$$C = \frac{\text{dose}}{V_1}\left[\frac{k_{31}(k_{20}+k_{21}-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t} + \frac{k_{31}(k_{20}+k_{21}-\alpha)}{(\gamma-\alpha)(\beta-\alpha)}e^{-\alpha t} + \frac{k_{31}(k_{20}+k_{21}-\gamma)}{(\gamma-\beta)(\alpha-\beta)}e^{-\beta t}\right]$$

Intravenous administration:

$$C = \frac{\text{dose } F_L}{V_1}\left[\frac{(k_{30}+k_{31}-\gamma)(k_{20}+k_{21}-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t} + \frac{(k_{30}+k_{31}-\alpha)(k_{20}+k_{21}-\alpha)}{(\gamma-\alpha)(\beta-\alpha)}e^{-\alpha t} + \frac{(k_{30}+k_{31}-\beta)(k_{20}+k_{21}-\beta)}{(\gamma-\beta)(\alpha-\beta)}e^{-\beta t}\right]$$

In these equations, γ, α, and β are the roots[1] of the transformed equation

$$0 = (s+\gamma)(s+\alpha)(s+\beta)$$
$$= (s+k_{30}+k_{31})(s+k_{10}+k_{12}+k_{13})(s+k_{20}+k_{21}) - k_{12}k_{21}(s+k_{30}+k_{31}) - k_{13}k_{31}(s+k_{20}+k_{21})$$

[1] When hypothetical values of the microconstants based on their physiological components are substituted into the transformed equation, the roots may be determined by iteration of synthetic division. Decreasing values of s (0 to $-\infty$) are iterated with a programmable calculator until all three values of s that satisfy the equation, $F(s) = 0$, are found. Unfortunately, the presence of k_{20} and k_{30} in the model precludes the calculation of the microconstants from actual data. In the three-compartment model of Gibaldi and Perrier (6), the elimination of a drug is represented solely by k_{10}. Thus, their method would overestimate the values of k_{31}, k_{21}, and k_{10} and would provide no adequate way of estimating k_{20} or k_{30}. However, simulations are still possible by substituting reasonable numerical values of the various microconstants into the equation.

Biological Effects of Nonalkaloid-Containing Fractions of *Erythroxylon coca*

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Abstract □ Water soluble nonalkaloid fractions of *Erythroxylon coca* were screened in mice for their effects on oxygen utilization and central nervous system (CNS) activity. The fractions were screened in dogs for cardiovascular, blood glucose, and respiratory changes. No CNS effects were demonstrated in mice; however, there was a reduction in the oxygen utilization rate. Intravenous administration of the extract to dogs produced hyperglycemia, a reduction in heart rate, and a decrease in blood pressure. No substantial change in the respiratory rate and tidal or minute volumes were observed.

Keyphrases □ *Erythroxylon coca*—biological effects of nonalkaloid-containing fractions □ Nonalkaloids—fractions in *Erythroxylon coca*, effects □ Oxygen utilization rate—effects of *Erythroxylon coca* on oxygen utilization rate, mice, dogs □ CNS activity—effect of nonalkaloid-containing fractions of *Erythroxylon coca*

The plant *Erythroxylon coca* has been used in the cultures of many Latin American societies for medicinal, nutritional, and religious purposes for centuries (1). The pharmacological and possible psychological effects of coca have made its use by natives commonplace. It is reported to aid them in performing strenuous work and in coping with the harsh mountain environment. While cocaine is not the only biologically active compound in the coca plant, it is the principal alkaloid and the most studied of the active compounds. Alkaloid free fractions of the coca leaf extracts recently have been shown to reduce food consumption but do not show alterations in locomotor activity (2). Cocaine, at doses ranging from 3.45 to 27.6 mg/kg, produced dose-related increases in locomotor activity and decreases in food consumption (3).

It has been reported that chewing coca leaves causes the Latin American native to be more resistant to cold and fatigue and decreases the need for food and sleep (4, 5). Most of these effects have been considered to be related to cocaine, since cocaine has been shown to increase the heart rate and blood pressure and to elevate blood glucose levels (6).

The present study was undertaken to determine whether cocaine-free extracts would produce changes in oxygen utilization, blood glucose levels, respiration, and/or cardiovascular effects that could be related to the alleged enhancement of physical stamina in humans.

EXPERIMENTAL

Test Compounds—Coca leaves (*Erythroxylon coca*)[1] were obtained from Tingo Maria, Peru, and extracted as described previously (2). Briefly, the crude ethanol extract of coca leaves was partitioned between water (fraction A) and chloroform (fraction B). Fraction A was made cocaine free by dissolving in ammonium hydroxide solution and extracting 10 times with chloroform. Each chloroform fraction was subjected to GLC analysis. These fractions each showed the absence of cocaine peaks. Fraction A was further partitioned between butanol and water yielding two fractions: fraction C, the butanol phase; fraction D, the water phase. The fractions were dried on a rotary evaporator at 40°.

All test solutions were prepared in distilled water from dried fractions immediately prior to testing.

Test Animals—The mice were male ICR Swiss, weighing 26–30 g[2]. They were housed in shoe box caging on pelleted corncobs[3] with food[4] and water freely available. Food was withheld overnight prior to testing. The mice were maintained in an environment of 21 ± 1° and a 12 hr light–dark cycle.

[1] The plant material was obtained through Mr. Ing Alberto Trelles Barnett, Impresa Nacionale de la Coca, Lima, Peru, and through the United States Department of Justice and the United States State Department. It was identified as *Erythroxylon coca* by Dr. M. W. Quimby, Department of Pharmacognosy. Voucher specimens are stored in the drug plant herbarium at the School of Pharmacy, University of Mississippi, University, MS 38677.

[2] Harlan Industries, Cumberland, Ind.

[3] San-i-cel, Paxton Processing, Paxton, Ill.

[4] Purina Laboratory Chow 5001, Purina Mills, St. Louis, Mo.

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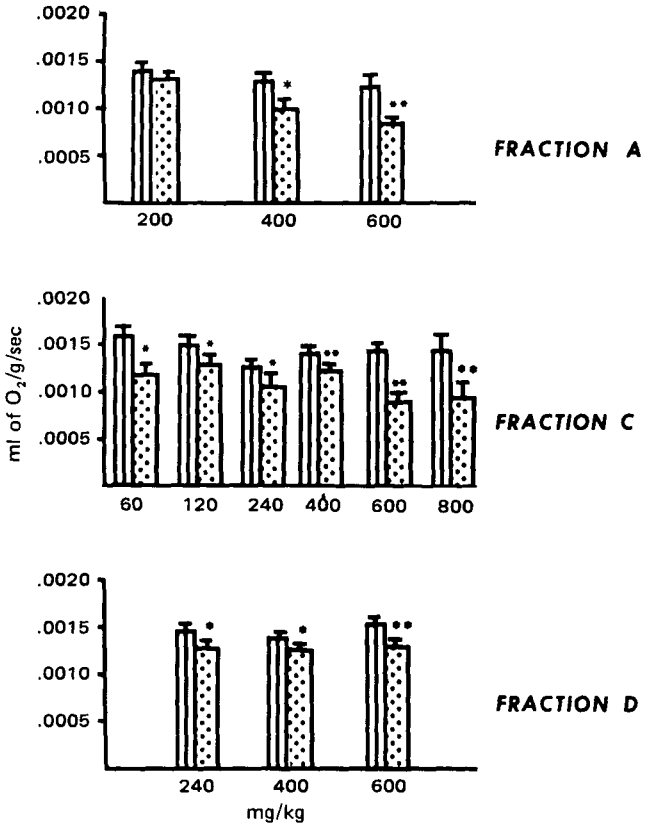

Figure 1—*Comparison of the effects of fractions A, C, and D on oxygen utilization in mice. Key: vertical bars represent* SEM (n = *12); (▥) vehicle; (∴) drug.* * p < *0.05.* ** p < *0.01.*

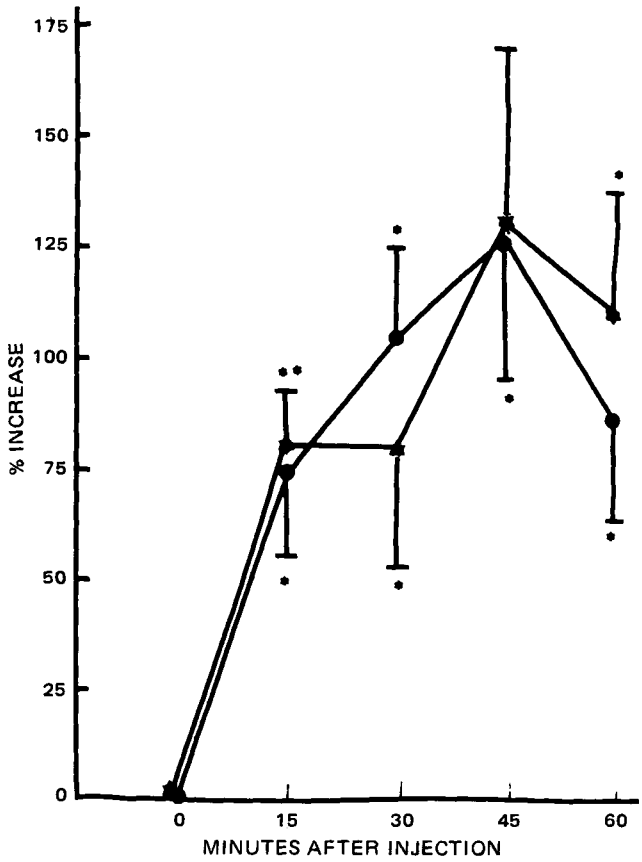

Figure 2—*Mean percent increases of plasma glucose levels in dogs following a single dose of 200 mg/kg, iv of fraction C (anesthetized* n = *4; conscious* n = *6). Key: vertical bar represents* SEM; *(★) anesthetized; (●) conscious.* * p < *0.05.* ** p < *0.01.*

The dogs[5] were conditioned, mongrel, adult males weighing 10–12.5 kg; vaccinated against distemper, hepatitis, and leptospirosis[6]; checked for internal parasites; and treated[7] as necessary. All dogs were free of heartworms and had two normal hemograms over the 3-week conditioning period.

Oxygen Utilization Screen—The oxygen utilization apparatus consisted of three widemouth 946 ml jars glued[8] to a 25 × 40 × 1.5-cm aluminum platform. Pelleted corncob bedding was placed in the bottom 2–3 cm of each jar to absorb urine. The platform and jars were placed in a constant temperature water bath[9] with water covering ~75% of the outer jar to maintain the temperature at 25°. This was done to minimize temperature change inside the jar during testing. A 4-cm piece of copper tubing (7-mm o.d.), fitted with a 3-way stopcock, was attached to the lid of the jar. This allowed the addition of air to the jar as well as attachment to a pressure transducer without breaking the jar's seal. A 7 × 7-cm nylon net bag filled with soda lime was suspended from the underside of the lid to absorb the carbon dioxide produced by the mouse. Ten mice were dosed intraperitoneally with either drug or vehicle. Five minutes after dosing, the mice were placed in the jar and the chamber was sealed. Twenty milliliters of air was added to the chamber and the resulting air pressure and the time required to return to baseline were recorded[10]. This allowed the determination of the amount of oxygen per gram per second utilized by the mouse using the following:

$$\text{ml } O_2/\text{g/sec} = \frac{20\ (\text{ml } O_2)}{\text{time (sec)} \times \text{wt of mouse (g)}} \qquad \text{(Eq. 1)}$$

Open Field Locomotor Screen—Locomotor activity was measured in an open field arena which consisted of 1 m^2 box with the floor marked off into 25 squares. The top was open and lighted by an overhead fluorescent lamp for better observation. Open field sessions were 5 min long and began 5 min following intraperitoneal injections. Treated and vehicle controls were run alternately to erase diurnal variation. Activity was measured by counting the number of squares the mouse entered during the test period. Statistical analysis was accomplished using the Mann-Whitney nonparametric test (7).

Respiratory and Cardiovascular Screen—The dogs used in the respiratory, cardiovascular, and blood glucose studies were anesthetized with pentobarbital (30 mg/kg). The femoral artery and vein were cannulated and a tracheotomy performed. Electrocardiograph[10] (ECG) (lead II), femoral arterial blood pressure[10], and respiratory parameters[11] (rate, tidal volume, minute volume, and oxygen uptake) were recorded. Arterial blood pO_2, pCO_2, and pH were measured at 15-min intervals[12].

Blood glucose levels[13] were determined at 15-min intervals on venous blood. Control parameters were recorded, then a single dose of fraction C was administered (200 mg/kg, iv). Blood pressure and heart rate were monitored constantly, while other samples and measurements were taken at 15-min intervals. Statistical evaluation of the data was performed using Student *t* test (8).

RESULTS AND DISCUSSION

In the partitioning of fraction A, ~26% of the starting material is removed in the butanol fraction. Five major and five minor constituents were detected in the butanol fraction by TLC.

The effects of fractions A, C, and D on the rate of oxygen utilization are shown in Fig. 1. Fraction A reduced the rate of oxygen utilization at doses ranging from 400 to 600 mg/kg, while fraction C reduced oxygen utilization at doses ranging from 60 to 800 mg/kg. Fraction D reduced the rate of oxygen utilization at 240 mg/kg, but lower doses were not tested. Partitioning fraction A between butanol and water successfully separated all other pharmacological activity into the butanol phase (fraction C).

Oxygen utilization rates are of interest since South American natives claim that chewing coca improves their ability to perform strenuous work, especially at high altitudes (1). Even though a decreased locomotor ac-

[5] Obtained from Memphis Animal Shelter, Memphis, Tenn.
[6] Decline-HL, Dellan Labs, Omaha, Neb.
[7] Telmintic, Pitman-Moore, Inc., Washington Crossing, N.J.
[8] Expoxe, Balkamp Ind., Indianapolis, Ind.
[9] Labline Instruments, Melrose Park, Ill.
[10] RM Dynograph, Beckman Instruments, Schiller Park, Ill.
[11] Collins Respirometer, Warren E. Collin, Inc., Braintree, Mass.
[12] Model 213 pH Blood Gas Analyzer, Instrumentation Laboratory, Lexington, Mass.
[13] Glucose Analyzer, Beckman Instruments, Schiller Park, Ill.

Table I—Effect of Fractions C and D on Open Field Activity in Mice

Compounmd	Dose	Counts/5 min ± *SEM*
Vehicle	0.5 ml	150.45 ± 12.0
Fraction C	200 mg/kg	117.8 ± 8.86[a]
	400 mg/kg	51.0 ± 9.25[b]
	600 mg/kg	42.1 ± 9.99[b]
Vehicle	0.5 ml	129.1 ± 16.1
Fraction D	200 mg/kg	138.2 ± 14.5
	400 mg/kg	175.20 ± 23.44
	600 mg/kg	179.60 ± 25.80

[a] $p < 0.05$. [b] $p < 0.001$.

tivity would decrease oxygen utilization, gross observation (9) of activity showed no difference between animals treated with fraction A and control animals. Additionally, fractions C and D produced a decrease in oxygen utilization (Fig. 1) while only fraction C produced a decrease in activity in the open field experiment (Table I). These data would indicate that the decrease in locomotor activity was not the only cause of decreased oxygen consumption.

A potent hyperglycemic effect was seen both in the anesthetized and conscious dogs treated with 200 mg/kg of fraction C. Blood glucose levels reached their peak 45 min after dosing and began to decline after 60 min (Fig. 2). This could explain a ready energy source which would improve the short-term ability to do work and alleviate hunger. The blood glucose levels begin to decline in 1 hr. This is the approximate length of time that chewing of coca leaves is reported to give stimulatory effects. The increased blood glucose is most likely due to a direct effect on glucose metabolism since no sugars remain in fraction C after extraction and separation. Therefore, it would seem that prolonged use of coca, by people on energy deficient diets, could lead to a rapid loss of the body's energy stores. However, histologically[14] no effect was observed on liver glycogen stores in mice 30 min postdose with 120 mg/kg of fraction C. These same mice showed significantly elevated blood glucose levels.

Since hard work or heavy exercise tends to increase cardiac output and mean arterial pressure, the sustained hypotensive effect and decrease in heart rate could be partially responsible for the enhanced work performance and endurance experienced by natives using coca.

Five minutes after injection of 200 mg/kg of fraction C, the heart rate of anesthetized dogs decreased from a mean of 200 to 125 bpm. The rate had not returned to baseline after a 60-min period (Fig. 3). Mean blood pressure dropped from 160 to 100 mm Hg concomitant with the initial fall in heart rate. Both systolic and diastolic pressures remained 20–30 mm Hg below baseline during the remainder of the test period (Fig. 4). There were no significant changes in oxygen uptake, tidal volume, minute volume, pO_2, pCO_2, or arterial blood pH; however, there was an initial increase in respiratory rate (panting). Electrocardiographic tracings

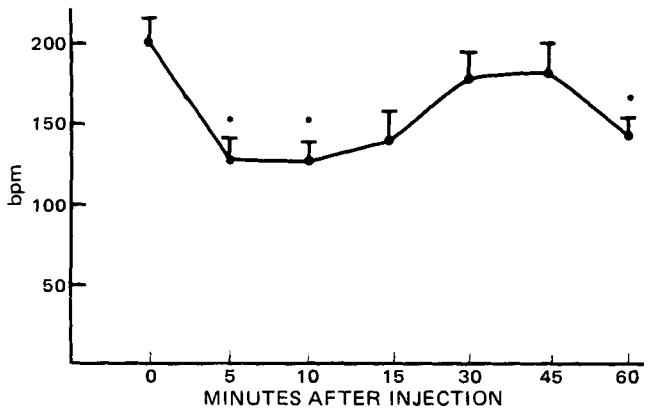

Figure 3—*Change in heart rate (beats per minute, bpm) of anesthetized dogs following a single dose of 200 mg/kg, iv of fraction C (n = 5). Vertical bar represents* SEM. * $p < 0.05$.

[14] Periodic acid schiff (Harleco) stain for glycogen, American Hospital Supply, Memphis, Tenn.

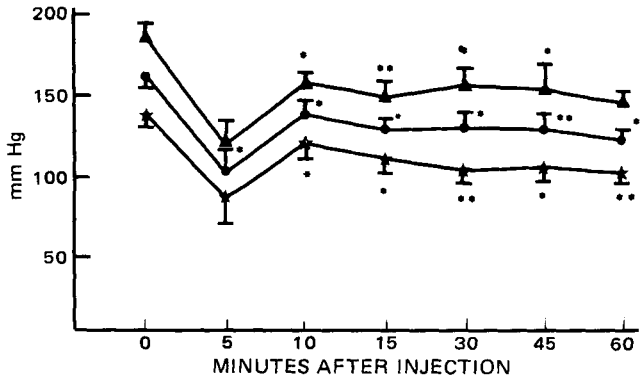

Figure 4—*Systolic, diastolic, and mean arterial blood pressure changes in anesthetized dogs following a single dose of 200 mg/kg, iv of fraction C (n = 5). Key: vertical bar represents* SEM; (▲), *systolic;* (★), *diastolic;* (●) *mean arterial.* * $p < 0.05$. ** $p < 0.01$.

frequently showed an inversion of the T wave, which returned to pretreatment polarity 20 min postinjection. Since pO_2 values remained stable during this period, the T wave inversion would not appear to be a hypoxic effect. No other ECG alterations were noted. No autonomic, CNS, hyperglycemic, or cardiovascular effects were obtained with fraction D.

A single 200-mg/kg dose, iv of fraction C given to conscious dogs produced a dramatic effect on the autonomic nervous system. Shallow, rapid panting and increased salivation, swallowing, and defecation began immediately after injection. Cyanosis and inactivity were produced and lasted ~1 hr postdose. These effects, exclusive of defecation, appeared to be masked in the anesthetized animals.

Vomiting, defecation, and inactivity are not problems reported by humans chewing the coca leaf. Chewing would slow intake of the drug and could modify most of the autonomic effects since the fraction would be absorbed over a longer period of time. It also appears that some compounds in the extract may be rapidly bound or metabolized since most effects return to normal levels within 10–30 min following a single injection. However, the increased blood glucose levels, lower blood pressure, and decreased heart rate remain, indicating that other compounds in the extract are longer acting.

The biological effects produced by noncocaine-containing coca fractions are not identical to those seen after cocaine administration. However, these data suggest that noncocaine-containing fractions may contribute to the beneficial effects seen in subjects chewing coca leaves.

REFERENCES

(1) E. Carroll, *Cocaine*, *NIDA Research Monograph*, **13**, 35–45 (1977).

(2) J. A. Bedford, H. N. ElSohly, G. L. Nail, M. C. Wilson, and C. E. Turner, *Pharmacol. Biochem. Behav.*, **14**, 725 (1981).

(3) J. A. Bedford, D. K. Lovell, C. E. Turner, M. A. ElSohly, and M. C. Wilson, *ibid.*, **13**, 403 (1980).

(4) C. Gutierriz-Noriega and V. Zapata-Ortiz, *Rueta Farmacol. Med. Exp.*, **1**, 1 (1948).

(5) M. F. Risemberg, *Rev. Med. Exp.*, **3**, 132 (1944).

(6) S. J. Mule', "Cocaine: Chemical, Social and Treatment Aspects," CRC Press, Cleveland, Ohio, 1976, p. 190.

(7) S. Siegal, "Nonparametric Statistics for the Behavioral Sciences," McGraw-Hill, New York, N.Y., 1956, p. 31.

(8) A. L. Edwards, "Experimental Design in Psychological Research," Holt, Rhinehart, and Winston, New York, N.Y., 1968, p. 81.

(9) M. W. Dunham, T. S. Miya, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 208 (1957).

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