13. The values for thermodynamic parameters are readily evident from the activation data given in Table 1. Thus, for oxygenation : enthalpy, $\Delta H_2^\circ = \Delta H_2^* - \Delta H_{-1}^*$; entropy, $\Delta S_2^\circ = \Delta S_2^* - \Delta S_{-1}^*$; free energy, $\Delta G_2^\circ = \Delta H_2^\circ - T\ \Delta S_2^\circ$.
14. A recent note (*15*) reports a calorimetric enthalpy for the reaction, $[O_2IrCl(CO)(Ph_3P)_2]$ (crystals) → $[IrCl(CO)(Ph_3P)_2]$ (crystals) + O_2 (gas), as − 22 kcal mole^{-1} at 25°C; according to this result, the oxygenation of the crystals by the gas is endothermic. Because of the low rates of the reversible oxygenations (Table 1), calorimetric measurements on our systems are infeasible (courtesy of Dr. W. Partenheimer). We have, however, determined the temperature dependence of the equilibrium constants for Cl, Br, and I directly from spectral data. The K_2 decrease with increasing temperature, in agreement with the kinetic data (Eq. 3).
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18. By a procedure outlined for the estimation of the Ir–H bond strengths in $[H_2IrA(CO)(Ph_3P)_2]$ (*10*). The basic assumptions in the present case are that the O–O bond energy decreases on coordination from 118 kcal (free O_2) to 63 kcal in $[O_2IrCl(CO)(Ph_3P)_2]$ and 49 kcal in $[O_2IrI(CO)(Ph_3P)_2]$ (*19*).
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24. The rate data (k_2 and k_{-1}) have been obtained by monitoring the intensity of an absorption band (378 to 397 nm) in the electronic spectrum of $[IrA(CO)(Ph_3P)_2]$ under the following conditions. (i) Oxygenation was followed under a constant pressure (150 to 750 mm-Hg) of O_2, depending on the complex and the temperature. The starting concentration of the complex was about $10^{-4}M$; in solution the ratio O_2 : Ir ranged from 4 to 50 in different experiments. The disappearance of $[IrA(CO)(Ph_3P)_2]$ was first order in the complex (K_{obs}); separate experiments showed that the reaction was also first order in O_2. Thus, $k_2 = k_{obs}/[O_2]$. Note that these reactions go essentially to completion; the reverse reaction is negligible under the conditions cited. (ii) Deoxygenation was followed in deoxygenated solutions of $10^{-4}M$ $[O_2IrA(CO)(Ph_3P)_2]$ (constant $Po_2 \sim 0$ mm Hg). The reaction (k_{-1}) was first order in the complex. The activation parameters have been calculated from the kinetic constants determined at 15° to 60°C. Maximum standard deviations are, for k_2, k_{-1}, and K_2, ± 2 percent; for ΔH_2^* and ΔH_{-1}^*, ± 1 kcal (except ΔH_{-1}^*, ± 2.6 kcal for I); for ΔS_2^*, ± 4 eu; for ΔS_{-1}^*, ± 1.5 eu.
25. Some of these reactions (Cl, Br, I) have also been studied by infrared spectrophotometric (*26*) and volumetric [(*4*), oxygenation only] methods. The results for oxygenation (*4, 26*) agree, in general, with those given here, but the data for deoxygenation (*26*) differ from and appear to be less reliable than the present results.
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1 July 1971

Marihuana: Standardized Smoke Administration and Dose Effect Curves on Heart Rate in Humans

Abstract. *A spirometer was used to deliver marihuana and placebo smoke to human subjects. This procedure produced linear dose-effect curves on heart rate and replicable dose effects in individual subjects. No differences were observed between experienced and inexperienced smokers in responsiveness to heart rate increases produced by marihuana.*

Marihuana research in humans has been difficult to evaluate because of conflicting results (*1*). A major problem complicating the comparability and replicability of studies has been the lack of a standard way of administering doses of marihuana (*2, 3*). Smoking marihuana cigarettes introduces at least two major sources of error: first, considerable and indeterminable amounts of smoke are lost to the air, and second, there is no way of determining the actual amount of smoke inhaled by the subject. Giving marihuana orally carries no assurance that the same substances pharmacologically active in smoke are being administered (*2*).

In an effort to overcome these problems, we have developed a system to deliver a measured quantity of smoke to a subject. Using change in heart rate as the measured effect, we investigated various dosages of marihuana to determine the efficiency of this delivery system.

Ten subjects were used, four inexperienced with marihuana, and six experienced smokers. An experienced smoker is defined as one who is currently engaged in smoking marihuana at least once a week. Three of the inexperienced subjects had never had any contact with marihuana before, and one had smoked marihuana three times 6 months before. All subjects were experienced tobacco smokers.

The subjects were all males. Nine were between 24 and 30 years of age, and one inexperienced smoker was 45. They were all judged to be in good health on the basis of routine medical history, physical examination, complete blood count, urinalysis, chest x-ray, and psychiatric examination.

All subjects were advised of the nature of the experiment and that both marihuana and placebo would be administered to them. They were also advised of the possible dangers of marihuana before they signed forms consenting to be subjects (*4*).

The marihuana and placebo were obtained from the National Institute of Mental Health. The marihuana was assayed to contain 1.5 percent Δ-9-tetrahydrocannabinol. Placebo had been commercially prepared by multiple extractions with alcohol to remove most of the Δ-9-tetrahydrocannabinol. Dosages of marihuana administered were the smoke from the total combustion of 62.5 mg, 125 mg, 250 mg and 435 mg of marihuana. The doses of placebo were equivalent to those of marihuana, but since all produced similar reactions, they were combined for purposes of our analysis.

The basic apparatus consists of a 12-liter spirometer and a machined aluminum crucible or pipe attached to the tubing so that as the inside bell of the spirometer is raised, air is drawn through the crucible into the spirometer. When marihuana, suitably chopped for burning, is placed in the crucible and ignited while air is being drawn through it, all of the smoke produced is drawn into the apparatus. Since the spirometer collapses to only half its size, the smoke is diluted by one-half with air. Once combustion is complete, the aluminum crucible is quickly disconnected, and the subject, with respiratory mask in place, is connected to the spirometer and inhales the smoke from it. Essentially, this is a closed, partially collapsible system which contains a fixed amount of smoke, and from which no smoke is lost into the atmosphere. The subject receives the same amount of smoke each time he empties the spirometer. The dose can easily be changed by varying the amount of marihuana burned, resulting in different concentrations of the smoke. Placebo can be administered in the same fashion, insuring the possibility of a double blind for any given dose.

Other variables in administering smoke are the duration of inhalation and the interval between inhalations. These variables were brought under stimulus control by instructing the subject to breathe according to a series of four lights. (i) A "ready" light signals the approaching cycle for 5 seconds. (ii) An "inhale" light comes on for 5 seconds, during which the subject inhales continuously. (iii) This is followed by the "hold" light, during which the subject holds the smoke in his lungs. This has a 15-second duration, and, during the breath-holding, the technician turns a valve closing the spirometer connection and opening the

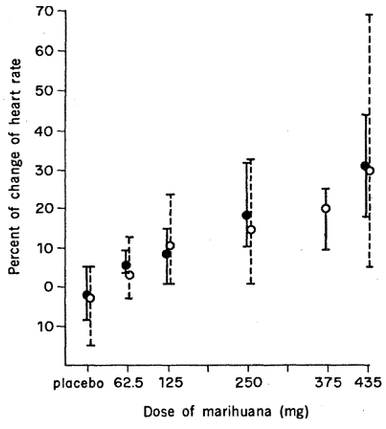

Fig. 1. Heart rate response to placebo and marihuana, expressed as percent of change from the baseline heart rate. Open circles and dotted lines, mean and range, respectively, of heart rate changes of experienced subjects. Solid circles and solid lines, mean and range of heart rate changes of inexperienced subjects.

respiratory pathway to the atmosphere. (iv) Thus, when the "exhale" light comes on, the subject empties his lungs into the atmosphere and breathes room air as he wishes for 35 seconds, after which the "ready" light signals the start of a new cycle. This closed system and the instructional control insures greater reliability in the dosage of marihuana than can be achieved with conventional smoking.

Since the system collapses to only one-half its size, the amount of inhaled smoke can be only one-half of the amount of marihuana combusted. For example, after the smoke from 500 mg of marihuana is drawn into the 12-liter spirometer, the subject can remove only 6 liters, or the equivalent of the smoke from 250 mg. A 250-mg smoke equivalent remains in the residual 6 liters. We have found that the spirometer can be rapidly refilled with room air and the subject can then take in an additional 6 liters, or one-half of the residual, or the equivalent of 125 mg of marihuana. In these two installments, a total dose of 375 mg can be thus administered. We have repeated this process still once more for a total dose of approximately 435 mg.

Most subjects can take in the 6 liters in three inhalations, or 3 minutes (1 minute for each cycle). This means a total of 9 minutes to administer the maximal 435-mg dose.

Subjects reported to the laboratory for 3 hours at weekly intervals. For each experiment, subjects had electrodes placed on their chests, and these were connected by cable to an E & M Physiograph in another room where the electrocardiogram and respiratory and tachygraphic tracings were recorded. An experimenter could monitor the subjects by closed-circuit television to note movements or other artifacts on the record. During the first 30 minutes of each session, baseline heart rate, electrocardiogram, and respiratory tracings were collected. A stable 10-minute segment of this baseline was used to calculate the percentage change in heart rate produced by the marihuana or placebo administered during that session.

Figure 1 shows the percentage change in heart rate as a function of marihuana dosage graphed separately for naive and experienced subjects. Over the dose range tested, heart rate can be observed to increase linearly as a function of dosage of marihuana. This is most evident when mean values are compared. As indicated on the graph, the measured effect is the change of rate compared to baseline values. This is expressed as a percentage change to compensate for the week-to-week variations in baseline. Each point on the graph was arrived at by taking the average of the minute-to-minute heart rate between 10 and 20 minutes after the beginning of administration of marihuana. The difference between this average and that of the same 10-minute segment of baseline was then divided by the average of that same 10-minute segment of baseline to obtain the final percentage change graphed in the figure. The period from 10 to 20 minutes after the beginning of administration of drug was selected for heart rate measurement because marihuana was found to have a definite and sustained effect on heart rate during that time segment.

No differences were found between inexperienced and experienced smokers in relation to heart rate increases produced by marihuana. Placebo produced no heart rate increase.

The variance between subjects in their responsiveness to marihuana was remarkably great and this accounts for the wide range at each dose as seen in Fig. 1. However, individual subjects showed linear increases in heart rate as a function of marihuana dosage and little variability in their response to a given dose of marihuana from one session to another.

In studying the interbeat interval, we observed a consistent effect of marihuana on cardiac rhythm. The most direct effect is the suppression of the normal sinus arrythmia. Since this nonspecific response is mediated by the vagus, it suggests that marihuana may have its effects on heart rate by altering normal autonomic tone. The diminution of sinus arrythmia was not attributable to changes in respiration.

In order to further study the effects of marihuana on autonomic tone, several subjects were asked to perform Valsalva maneuvers every 60 seconds during baseline and after various doses of marihuana. In short, marihuana suppressed the cardiac slowing during the Valsalva maneuver. This response was completely obliterated during peak effects at very high doses. Figure 2 shows a series of tachygraphic recordings at baseline and after the beginning of inhalation of the smoke from 435

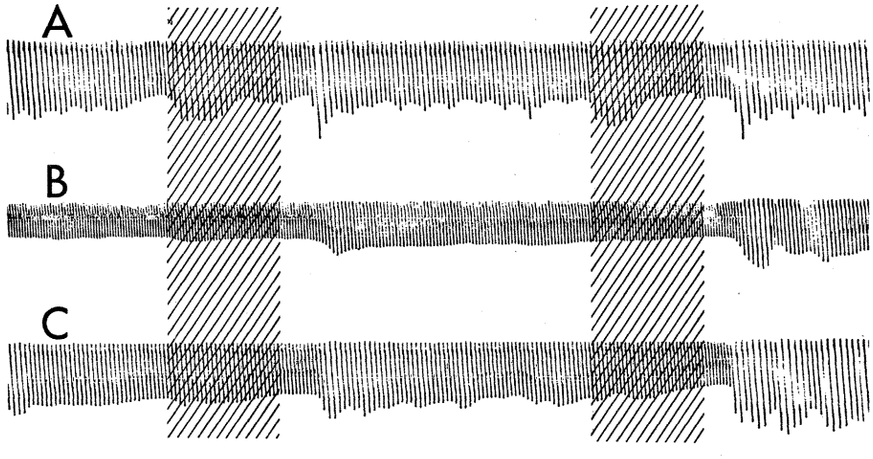

Fig. 2. Effect of marihuana on Valsalva maneuver. Cross-hatched areas are the period of inspiration and breath-holding. Cycle length is 1 minute. *A* is a baseline tracing. *B* was taken 10 minutes after administration of marihuana smoke was begun. *C* was taken 50 minutes later.

mg of marihuana. The vertical shaded areas indicate the period of breath-holding. Tracing A is baseline and it shows the typical cardiac slowing and gradual return. There is the additional slowing or "overshoot" for a few beats with exhalation, and this is followed by normal sinus arrythmia. Tracing B is a peak effect of 150 beats per minute at its height (90 at its lowest point). This was taken 10 minutes after administration of marihuana began. Clearly there is no response during Valsalva maneuvers; however, pulse slowing after exhalation does persist. Tracing C was taken 50 minutes after the dose and has an average pulse rate of about 100 beats per minute. The response during Valsalva maneuvers is still blocked, but the slowing of the pulse after exhalation is markedly enhanced. This is prolonged and at times appears to be followed by sinus arrythmia.

The maximum heart rates obtained in response to large doses of marihuana were in the range of 140 to 160 beats per minute. These rates correspond to heart rates seen in the absence of vagal tone and further suggest that marihuana alters heart rate by altering autonomic tone.

In conclusion, we have been able to use a spirometer to deliver reliable quantities of marihuana and placebo smoke to human subjects. The reliability of this procedure is attested to by the production of linear dose-effect curves and the replicability of dose effects in the same subject from one session to another. Using this system of administration of marihuana smoke, we observed no differences between experienced and inexperienced smokers in responsiveness to heart rate increases produced by marihuana.

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4 June 1971; revised 5 August 1971

Immunotherapy of Cancer: Immunospecific Rejection of Tumors in Recipients of Neuraminidase-Treated Tumor Cells Plus BCG

Abstract. *Firmly established methylcholanthrene fibrosarcomas in syngeneic mice will totally disappear if the hosts are treated with living tumor cells that have been exposed to* Vibrio cholerae *neuraminidase in vitro. The effect is magnified by the simultaneous injection of a nonspecific immunostimulant, BCG. The rejection of the methylcholanthrene tumor is immunospecific and can be induced only with tumor cells, treated with* Vibrio cholerae *neuraminidase, identical in type with the growing tumor.*

A number of techniques can be used to immunize animals to syngeneic tumors but only two immunological techniques have been successful in inducing the regression of firmly established, growing, solid tumors in syngeneic hosts—(i) the intralesional injection of living BCG organisms (*Mycobacterium bovis*, strain BCG) (*1*) and (ii) the inoculation of living tumor cells treated in vitro with *Vibrio cholerae* neuraminidase (VCN) (*2*). The present studies demonstrate that there is a synergistic action between BCG and tumor cells treated with VCN in inducing immunospecific regression of firmly established, solid-tissue, methylcholanthrene fibrosarcomas.

Two different fibrosarcomas (MC-42, MC-43) were induced with 3-methylcholanthrene in C3H/HeJ female mice (*3*). The tumors have been serially transplanted in syngeneic female mice without evidence of loss of antigen. In unimmunized mice, an inoculum of 120 MC-42 cells or 1280 MC-43 cells will kill 50 percent of the recipients. Spontaneous regression does not occur. The tumors are specific for the strain in which they arose and do not grow in allogeneic mice. These tumors are weakly immunogenic, that is, inoculation of the tumor followed by amputation leads to tumor-specific resistance against subsequent inoculation. The MC-42 tumor, however, does not immunize against the MC-43 or vice versa (*3*).

Suspensions of sterile tumor cells were prepared by pressing the tumor through successively smaller stainless steel screens in medium 199 (M199). No trypsin was utilized at any time. Viable cells (20,000) were injected subcutaneously into the lateral posterior flank of recipient mice. The mice were inspected daily to determine the day of appearance of the tumor, the largest diameter of a growing tumor was measured with calipers at biweekly intervals, and the day of death was recorded. The tumors had usually reached palpable size by day 8 and measured 0.5 to 1.0 cm by day 15 when challenge with BCG (*4*) plus cells treated with VCN (*5*) was started. Suspended tumor cells for the challenge were incubated for 1 hour with 25 units of VCN (*5*) per milliliter per 10^6 cells plus mitomycin C (25 μg/ml) to prevent the growth of the tumor challenge. The cells were then washed three times, mixed with BCG or M199, and inoculated at sites distant from the growing tumor. The BCG (*4*) was either mixed with the tumor cells (1 : 1) or injected alone into the tumor nodule itself, or at another site. A single injection of BCG, or MC-42 cells exposed to heat-inactivated VCN (*5*), plus mitomycin C, or any combination of these treatments did not produce total regression in any of the firmly established MC-42 tumors. In contrast, a single injection of MC-42 tumor cells exposed to VCN plus mitomycin induced the regression of 2 out of 7 tumors. When the VCN-treated tumor cells were injected in combination with BCG, 16 out of 56 tumors totally regressed. The site of the BCG injection had some influence on the response of the tumors. If the BCG was injected directly into the tumor mass and the VCN-treated tumor vaccine was injected elsewhere, only 1 out of 14 tumors regressed. However, if the BCG was injected at a separate location from the injection of VCN tumor vaccine, or mixed with VCN tumor, 15 out of 42 tumors totally regressed and the animals survived indefinitely. Mitomycin-treated tumor cells never produced palpable tumors.

This experiment was repeated with the use of a total of six injections of BCG or 10^6 tumor cells, or both, on alternate days beginning 15 days after the inoculation of 20,000 normal MC-42 tumor cells. Regression of the firmly established tumor was never induced in animals that received no treatment or in animals who received tumor cells that had been incubated with heat-inactivated VCN, even if those injections