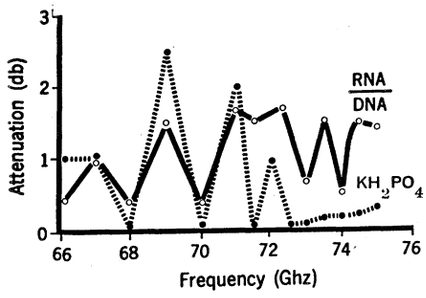

Fig. 5. The differential microwave absorption spectrum at 90 percent relative humidity of isolated RNA and DNA and the absorption spectrum of KH_2PO_4.

it was not exactly the same (Fig. 5). No difference in the absorption spectra of RNA isolated from normal and tumor cells was observed, but extracted tumor DNA, free of RNA (*7*), did exhibit increased attenuations at 69, 71, and 75 Ghz over normal DNA. The magnitudes of these increases, however, were not as great as those observed with intact cells.

The attenuation of microwaves within the frequency band used seems to be due largely to the rotation of water molecules adsorbed to certain groups of macromolecules. Since the makeup of water lattices should reflect the structure of macromolecules, microwave spectroscopy may allow the structure of in vivo individual or complexes of macromolecules to be determined. The results obtained suggest that attenuation between 66 and 76 Ghz is due to the rotation of water molecules adsorbed to P=O, C–O–[P], C–O–C, and sugar hydroxyl groups and, therefore, the absorption spectrum in this frequency band appears to reflect the hydration patterns of nucleic acids within the cell. Since a difference between spectra of RNA isolated from normal and from tumor cells could not be detected, the increased attenuation by tumor cells at 69, 71, and 75 Ghz was thought, at first, to be due to an increase in chromosome number or quantity of DNA per cell. This was ruled out, however, because differences were still apparent after all the spectra of the cells were normalized to the attenuation at 67 Ghz, a frequency attenuated equally by tumor and by normal DNA, and their component parts. There seem to be two possible explanations, therefore, for these results. (i) The RNA/DNA ratio in tumor cells may be different from that in normal cells and (ii) the RNA/DNA ratio may be the same in both cell types but the quantities of specific kinds of macromolecular groups are different. The former supposition does not seem to account fully for the results obtained, for, if it did, the differential absorption of tumor over normal cells at 73 and 74 Ghz would be expected to be less than unity (Fig. 5), not greater than unity as was found (Figs. 1 and 2). Therefore, both factors seem to play some role in the differences observed.

As far as the types of groups involved in these phenomena are concerned, the differential spectra of tumor over normal cells suggests that the former cell type has fewer P=O groups and more C–O–[P], C–O–C, or sugar hydroxyl groups than the latter. From infrared spectroscopy, the DNA extracted from tumor cells appears to be a much more rigid structure than normal DNA in that it does not have the same flexibility to alter its structure when its hydration lattice is changed (*7*). One way in which extra rigidity together with a decrease in P=O and an increase in C–O–[P], C–O–C, and sugar hydroxyl groups could occur is by small molecules, or even extra pieces of either RNA or DNA, becoming covalently bonded to the P=O groups present in one of the outer grooves of normal double-stranded DNA molecules.

S. J. WEBB

Department of Bacteriology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

A. D. BOOTH

Department of Electrical Engineering, University of Saskatchewan

References and Notes

1. D. E. Dodds, thesis, University of Saskatchewan, Saskatoon, Canada (1968); S. J. Webb and D. E. Dodds, *Nature* **218**, 374 (1968).
2. S. J. Webb and A. D. Booth, *Nature* **222**, 1199 (1969).
3. E. M. Bradbury, W. C. Price, G. G. Wilkinson, *J. Mol. Biol.* **3**, 301 (1961).
4. M. Falk, K. A. Hartman, R. C. Lord, *J. Amer. Chem. Soc.* **85**, 387 (1963).
5. S. J. Webb and M. D. Dumasia, *Can. J. Microbiol.* **14**, 841 (1968).
6. S. J. Webb and J. S. Bhorjee, *Can. J. Biochem.* **46**, 691 (1968).
7. S. J. Webb and R. Bather, *Phys. Med. Biol.* **15**, 271 (1970).
8. We thank the National Research Council for supplying some of the microwave components and J. S. Webb for technical assistance.

29 April 1971

Morphine Tolerance and Dependence Induced by Intraventricular Injection

Abstract. *Injection of small quantities of morphine into the cerebral ventricular system of awake, relatively unrestrained, monkeys depressed or abolished operant food-reinforced lever pressing. After repeated injections progressively higher doses of morphine were needed to depress responding. Also, dependence could be demonstrated in these animals by precipitating specific abstinence signs with an antagonist.*

Repeated administration of opiate narcotics results in tolerance and dependence. The first is defined usually as a progressive diminution of the effects of successive doses of a drug or, conversely, the need for gradual increases in drug dosage to maintain the original intensity of effects. Dependence is defined by its consequence: the development of a specific physical abstinence syndrome following withdrawal of the agent. This syndrome is readily abolished by readministration of the drug or its surrogates (*1*). While it is generally agreed that most of the functional changes leading to these phenomena probably develop within the central nervous system (*1*), neither the identity of the biochemical disturbances responsible, nor their localization in neural structures, is yet clearly established (*2*). One of the reasons for the relative lack of knowledge concerning these two problems is that most investigators have used methods of narcotic administration (oral, subcutaneous, intravenous, and so forth) which make the entrance of the drugs into brain tissue contingent upon its uptake into extracerebral tissues first, and then upon the uptake properties of the "blood-brain barrier."

We have carried out a series of experiments in monkeys with cannulas implanted permanently in their cerebral ventricular system. Morphine—the prototype of the narcotic opiates—and certain antagonists (*3*) were thus mixed with the cerebrospinal fluid bathing the inner and outer surface of the brain. Our original purpose was to devise a method for efficiently conveying morphine to the brain without the technical difficulties of prolonged vascular catheterization. Soon it became apparent that this approach was not only satisfactory in that regard but that tolerance and dependence developed in these animals

after repeated administration of minimal amounts of the drug. Our subjects were six young stump-tailed macaques (*Macaca speciosa*), of both sexes, and of weights ranging between 3.5 and 5.0 kg. They had received no drugs of any kind previously. They were implanted with cannulas, made of 23-gauge hypodermic needle tubing, placed with their tips in the body of the lateral ventricles. These cannulas were affixed to the skull surface and to a protective fitting by expansion rivets and dental acrylic cement. The monkeys were trained to press an illuminated button as an operant response for food reinforcement. A fixed ratio of response to reward (10 : 1) was used. Some animals were rewarded with solid banana pellets and some with artificially sweetened and flavored water, under suitable deprivation conditions. No differences in results were noted between these two procedures. In each session, after stable pressing had started, morphine (as morphine sulfate) was injected in 0.01-ml increments spaced 1 to 2 minutes apart, starting 10 to 15 minutes after the onset of stable responding, and was continued until the animal slowed down sharply or stopped pressing the response button altogether. This depression was our quantitative measure of the effect of the drug. Each cannula was connected by a fine plastic tube to a remotely controlled solenoid injector ("lambda pump," Harvard Apparatus Co.) which delivered 0.01 ml of solution with each stroke. The morphine sulfate solution contained either 15 or 50 mg of the sulfate salt per milliliter and was sterilized before use. The higher concentration was used after tolerance had developed, to keep the total volume injected in each session under 1.0 ml. The plastic tube was protected by an outer steel spring or by a flexible copper mesh hose, which also mildly restrained the animals within the testing box. The testing sessions were alternated so that the animals received either morphine or saline solution. This was done partly to provide controls, but also because anorexia became a serious problem if morphine was given daily. This was particularly the case in the early days or weeks of drug administration and became much less obvious in time, but we have not quantified this apparent tolerance. Figure 1 shows the progressive increase in drug dosage required to eliminate responding in different animals, and the disappearance and redevelopment of tolerance in one of them. Saline, in doses up to 1.0 ml per session, failed to modify lever pressing at all.

Morphine dependence can be tested either by termination of drug administration or by injection of an "opioid antagonist" such as nalorphine or naloxone. The development of specific physical symptoms (that is, the "abstinence syndrome") after injecting the antagonist is definite evidence for dependence (*3*). For reasons which are rather unclear to us, morphine withdrawal produces a rather mild abstinence syndrome in stump-tail monkeys. Also, the abstinence syndrome thus obtained is spread over a relatively long time. These reasons prompted us to use nalorphine precipitation instead. After a minimum of five doses of intraventricular morphine sulfate spread over 10 to 14 days, nalorphine triggered definite signs of abstinence within 15 minutes after injection, regardless of whether it was administered intraventricularly (0.1 mg total dose) or subcutaneously (1 mg/kg). The precipitated abstinence syndrome consisted of hyperactivity, hyperirritability, sialorrhea, retching, vomiting, diarrhea, and contorted posturing suggestive of abdominal and/or muscular pain. It was readily terminated by intraventricular or subcutaneous morphine. The same dose of nalorphine did not produce any such changes in the same monkeys prior to the start of morphinization.

These findings clearly indicate that tolerance and dependence to morphine are readily established by repeated intracranial injection of much smaller quantities of this narcotic than are required for producing similar effects with subcutaneous injections, although, as can be seen from Fig. 1, the rate of development of tolerance varied among individuals. Not shown is the fact that the severity of the abstinence syndrome also showed individual variations, as is also the case with morphine given through other routes. In the same species and under comparable conditions the subcutaneous doses of morphine—injected three times daily instead of every other day—were 15 to 20 times larger and had to be sustained 2 to 3 weeks before a strongly positive nalorphine response could be elicited. The intraventricular effective doses of nalorphine were also comparatively much smaller than the subcutaneous equivalents. The reason for the differences in effective dosage between parenteral and intraventricular morphine is relatively easy to understand: morphine is quite inefficiently taken up by brain tissue

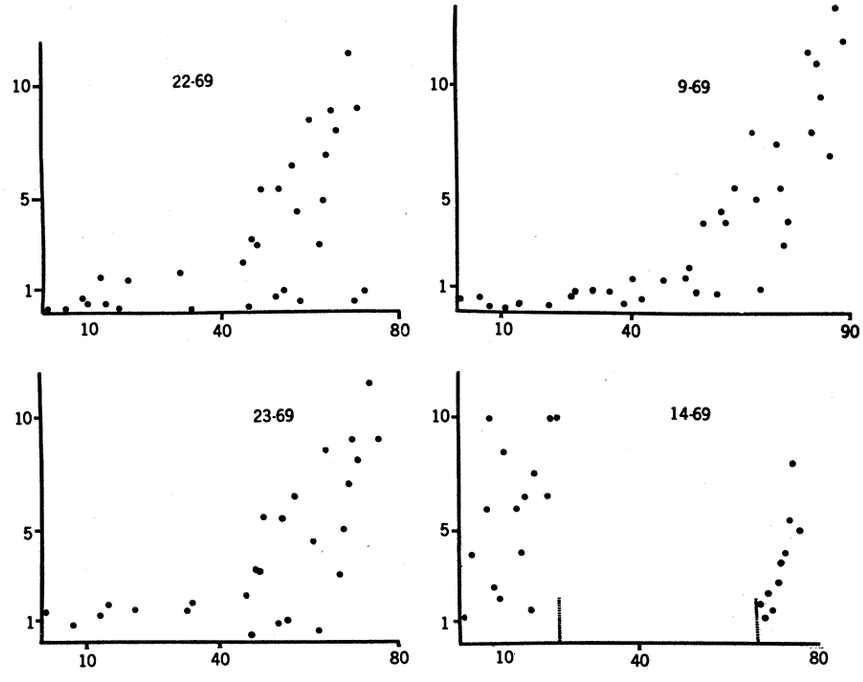

Fig. 1. Records from four different animals, showing the increases in dose of intraventricular morphine required to stop lever pressing. Only the drug runs have been included, not the saline controls. Abscissas: calendar days from the first dose of morphine sulfate. Ordinates: morphine dose in milligrams (total) per session. In monkey 14-69 the drug was discontinued for 42 days and then restarted, with apparent reduction of the initial tolerance.

from the circulation, in contrast to its quick uptake and disposition by visceral organs. For relatively equivalent doses, however, the duration of behavioral depression (especially in the early states of morphinization) was much greater after intraventricular injection, and this would indicate a far more prolonged contact of the drug with its receptors in brain. Hug (*4*) has shown that morphine is removed fairly ineffectively from the cerebrospinal fluid into the bloodstream, thus making this long contact possible. It is well known that the establishment of dependence and the development of tolerance are related to the presence of sustained high levels of the narcotic in blood (which will mean also relatively high levels in brain tissue) following repeated subcutaneous or intravenous injection (*1*). For the reasons given above, this may be less necessary when the intraventricular route is used. More important methodologically than the dosage differences is the fact that the cannulas rarely became obstructed and were then easily cleaned, they could not be removed by the animals, and it was not necessary to keep them flushed continuously or to maintain the animals under anticoagulant treatment. These are substantial practical advantages over chronic intravenous catheters (*5*). We have not seen any bacterial infections deriving from the cannulas, even after several months. We used only simple aseptic technique for implantation and injection, and protected the cannulas by a metal cap on the fitting around them. The only serious problem encountered was anorexia, concurrent with prolonged behavioral depression, which developed after each of the first few doses of morphine, and which we treated by gastric tube feeding with a high-calorie liquid diet when necessary.

The findings reported here lend support to the suggestion that some of those neural structures adjacent to the ventricular system (*6*) are major sites of action of the narcotic.

E. EIDELBERG
C. A. BARSTOW
Division of Neurobiology, Barrow Neurological Institute of St. Joseph's Hospital and Medical Center, Phoenix, Arizona 85013

References and Notes

1. G. A. Deneau and M. H. Seevers, *Advan. Pharmacol.* **3**, 267 (1964).
2. J. Cochin, *Fed. Proc.* **29** (1), 19 (1970).
3. W. R. Martin, *Pharmacol. Rev.* **19**, 463 (1967).
4. C. C. Hug, Jr., in *Narcotic Drugs, Biochemical Pharmacology*, D. H. Clouet, Ed. (Plenum, New York, 1971), pp. 122–136.
5. J. C. Woods and C. R. Schuster, *Int. J. Addict.* **3**, 231 (1968).
6. V. J. Lotti, P. Lomax, R. George, *J. Pharmacol. Exp. Ther.* **150**, 135 (1965); *ibid.*, p. 420; E. Eidelberg, M. L. Bond, C. A. Barstow, reported to the NAS–NRC Committee on Problems of Drug Dependence, Toronto, 1971; A. Herz, K. Albus, J. Metyš, P. Schubert, H. J. Teschemacer, *Neuropharmacology* **9**, 539 (1970).
7. Supported by grant MH 14588-03 of the National Institute of Mental Health and by the Barrow Neurological Foundation. We gratefully acknowledge the advice of Dr. A. S. Schwartz.

6 July 1971

Sex Attractant Pheromone of the House Fly: Isolation, Identification and Synthesis

Abstract. *A sex pheromone isolated from the cuticle and feces of the female house fly attracts the male fly; it has been identified as (Z)-9-tricosene. Chemical and biological comparisons of the natural and synthesized compounds show that they are identical.*

We report the first isolation, identification, and synthesis of a sex attractant pheromone of the common house fly, *Musca domestica* L. The compound, for which the name muscalure is proposed, is (*Z*)-9-tricosene.

The house fly is a danger to the health of man and animals principally because it breeds in manure, garbage, and fermenting crops and carries and spreads typhoid, dysentery, diarrhea, cholera, yaws, trachoma, and many other diseases (*1*). It also serves as an intermediate host of roundworms and tapeworms. The mating behavior of this worldwide pest has been studied extensively (*2*), the studies dating back to Aristotle (*3*). The male is sexually aggressive and the flies are very prolific breeders, so that the prospect that the insect's own sex pheromone could be used to repress its reproductive potential has considerable appeal.

Although olfactorily attractive pheromones of dipterous insects have been sought for many years, none have been isolated or identified. Presence of a house fly sex pheromone has been noted, because live or dead female flies were found to be attractive to males (*4*). Other studies revealed that male flies were also attracted to fly feces and lipid extracts of fly feces (*5*), and cuticular lipids (*6*).

Fractionation of cuticular and fecal lipids disclosed that the attractant, a hydrocarbon, was produced by only sexually mature females (*7*) for the attraction of only sexually mature males (*6*). Accordingly, the attractant was obtained from sexually mature, laboratory-reared female house flies (Orlando Regular strain) by surface washing to remove the cuticular lipids with hexane or ether. The concentrate was chromatographed on a silicic acid column, and the active fraction was eluted with hexane; elution with more polar solvents has yielded no active material (*7*). Thin-layer chromatography (TLC) of the active percolate on silica gel–silver nitrate plates (*8*) with 1 percent ethyl ether in hexane gave four zones. Chromatographic mobility of the zone with most of the activity (R_F 0.70) was consistent with that of a long-chain monoolefin; R_F values of the other zones were consistent with paraffins (R_F 0.95) and polyolefins (R_F 0.25, 0.10). Larger amounts of the monoolefin fraction were obtained by column chromatography (*9*), and gas chromatography (GC) of this fraction (*10*) gave peaks corresponding to 22 percent C_{23}, <1 percent C_{25}, 65 percent C_{27}, 10 percent C_{29}, and 3 percent C_{31}. Materials from the three major peaks were collected by preparative GC (*11*), and the C_{23} material was by far the most attractive. It gave a single peak on poly(diethylene glycol succinate) (DEGS), OV-17, and SE-30 columns (*10*) with retention times [Kovats indices (*12*)] that suggested a straight-chain monoolefin. Its mass spectrum showed a molecular ion at *m/e* 322 corresponding to $C_{23}H_{46}$. Its activity was lost, and its GC peak disappeared on treatment with 5 percent bromine in carbon tetrachloride, verifying the presence of a double bond. Position of the double bond was determined by microozonolysis of a 10-μg sample followed by GC of the products (*13*); retention times of the peaks coincided with those of nonanal and tetradecanal. Configuration of the double bond was established as (*Z*) by TLC on silica gel–silver nitrate plates (*14*). Absence of branching was confirmed by instantaneous hydrogenation (*15*) of 100 μg of the olefin followed by combined GC–mass spectrometry of the saturated product (*16*); the mass spectrum matched that of *n*-tricosane.

The data indicated that the sex at-