts of the capillaries than that of moist cigarettes. The smoke of dry cigarettes will condense on the respiratory walls in greater quantities and will remain longer with greater injury of the cell wall than the smoke of moist cigarettes. This may explain why there is a higher death rate of lung cancer in England, Austria and Finland compared to Japan where smoking is at the same level but heating facilities are not the same. In the winter time it is common to see Europeans smoking dry cigarettes (29).

In relation to pregnancy, it has been observed that more premature babies are born from mothers that are smokers (22).

Nicotine passes easily through cell membranes, so it crosses the placenta into circulation. Sontag et.al. observed that after smoking, not only does the mother's heart beat increase, but also the one of the child.

Research shows sex dependent metabolism of nicotine in non-smoking humans and that smoking causes alterations in nicotine metabolism. More nicotine but less cotinine is excreted by female non-smokers than by male non-smokers (4).

II. PURPOSE OF THIS STUDY

The Indians in the highlands of Bolivia and Peru chew coca leaves, besides this fact they also have a poor diet and consume alcohol in different forms (chicha, beer, and pisco). It was the purpose of this study to determine (using a rat model) whether the consumption of the cocaine in the coca leaves or the poor diet that these people have, has any significant effect on the consumption of alcohol.

The other part of the study was concerned with the nicotine effects upon alcohol consumption, since many smokers also drink. Also diet was tested to see if there was any major effects of nicotine on the alcohol consumption of rats fed a poor or good basal diet.

CHAPTER II

EXPERIMENTAL PROCEDURES

I. MATERIALS

Animals: Male weanling Sprague-Dawley albino rats (122) weighing between 45-60 grams were used for the experiment. All of them were individually housed and given a free choice ad libitum of water and a 10% alcohol solution to determine the individual pattern of drinking before the experimental phase was started. After five weeks they were divided in six different groups according to their drinking pattern. Each group had twelve animals.

Animal Housing: Hoeltge individual cages were used to house the animals. Each cage was provided with two graduated drinking tubes with bent stainless steel delivery tubes. Above the funnels an adjustable shelf prevented the animals from defecating or urinating into the funnels.

Fluids: The animals were given a free choice, tap water or a 10% ethanol solution (v/v) to drink. In order to differentiate the two fluids a few drops of red food coloring was added to the alcoholic solution.

Drugs: Three different solutions were used for this experiment: 1. A solution of cocaine hydrochloride

2. A solution of Nicotine

3. A 0.9% sodium chloride solution (saline sol.)

The concentration of the first two solutions were changed during the experiment as the weight of the rats increased. Once the rats were divided into six different groups, these solutions were injected intraperitoneally once every other day. Of the six groups two received injections of cocaine, another two received nicotine injections and the last two received a saline solution.

Pre-experimental diet: A purified basal diet with minimum levels of vitamin mix (0.12%) and minerals (2.5%) was used to encourage alcohol consumption. Coffee was not included for this purpose as it has been done in the past in some of the alcohol studies as the effects of caffeine might have an influence on the outcome of the results while using cocaine and nicotine.

Experimental diets: The same basal diet with a content of 0.12% vitamin mix and 2.5% minerals was used as a poor quality diet. The good quality diet had higher levels of vitamin mix 1% and minerals (5%).

Vitamins: A vitamin fortification mixture from Nutritional Biochemicals was added to the purified basal diets.

Minerals: Hegsted salts were added to the diets at levels specified above.

TABLE II

COMPOSITION OF POOR QUALITY BASAL DIET

Ingredient	Amount Grams	Percent of Diet
Starch	1901.4	63.4
Crisco	600.0	20.0
Promin D	420.0	14.0
Minerals	75.0	2.5
Vitamin Mix	3.6	0.12

TABLE III

COMPOSITION OF GOOD QUALITY BASAL DIET

Ingredient	Amount Grams	Percent of Diet
Starch	1800	60.0
Crisco	600	20.0
Promine D	420	14.0
Minerals	150	5.0
Vitamin Mix	30	1.0

TABLE IV

THE VITAMIN MIXTURE AT 1% AND 0.125% OF THE DIET*

Vitamins	NRC Rat Req. mg/100 gm	Amt. Supplied mg/100 gm	Percent of Req. Supplied 1%	0.125%
Thiamine hydrochloride	0.125	1.0	800	100
Riboflavin	0.250	1.0	400	50
Calcium pantothenate	0.800	3.0	375	47
Niacin	1.500	4.5	300	38
Folic Acid	none	none		
Biotin	none	none		
Pyridoxine HCL	0.120	1.0	833	104
Inositol	none	5.0		
Choline chloride	75.000	75.0	100	12.5
Ascorbic Acid	none	none		
Vitamin B_{12}	0.500	1.0 mcg	200	25
Alpha tocopherol	6.000	5.0	83	10
Menadione	0.010	none		
Vitamin A concentrate	200 IU	4500 mcg	2250	281
Vitamin D concentrate	?	250 mcg		

*From Smithson C., Effects of dietary Factors and Morphine Administration on Voluntary Alcohol Consumption and Dopamine Metabolism in Rats. Aug. 1972.

TABLE V

THE HEGSTED MINERAL SALTS AT 5% AND 2.5% OF THE DIET*

Mineral	NRC Rat Req. mg/100 gm	Amt. Supplied mg/100 gm	Per Cent of Req. Supplied 5%	2.5%
Calcium	600.00	687.00	114	57.0
Phosphorus	500.00	354.00	71	35.5
Potasium	180.00	726.00	403	201.5
Sodium	50.00	329.00	658	329.0
Chlorine	50.00	508.00	1016	508.0
Magnesium	40.00	50.40	126	63.0
Iron	2.50	14.20	568	284.0
Manganese	5.00	6.15	122	61.0
Iodine	0.015	3.06	20400	10200.0
Zinc	1.20	0.60	500	250.0
Copper	0.50	0.38	76	38.0
Ca:P	1.2:1	1.94:1		

*From Smithson C., Effects of dietary Factors and Morphine Administration on Voluntary Alcohol Consumption and Dopamine Metabolism in Rats. August 1972.

II. COLLECTION OF DATA

Body Weight: The rats were weighed weekly at the same time of the day (8:00 A.M.). The weight gain per 100 grams per rat during five weeks was calculated.

Fluid Consumption: The amount of fluid consumption was calculated weekly by substracting the predetermined evaporation constant; the remaining fluid in the drinking tube and the collecting bottle from the total amount given to the animal. The evaporation constant for alcohol was 6 ml. and 5 ml. for water per week. Weekly computations for alcohol and water per 100 grams of body weight were made.

III. DESIGN OF STUDY

One hundred and twenty two male Sprague-Dawley rats weighing 45-60 grams each were housed individually in cages equipped with two graduated drinking tubes, one containing water and the other a 10% ethanol solution. The animals were placed on a poor quality basal diet and offered a fluid choice of water and 10% ethanol solution ad libitum for five weeks. The drinking tubes were alternated so that the alcohol was on the right side one week and on the left the next in order to avoid place preferences. After this period the highest and lowest alcohol consumers were discarded, six groups of twelve animals each were then formed by partially

random selection. Then for another five weeks three of the six groups of animals were given a poor basal diet and the other three, a good basal diet continuing with the same choice of fluids.

The following are the experimental groups:

GROUP	DIET	INTRAPERITONEAL INJECTION
I	Poor quality basal diet	Cocaine
II	Good quality basal diet	Cocaine
III	Poor quality basal diet	Nicotine
IV	Good quality basal diet	Nicotine
V	Poor quality basal diet	Saline
VI	Good quality basal diet	Saline

Accurate measurement of fluid intake was obtained by collecting the dripping from the drinking tubes in fluid waste collection bottles, also evaporation constants for alcohol and water were used in calculating the weekly consumption of both fluids; these evaporation constants were determined in a previous study in this laboratory by Marsh (1967). A mean evaporation constant of 6ml. of alcohol and 5ml. of water per week were used.

Once every other day 30 mg per kilogram of body weight of a solution of cocaine hydrochloride was given intraperitoneally to groups I and II; groups III and IV received 5 mg per kilogram body weight of a nicotine solution also intra-

peritoneally. The last two groups V and VI were used as controls, and were injected intraperitoneally with a saline solution. B-D YALE TUBERCULIN syringes and 25 Gauge needles were used for the injections. The volume of fluid that each animal received at each injection was 2 ml.

IV. ANALYSIS OF DATA

Mean and standard error of alcohol and water intake per 100 grams of body weight were calculated for each rat, then the corresponding mean for five weeks per group was computed. The same procedure was used to calculate weight gain for the five week period. The experimental diets were available to the animals ad libitum, food intake was not recorded.

The F Test based on a general linear model was used to determine the difference of alcohol and water intake, and also weight gain among groups, taking into account the effect of diet and interaction between diet and group. Pre-alcohol consumption was used as a covariate. Since interaction between diet and group was significant in the nicotine study while testing weight gain, a T test was run to determine if there was a significant difference between nicotine and its control on the poor basal diet and nicotine and its control on the good basal diet.

TABLE VI

EFFECT OF COCAINE AND DIET ON ALCOHOL CONSUMPTION OF RATS

Supplement	10% Ethanol Consumption in ml/100 gr Body weight/week during a five week period	
	Poor Diet	Good Diet
Control	32.9 ± 4.0*	16.4 ± 2.9
+ Cocaine	27.6 ± 4.8	15.5 ± 2.9

Difference between Cocaine and Control groups was not significant ($P > 0.05$)

Difference between Poor and Good Diet groups was significant ($P < 0.001$)

* Mean $\pm$ Standard error of Mean

TABLE VII

EFFECT OF NICOTINE AND DIET ON ALCOHOL CONSUMPTION OF RATS

Supplement	10% Ethanol Consumption in ml/100 gr Body weight/week during a five week period	
	Poor Diet	Good Diet
Control	32.9 ± 4.0*	16.4 ± 2.9
+ Nicotine	28.6 ± 4.5	19.3 ± 2.7

Difference between Nicotine and Control groups was not significant ($P > 0.05$)

Difference between Poor and Good Diet groups was significant ($P < 0.001$)

*Mean ± Standard error of Mean

TABLE VIII

EFFECT OF COCAINE AND DIET ON WATER CONSUMPTION OF RATS

Supplement	Water Consumption in ml/100 gr Body weight during a five week period	
	Poor Diet	Good Diet
Control	26.9 ± 3.9*	39.6 ± 3.0
+ Cocaine	24.1 ± 5.8	51.4 ± 2.7

Difference between Cocaine and Control groups was not significant ($P > 0.05$)

Difference between Poor and Good Diet groups was significant ($P < 0.001$)

* Mean ± Standard error of Mean

TABLE IX

EFFECT OF NICOTINE AND DIET ON WATER CONSUMPTION OF RATS

Supplement	Water Consumption in ml/100 gr Body weight during a five week period	
	Poor Diet	Good Diet
Control	26.9 ± 3.9*	39.6 ± 3.0
+ Nicotine	28.6 ± 4.3	41.7 ± 3.0

Difference between Nicotine and Control groups was not significant ($P > 0.05$)

Difference between Poor and Good Diet groups was significant ($P < 0.001$)

* Mean $\pm$ Standard error of Mean

TABLE X

EFFECT OF COCAINE AND DIET

ON WEIGHT GAIN OF RATS

Supplement	Weight gain in grams/week during a five week period	
	Poor Diet	Good Diet
Control	6.33 ± 0.43*	11.03 ± 0.78
+ Cocaine	5.12 ± 0.67	9.4 ± 0.91

Difference between Cocaine and Control groups was not significant ($P > 0.05$)

Difference between Poor and Good Diet groups was significant ($P < 0.001$)

* Mean $\pm$ Standard error of Mean

TABLE XI

EFFECT OF NICOTINE AND DIET

ON WEIGHT GAIN OF RATS

Supplement	Weight Gain in grams/week during a five week period	
	Poor Diet	Good Diet
Control	6.31 ± 0.43*	11.08 ± 0.78
+ Nicotine	8.29 ± 0.79	9.44 ± 0.29

Difference between Nicotine and Control groups was not significant ($P > 0.05$)

Difference between Poor and Good Diet groups was significant ($P < 0.001$)

Interaction between diet and group was significant ($P < 0.001$)

With a T test, difference between nicotine group and its control on poor diet was significant ($P < 0.05$)

With a T test, difference between nicotine group and its control on good diet was not significant ($P > 0.05$)

* Mean $\pm$ Standard error of Mean

CHAPTER III

RESULTS AND DISCUSSION

Data for the mean alcohol and water consumption per 100 grams of body weight per week during a five week experimental period are shown in Tables VI, VII, VIII, and IX. Weight gain per week during the same period is given in Tables X and XI. Results show that the alcohol consumption of rats given a free choice of a 10% ethanol solution or tap water did not increase when injected once every other day with a solution of cocaine hydrochloride ($P > 0.05$).

The amount of cocaine given (30 mg/Kg body weight), was equivalent to 6 grams of coca leaves per kilogram of body weight, considering that the content of cocaine in Erythroxylon coca is 0.5% (38). The amount consumed daily by the Indians in Peru is 2.74 - 4.57 mg of cocaine per kilogram of body weight (for a person weighing 54.7 Kg.). This would be about 30-50 grams of coca leaves or 150-250 mg of cocaine.

One thing that should be mentioned is that while running the experiment eight rats in the cocaine study died right after receiving the corresponding intraperitoneal injection, this could be due to the action of alcohol upon the drug since the dose tested for laboratory work is not tested under such circumstances. The speed of the injection should be watched, it was noticed that if it was slowed the animal

would not go into convulsions, as it was the case with a quick administration. The first 3 rats died when they were injected twice a day with the same dose of cocaine (30 mg/Kg body weight each time). So after two days it was decided to inject only once a day.

As seen in Tables VI and VII, when alcohol consumption was studied under the influence of nicotine, no significant increase in alcohol consumption was observed ($P > 0.05$).

The amount of nicotine given was 5 mg/Kg body weight. Considering that each cigarette contains 20-30 mg of nicotine (15, 7) a person weighing 54.7 Kg that smokes a package of cigarettes a day would be getting 400 - 600 mg of nicotine or 7.3 - 10.9 mg per kilogram of body weight. However it is estimated that only 2.5 - 3.5 mg per cigarette are actually absorbed (2). This is equivalent to 50-70 mg per package or 0.91 - 1.28 mg per kilogram of body weight. A smoker in order to get the amount of nicotine injected into the rats would have to smoke four to five and a half packages a day.

The amount of water consumption also showed no significant difference in both the cocaine and nicotine studies (Tables VIII and IX) when compared with the controls ($P > 0.05$).

On the other hand there is a significant difference between diet groups ($P < 0.001$), rats in the cocaine study on a poor basal diet were found to be consuming more alcohol than water compared to rats on a good basal diet. This was also observed in the nicotine study. Similar results were reported by Marsh(26), Emmerson (2) and Smithson (33) in

studies done in this laboratory.

As far as weight gain is concerned, rats in the cocaine groups showed no significant difference when compared to the controls ($P > 0.05$). In this same cocaine study rats on a poor basal diet gained significantly less weight compared to the rats on the good basal diet ($P < 0.001$). In the case of the nicotine study there was no significant difference between nicotine groups and the controls ($P > 0.05$). In this same study again rats with a poor basal diet showed significantly less weight gain than the rats on the good basal diet ($P < 0.001$). One thing that was noticed in the nicotine study while testing for weight gain was that interaction between diet and group was significant ($P < 0.001$). The group effect between nicotine and its control on the poor basal diet was not the same as the group on the good basal diet. The interesting thing to note is that rats in the nicotine group on the poor basal diet were gaining apparently more weight than the control, and the reverse occurs with rats on the good diet, (+ nicotine) where the controls were apparently gaining more weight.

Therefore a T test was done comparing those groups. No significant difference was seen between the nicotine group and its control on the good diet ($P > 0.05$). On the other hand there was a significant difference between the nicotine group and its control on the poor diet ($P < 0.05$). Rats injected with nicotine in this group gained more weight compared to their controls, although this is statistically significant,

the difference between these last two groups is not of practical importance. In laboratory work if the difference is less than five grams, its not considered of practical significance.

We know that nicotine has many detrimental effects, it would be interesting to measure the alcohol dehydrogenase in rats given nicotine, to see if it is inhibited as in the yeast cells, and to what extent. If nicotine to a certain degree inhibits alcoholic dehydrogenase, this may be the reason why rats on nicotine don't drink more alcohol than the controls. However, previous observations in humans indicate that the more a person smokes the higher the tendency of alcohol consumption (30). We recognize that in this study a large amount of nicotine was injected into the rats at once, while the smoker gets the drug at different intervals.

In the case of the cocaine study, it would be interesting to test cocaine using different doses, and dividing the daily dose in such a way that it would be given several times a day instead of only once, as it was in the case of our study. Also cocaine could be given orally instead of being injected, since that is the way that the Indians in South America consume the drug. Of course in this case many difficulties will have to be overcome.

CHAPTER IV

SUMMARY

The effect of cocaine, nicotine and diet on voluntary alcohol consumption in male Sprague-Dawley rats was studied. Alcohol and water intakes were measured weekly and reported as ml of a 10% solution of alcohol or water ingested per 100 grams of body weight. Also weight gain was recorded. A five week pre-experimental period was designed to determine individual tendencies to drink and thus to eliminate the high and low drinkers from the study. Following this period, the experimental phase was run for five weeks.

No difference was found in alcohol intake when either cocaine or nicotine was injected intraperitoneally into the rats ($P > 0.05$). However rats on a poor basal diet were ingesting significantly more alcohol than water and gained less weight than rats on a good basal diet ($P < 0.001$).

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EFFECTS OF COCAINE , NICOTINE AND DIET ON VOLUNTARY ALCOHOL CONSUMPTION IN RATS

by

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in the Field of Nutrition

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ABSTRACT

The consumption of alcohol is a problem not only in this country (U.S.), but also in many other places in the world. Thus we find the Indians in Peru, Bolivia and other neighboring countries (Ecuador and northern part of Argentina) consuming alcohol to the point that they become alcoholics, in addition to this they also chew coca leaves, plant from which cocaine is extracted.

In an effort to establish the cause for this behavior, we put Sprague-Dawley albino rats on either a poor or good basal diet. Once every other day the rats were injected with a solution of cocaine.

From the results obtained by statistical analysis we conclude, that cocaine at the level used does not affect the intake of alcohol in rats. Diet as shown previously does make a difference. Rats on the poor basal diet tended to drink however more alcohol than water, and gained less weight than rats on the good basal diet.

Since we know that these people (Indians) have a deficient diet, we could assume that alcohol may be one of their sources of obtaining the calories they need. In this study we see that cocaine does not increase the intake of alcohol, this could be due to its well known anorectic effects, as the Indians themselves state that this is one of the reasons they chew coca leaves.

The other part of this study is concerned with nicotine. Since many smokers also drink, the effects of this drug upon alcohol consumption were tested under the same conditions as cocaine. From our study we found that injection of nicotine into the rats did not affect alcohol consumption. Also in this case rats on the poor diet tended to drink more alcohol than water, gaining less weight than rats on the good diet.