THE ACTIONS OF NICOTINE ON CENTRAL NERVOUS SYSTEM FUNCTIONS[1]

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I. INTRODUCTION

Nicotine is both a neuropharmacological agent in its own right, and a pharmacological tool. Its properties and effects on the central nervous system would command only an academic interest were it not the principal ingredient of a plant used by millions of persons; but even if it were not for tobacco, nicotine would still be of inestimable importance as a tool in biological research. From its utilization as a tool, everything we know or may learn of the actions of nicotine on the central nervous system (CNS) is of potential value; however, so far as tobacco-smoking is concerned, much of what we already know of the neuropharmacological and other actions of nicotine is clearly *irrelevant*. The reasons behind this irrelevancy are, first, the quantitative matter of dosage, and secondly, the qualitative fact that nicotine is not the only, though it would seem to be the indispensable, factor, pharmacological or otherwise, involved in tobacco use. It appears reasonable to conclude that small doses of nicotine are used, so to speak, in tobacco-smoking for the primary purpose of CNS stimulation, while large doses are employed in the laboratory primarily to produce ganglionic paralysis.

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[Lewin (114) offered the classic formulation of the former concept, as Langley and Dickinson (109) are responsible for the classic statement regarding the ganglionic action of nicotine.] There is this to be added, however: doses or concentrations of nicotine of the order of those producing ganglionic (or in fact any other kind of) depression or paralysis are never involved in normal smoking by normal habitual smokers (111, p. 53), and it is primarily experiments involving such doses or concentrations which are irrelevant to the pharmacology of tobacco-use; while, on the other hand, experiments, whether on man or animals, involving what we may term "smoking" amounts of nicotine are a part, and by no means the unimportant part, of the pharmacology of nicotine.

Passing to the relationship between tobacco-use and the pharmacology of nicotine in its qualitative aspects, we may at first thought consider that since the doses of nicotine supplied in the course of normal tobacco-use are of an order of magnitude producing, or capable of producing, pharmacological effects in the individual user at least, experiments involving the use of tobacco-smoke, whether "forced" in animals or "voluntary" in man, form a valid part of the pharmacology of nicotine. This, however, does not at all follow, not only because tobacco-smoke contains many other ingredients, known and unknown, with distinct pharmacological actions of their own, but also because the dose of nicotine contained in the smoke and exerting its own particular pharmacological action within the organism is not really ascertainable, though they are often estimated. Thus, there are objections, both qualitative and quantitative in nature, to including the results of experiments with tobacco-smoke in any limited review of the pharmacology of nicotine, at least without so many qualifications that space, even more than scientific logic, forbids. There would seem to be, however, some justification for including in such a review brief mention of experiments in which the results of tobacco-smoking, voluntary or involuntary, *appear* to be identical to those of experiments involving equivalent doses of nicotine. In these instances, it may be tentatively concluded that the observed effects of tobacco-smoke or smoking were due essentially to the nicotine content of the smoke; however, it may never be concluded with assurance that these effects were not subtly modified (beyond the range of current recording equipment, or in ways for which no recording is currently possible or even conceivable) by tobacco-smoke constituents other than nicotine.

In summary, the pharmacology or toxicology of tobacco-smoking and the pharmacology and toxicology of nicotine are not identical, and often are not even comparable. Since we are concerned in this review with the neuropharmacology of nicotine, the results of "smoking" experiments will not, except in special circumstances, be mentioned, and then only if these *seem* to be equivalent to results obtained in controlled, quantitative experiments with nicotine. For an account of such "smoking" experiments in general and in particular, the reader is referred to the reviewers' monograph on tobacco (111), in which, incidentally, most of the neuropharmacology of nicotine up to about mid-1959 also appears, though in that deliberately non-critical fashion appropriate to a comprehensive historical review of a dynamic subject. The present critical review is designed

usefully to fill the gap between the authors' "comprehensive account of the world literature" and the simple (and true) statement that the neuropharmacological action of nicotine is "stimulation, followed by depression." This "gap" may also be described in other terms as "mechanism of action"—*lacking* in the simple statement, *buried* in the monograph.

II. SPONTANEOUS ACTIVITY

A study of the effect of daily nicotine injection (5 to 7.5 mg/kg) on the activity of white rats in revolving cages revealed that the drug produced a striking decrease in voluntary activity, the extent of the drop varying with the individual animal (157). On cessation of injections, restoration of activity occurred.

The effect of single and repeated doses of nicotine on the activity of male rats in spring-suspended cages equipped for recording vibrations was reported by Kuschinsky and Hotovy (104). After 0.25 mg/kg subcutaneously, stimulation lasting one-half to two hours was recorded, the movements having the same appearance (running movements) as those following methamphetamine or caffeine. With larger (0.5 mg/kg) doses, the effect was greater and of longer duration; with smaller doses, it was less, and also the reaction of the animal was different, 0.05 mg/kg often causing an initial sedative effect for a period of about ten minutes. Following the disappearance of the stimulating effect of a dose of nicotine, a second injection again produced the same effect; no weakening of the stimulating effect was apparent in animals injected daily for several months with large doses of nicotine. The threshold dose for nicotine stimulation was found to be 1/640th of the average lethal dose of 32 mg/kg. A stimulant dose of methamphetamine followed by a stimulant dose of nicotine resulted in an additive effect.

It has been suggested that the sand-digging response of rats was a valid measure of activity (87). However, it may be questioned whether injection of 6 mg of nicotine sulfate/kg, which caused the animals to undergo a grand mal convulsion of one-half to one minute duration, following which they remained inactive for five to twenty minutes (22), demonstrated a valid pharmacological action of nicotine, rather than a non-specific, and certainly not unnatural, response to a severe convulsion induced by any means. Control injections of saline were immediately excitatory to the sand-digging activity.

An improved apparatus for measuring the motor activity of mice has recently been described by Bonta *et al.* (11), who also published a table of results obtained with their apparatus following intravenous injection of 0.18 to 0.43 mg of nicotine/kg; this table showed graded increases in motor activity with increasing dosage.

Analysis of these activity experiments indicates that "small" doses of nicotine are stimulant to spontaneous activity, while "larger" doses are depressant. Since the central nervous system is so delicately adjusted, and so susceptible to the effects of neurotropic drugs such as nicotine, it would seem highly desirable to investigate the actions of such agents on "spontaneous activity" and other functions of the organism-as-a-whole within the range of minimal effective

dosage. In animals, as well as in man, we may surely believe that the optimal effects of neuropharmacological agents lie within this range of "minimal" dosage, not within that of "maximal" or supra-maximal dosage.

III. CONDITIONED REFLEXES

The stickleback fish is attracted by red colors, but is insensitive to yellow (9). After a period of feeding the fish red worms with a yellow forceps, merely placing a yellow forceps in the water sufficed to attract the fish. With the conditioned reflex thus established, the fish were placed for twenty-four hours in water containing 0.002 ml of nicotine/l; they were then attracted to red worms placed directly in the water, but were indifferent to the yellow forceps. After removal from the nicotine solution into normal river-water, the fish recovered their conditioned reflex one day later.

In adult and growing rats, a definite conditioned salivary reflex was said to have followed a few injections of nicotine (6). As the injections were continued, the conditioned reflex was extinguished or inhibited in the growing rats, but not in the adult animals. Evaluation of this observation does not seem possible from the few details that accompanied its description. In recent experiments using this species, Geller *et al.* (66), by rewarding with food those lever-pressing responses spaced twenty to twenty-two seconds apart, trained hungry rats to time precisely. No significant effect occurred until the third day following a test dose of 0.1 to 0.25 mg nicotine bitartrate/kg, at which time there appeared a flattening of the inter-response time distributions, an increase in the average response rate, and a marked decrease in the total number of re-enforcements (food rewards) obtained. At this time, the animals were observed to be somewhat agitated in the experimental chamber, but not in the home cages. No such agitation was observed on the day of drug injection. Since all, or almost all, of the radioactivity from C^{14}-randomly-labeled nicotine administered to rats was excreted in the urine within sixteen hours (65; see also 111, pp. 19 ff.), it seems difficult to ascribe the above-reported effects to any pharmacological action of a single injection of nicotine three days previously. A more likely conclusion would seem to be that the animals were affected by some other part of the experimental procedure rather than by the nicotine *per se*: witness the "agitation" correlated with the "experimental chamber" but not with the "drug injection." Further support for this conclusion is afforded by experiments reported by Mercier and Dessaigne (123) on rats trained to discriminate between colors, from which it appeared that the rat adapts rapidly to initial perturbations produced by nicotine.

Novikova (134) studied the action of nicotine on conditioned reflexes in 2 dogs previously conditioned to food-reflex salivary secretion in response to the sounds of bell and whistle, to light, and to a "toucher" fixed on the left hip. Subcutaneous injections of 0.02 to 0.2 mg nicotine/kg resulted more or less in inhibition of the food-conditioned reflexes. Under normal conditions, both dogs exhibited a distinct relationship between the strength of the conditioned stimulus and its secretory effect: strong stimuli (bell and whistle) produced, as a rule, a

more intense conditioned secretion, whereas light, being the weakest stimulus used, gave a small secretion. After injection of a moderate dose of nicotine, this relationship was lost, and all stimuli gave reflexes of the same intensity. A paradoxical phase was also observed, in which physiologically weak stimuli produced a more intense effect than physiologically strong ones. Increase in the dose of nicotine always resulted in increasing inhibition, up to a total loss of conditioned food-reflexes; 0.2 mg/kg produced a strong inhibition of conditioned food-reflexes to all stimuli and also produced well-marked symptoms of nicotine poisoning. Doses of 0.1 and 0.15 mg nicotine/kg, which led to deep inhibition of conditioned reflexes, also produced an increase in the secretory response to the unconditioned stimulus of feeding; this was said to prove that these doses of nicotine exerted a stimulating influence on the centers of unconditioned reflexes. The effect of large doses of nicotine was to produce an inhibition of the functions of the cerebral cortex, and not paralysis of the "center" of salivation. Cortical inhibition increased with the dose of nicotine, leading to the equalization phase, to the paradoxical phase, and finally to total loss of conditioned food-reflexes. The inhibition of conditioned reflexes by nicotine was regarded as being due to the development of parabiosis in the cortex, or else to "extra-liminar inhibition" in Pavlov's sense of the word. Nicotine creates such a state of increased cortical excitability that physiologically strong stimuli become supramaximal; owing to this, the conditioned reflex is either absent or insignificant (paradoxical phase), and with more severe poisoning, moderate or weak stimuli also become supramaximal, and produce no effect (phase of inhibition).

IV. LEARNING

It has been reported that nicotine reduced the performance of white rats in a maze, with respect to time required to run the maze, number of errors, or both (54, 55, 56, 120, 121). The smallest effective single dose was 0.02 mg nicotine tartrate, which was said to have had a persistent effect (121), again, an observation not easily conformable to what we know of the speed and completeness of nicotine metabolism and excretion. In chronic experiments, in which the animals received daily injections of 0.05 to 1 mg nicotine subcutaneously or intraperitoneally over many months (54, 55), progressively poorer performances were observed.

In the recent study by Mercier and Dessaigne (123) of the effect of nicotine on rats trained to discriminate between colors, doses of 0.5 and 0.75 mg/kg depressed performance on the first day but not on the second. Increasing the daily dose to 1.5, and then to 2 mg/kg subcutaneously, depressed performance for the first four days, with return to normal on the fifth day of injection, while with a dose of 2.5 mg/kg, a relative tolerance was reached by the fifth week. Following a rest of fifteen days, 2 mg/kg dropped performance to zero, but recovery to 80 and 100% occurred in the next two days; a similar result occurred with 2.5 mg/kg.

It seems possible that these effects on performance were not caused by specific interference with the learning process but can be explained by non-specific

effects on the general state of health of the animals. At least, this is a point that should be closely evaluated in the design and conduct of this type of experiment.

V. HIGHER CEREBRAL FUNCTIONS

A. Sensorimotor functions

A feature of most of the reported experiments on the effect of nicotine on functions of the sensorimotor areas is the appallingly high concentration of the nicotine solutions used, concentrations which would never be reached following systemic administration of even lethal doses; concentrations, furthermore, often sufficient to produce local (non-specific) destruction of tissue by the alkaline nicotine. Whether the results of such experiments should be accorded serious consideration is doubtful; at best, such experiments are object-lessons in "how not to do it."

Baldi (5) applied 10% nicotine to exposed motor areas of dogs, and never observed movements in limbs. Testing a motor point with minimal electrical stimulation before and after nicotine led in one case to an exaggeration of excitability and, in other experiments, to lowering of excitability. Amantea (2) applied a disc of blotting paper containing 5% nicotine directly to the cortical centers of the sigmoid gyrus of unanesthetized dogs, and found that nicotine, like morphine, picrotoxin, and strychnine, produced an initial decrease and gradual increase in the threshold of excitability, characteristic cutaneosensory modifications (hyperesthesia of given cutaneous areas), and typical motor phenomena (clonus of the muscular group corresponding to the center being tested). Moussatché (129) applied 2, 10, and 50% nicotine to loci of the motor areas in trephined unanesthetized dogs, and reported that persistent clonic contractions were produced, and that paralysis never supervened.

Rizzolo (145, 146, 147, 148) studied the effect in unanesthetized dogs of application of 1% nicotine to the surface of the cortex and on the underlying white substance; the applications were made on predetermined optimum motor points for the movement (flexion or extension) of the forelimb and movements of the eyelids, and the chronaxie was used as the measure of excitability. The initial application of nicotine to the cerebral cortex always caused a 25 to 50% decrease of the chronaxie from the control value; after the second application, the chronaxie usually continued to decrease, but occasionally there was an increase to above the control value; the third application was always accompanied by an increase in chronaxie to about double the control. Application of the nicotine to the white matter (with the overlying gray substance removed) at a motor point modified the chronaxie of that motor point; the initial application caused a decrease of 23 to 39% from the initial chronaxie value; the second application caused a 33 to 57% decrease; the third application, on the other hand, increased the chronaxie by 25 to 43%; and with the fifth application, the chronaxie increased about 86% above the original value. Application of nicotine to a part of the corona radiata about forty-five minutes after the ablation of

the overlying cortical gray substance caused in most instances the appearance of both clonus and generalized epileptiform convulsions. When the nicotine-treated gray matter was removed, the white matter underneath was found to have the same chronaxie value as did the gray matter before treatment with nicotine (146). In another somewhat similar study on dogs, A. and B. Chauchard and P. Chauchard (25) applied a solution of nicotine (1 drop in 5 ml of Ringer's solution) to the cortical motor zone for extension and flexion of the toes. The normal values for the motor chronaxies corresponding to the foot opposite the hemisphere studied were extension, 20, and flexion, 40. Following application of nicotine, the chronaxie was increased and equalized at a level of 70, but quickly dropped to the normal level, the fall corresponding to the appearance of cortical inexcitability. Weaker dilutions of nicotine (1 drop in 20 ml) also augmented cortical and peripheral chronaxies.

B. Brain potentials

The effect of nicotine on brain potentials was first seriously investigated by Libet and Gerard. The olfactory bulb of the isolated frog brain maintains a remarkably regular potential rhythm (115). This preparation bathed in 0.5% nicotine gave very regular large slow waves, although the drug paralyzed central synaptic transmission, indicating that neurones can beat and synchronize without neural stimulation. Local application of 0.5% nicotine to the exposed optic lobes in a freshly amputated frog head eliminated within six minutes visual action potentials in the optic lobes evoked by shining light in the eyes, while leaving unchanged the action potentials in the optic nerve (116). Nicotine also prevented changes in olfactory bulb potentials otherwise produced by stimulation of the olfactory nerves. The characteristic waves produced by nicotine in the isolated frog brain were prevented by the simultaneous action of iodoacetate (67, 68). Application of 0.01 to 0.5% caffeine to the isolated frog brain had as its striking action the initiation of powerful diphasic waves of 0.2-second duration, which originated mainly in the bulb, and spread over the entire hemisphere; a third wave lasting one to four seconds, and persisting when the others had disappeared, spread at about 1 cm per second; the main diphasic wave travelled 5 cm per second at 20°C. Nicotine did not block these spreading waves, but did slow their movement. Nicotine greatly altered the electrical rhythm of isolated frog brain; in 0.5% solution, the 6 per second waves were slowed to 4 per second after some three minutes, and then discontinuously to 2 or 1 per second after five to ten minutes. The amplitude was increased to 300 microvolts or more, and the wave form altered from a roughly sinusoidal one to bizarre skewed forms. Since nicotine blocks synaptic conduction and yet produces extremely regular asymmetrical waves, its action was said to afford especially strong evidence for the conclusion that the recorded potential was that of a single unit magnified by the synchronous activity of many such units. Nicotine acted very powerfully; Gerard and Libet never failed to obtain the usual potentials, even when the drug was applied to a "stale" brain, the spontaneous waves of which had largely disappeared. When combined with 0.004 M iodoacetate,

however, the action of nicotine on brain waves was completely eliminated, and the feeble rapid potentials of the iodoacetate alone appeared. Gerard and Libet (68) suggested that the action on cell potentials of nicotine, which increases lactic-acid production (4), is abolished by iodoacetate, which prevents glycolysis.

Pick and Unna (139) reported that injection of 3 to 15 mg of nicotine per kg in spinally pithed frogs caused an immediate acceleration of the brain waves from 5 or 6 to 10 or 15 per second; this phase, probably due to a stimulation of the central sympathetic synapses, lasted for about ten minutes, and was followed by a gradual decrease in the frequency of the waves; fifteen to twenty minutes later, no electrical potentials could be recorded, although the electrocardiogram remained unaltered. These findings differed from those of Gerard and Libet (68), who, as noted above, observed on the isolated frog brain an increased amplitude of the potentials, but unaltered or decreased frequency of the waves following nicotine. Pick and Unna thought it possible that this phase of "parasympathetic" stimulation of the olfactory lobes might have been masked in their own experiments by the "sympathetic" stimulation of the hemispheres of the frog. A combination of monoiodoacetic acid and nicotine shortened the phase of increased electrical activity which followed nicotine alone, and led to a more rapid decrease and disappearance of the electrical potentials than did either substance alone.

Turning now to electroencephalographic (EEG) changes in mammals, Longo and Bovet (118) reported that intravenous injection of 2 mg of nicotine bitartrate per kg into curarized rabbits under artificial respiration produced a typical EEG picture of "grand mal," particularly noticeable in the motor cortex, which was the counterpart of the tonic-clonic contractions and the muscular tremors produced by nicotine in the normal rabbit. In order to eliminate the possible influence of peripheral actions of the drug, von Berger and Longo (175) then studied the action of nicotine on the EEG using Bremer's (13) "*encéphale isolé*" or "isolated brain" preparation of the rabbit, while blood pressure was maintained with a slow infusion of epinephrine. The action of nicotine on the EEG of the "isolated brain" of the rabbit did not differ from that in the unanesthetized curarized rabbit described above, and corresponded to the motor manifestations in the intact animals. Following the injection of nicotine, the modifications of the EEG presented various phases in time which could be synthesized into three periods; in the first, there was a disappearance of the spindles and the other normal components of the tracing, which were replaced by waves of 25 to 30 cycles/second at low voltage in the frontal lead, and waves of 5 or 6 cycles/second at voltages close to 100 to 150 microvolts in the parietal lead. This picture lasted for 20 to 60 seconds, and then passed into the second phase (convulsive attack), which occurred as a consequence of the accentuated synchronism of the first phase; the attack appeared in the form of spikes of voltages varying from 300 to 700 microvolts, particularly evident in the parietal lead; initially, the spikes were rapid (25 to 30 cycles/second), but they gradually diminished in frequency (down to 7 cycles/second) and became mingled with

the large slow waves. The third phase was that of cerebral silence, which occurred after a period of 40 to 120 seconds, the duration of the convulsive attack; this phase might be preceded by a period in which the spikes had disappeared or were very rare, and in which the large slow waves predominated (1.5 to 2 cycles/second, 400 to 500 microvolts). In a subsequent communication, Longo *et al.* (119) reported essentially similar results in EEG studies on 50 normal and "isolated brain" rabbits and on some cats and dogs.

According to Morocutti and Sergio (127), following intravenous injection of 2 mg nicotine/kg in rabbits, the electrocorticogram showed an initial brief phase of synchronization, followed by a discharge of "spikes" varying in voltage from 300 to 700 microvolts; in the third phase, slow waves usually appeared, averaging 2 cycles/second and 400 microvolts; finally, there might or might not be, according to the intensity of the crises, a phase of cerebral silence. The total duration of the crisis was, on the average, 120 seconds. According to Silvestrini (156), intravenous injection of 1.5 to 2.0 mg nicotine/kg in rabbits produced the following succession of phenomena: a desynchronization coming on immediately and lasting 30 to 50 seconds; a convulsive activity which reproduced fully the picture of an attack of grand mal seizures; and finally, electrical silence with more or less accentuated and irregular slow potentials. Doses of less than 1 mg/kg provoked only a prolonged desynchronization. The clonic and tonic contractions, as well as the muscular fasciculation characteristic of the motor excitation produced by 1 to 1.5 mg nicotine/kg, corresponded with the appearance in the EEG tracing of a convulsive pattern. The desynchronizing effect of nicotine was said to be due to excitation of the reticular formation, while the convulsive effect was ascribed to a general state of excitation, probably including the cortical neurones. Benactyzine and hydroxyzine did not block the nicotine desynchronization, but inhibited the convulsive activity completely in sufficient dosage. No parallelism was observed between this antagonism and the blocking of the arousal reactions from sensory (acoustic) stimulation. The effects of benactyzine and hydroxyzine on the motor reactions of nicotine were similar to those observed on the cerebral convulsive patterns. Scopolamine and atropine, although not preventing the convulsive manifestations, inhibited completely the desynchronization produced by nicotine.

Stumpf (164) has also described the actions on the EEG in rabbits. Immediately following intravenous injection of 0.5 to 3 mg nicotine/kg into awake, non-narcotized animals, changes were observed characteristic of an "arousal reaction" (regularizing and "rhythmizing" of the activity of the hippocampus). [Electrical stimulation of the brain-stem reticular formation is followed by behavioral and EEG changes of arousal in animals (128).] Subsequently, there appeared a discharge pattern, similar to that seen in convulsions, consisting of the same components as those observed in after-discharges following electrical stimulation of the hippocampus (regular types of peak discharges and multiple spike and wave complexes). At a later stage, the septum was also involved in these convulsive reactions. The first phase of the action of nicotine on the EEG could be abolished by lesions of the septum; after such lesions, the regular,

high activity of the hippocampus was replaced by a low-amplitude, high-frequency activity. Chlorpromazine (5 to 10 mg/kg) and hexobarbital (30 mg/kg) prevented the changes in the EEG initiated by nicotine, especially the convulsive discharge pattern. Scopolamine, however, was an ineffective nicotine antagonist. The action of nicotine on the EEG may be regarded as relatively specific; following injection of lobeline, cytisine, α-aminonicotine, α'-aminonicotine, α-amino-β'-bromnicotine, α'-amino-β'-bromnicotine, α-oxynicotine, or α'-oxynicotine, no similar activity of the hippocampus was observable. In a subsequent series of experiments, Dunlop *et al.* (47) investigated cortical, reticular, and hippocampal unit activity in adult male New Zealand rabbits immobilized with *d*-tubocurarine following intravenous injection of 3 mg nicotine bitartrate/kg. These doses induced hippocampal seizure activity in 44% of experiments, while 6 mg nicotine bitartrate/kg was 90% effective. Regularization of the EEG activity recorded from the microelectrode (*i.e.*, appearance of regular waves at 4 to 8.5 per second) occurred in 95% of all experiments. Of the three regions examined, the hippocampus had the lowest threshold for nicotine effects. Following nicotine injection, regularization of the EEG in all regions was accompanied by increased unit excitability, and, during seizures, the unit activity was depressed. Modification of neocortical and reticular unit activity, without any concomitant EEG changes in these regions, was occasionally recorded after nicotine injection. Further experiments indicated that these changes in unit activity were due to alterations in hippocampal excitation elicited by the drug. Electrical stimulation of the hippocampus, which evoked regularization and seizure discharges, similarly modified the reticular unit excitability; but no conclusive results could be obtained regarding the hippocampal influence on neocortical units.

According to Knapp and Domino (101), midpontine-transected cats and rabbits, which displayed EEG arousal in response to epinephrine and serotonin, were also found to show EEG arousal following small (10 to 20 μg/kg) doses of nicotine. In dissociation of this effect from adrenal catecholamine release, epinephrine-insensitive prepontine-transected cats and rabbits were found to retain their sensitivity to nicotine. Furthermore, in the midpontine-transected dog, in which epinephrine and norepinephrine do not cause EEG arousal, nicotine was effective in the same low doses, while the potent ganglionic stimulant, 1,1-dimethyl-4-phenylpiperazinium iodide, in dosage of 5 to 10 μg/kg did not cause EEG activation. Completely deafferented dog brains were prepared by pre-trigeminal transection, bilateral destruction of cranial nerves II and III, and the topical application of 2% lidocaine to the olfactory mucosa. In such preparations, nicotine still caused EEG arousal, indicating that the site of action of nicotine is within the central nervous system.

In cats anesthetized with ether, large doses of nicotine (50 mg/kg in divided doses given intravenously) failed to depress the reticular activating system (57). After the initial cardiovascular effects had subsided, the EEG voltage was somewhat lower, and the frequency higher, than before. Occasional convulsive spikes appeared. However, clear signs of further EEG activation were obtained on reticular stimulation.

Ochs and Hunt (135) examined the effect of nicotine and γ-aminobutyric acid (GABA) on cortical transmission; the possibility that these drugs act as synaptic blocking agents in the cortex was said to be indicated by their reversible block of propagation of spreading depression (SD). This was shown in the molecular layer preparation, where SD transmission continues although all cortical layers below the first are cut through. Nicotine was placed either on the recording point while direct cortical responses (DCR) were elicited, or into a well-type of recording electrode while recording DCR. Nicotine either had no effect on the DCR, or augmented the DCR almost twofold. An increase in the DCR after nicotine application could come about by a selective block of inhibitory synapses acting on apical dendrites. Gamma-aminobutyric acid either eliminated the DCR, or reversed it, and this was related to direct application or use of the well-electrode. This compound has been suggested as a specific blocking agent for excitatory synapses. Block of SD would then come about by block of inhibitory synapses (nicotine) or block of excitatory synapses (GABA). Alternately, nicotine may have an action on the release of or reaction to transmitter substance postulated in the "contiguity" theory of SD transmission. Gamma-aminobutyric acid could also have this effect, or more probably it acts in a generalized fashion on apical dendrites to interfere with transmission of SD and DCR.

There appear to be no reports of EEG changes in human subjects following nicotine administration in "smoking" dosage. Such investigations would be highly desirable, if only to corroborate the impression that the EEG changes observed during or following cigarette-smoking do not appear to be due to nicotine *per se*; although there is, of course, the possibility that any "pure" nicotine effects may be "covered up" by EEG changes brought about by the physical act of smoking or by other constituents of tobacco-smoke. A brief review of several recent studies on man will illustrate these points and possibilities.

Lambiase and Serra (108) recorded cortical electrical activity in 25 subjects before and after smoking one cigarette; in 80%, depression in voltage and an acceleration in frequency of the alpha rhythm were noted, but the form remained almost unchanged. These alterations were more consistent in subjects over 35 years of age, and were attributed to the double action of carbon monoxide and of nicotine, resulting in cerebral anoxia (indicated by depression of voltages) and release of epinephrine (indicated by acceleration of frequency). Electroencephalograms made by Wechsler (178) on 10 normal subjects before, during, and for ten minutes after smoking 3 regular-size cigarettes in ten minutes [*oversmoking*] revealed an intermittent flattening lasting one to thirty seconds. This pattern occurred only with puffing on the cigarette; however, it occurred also to a lesser degree in individuals who did not inhale, and in those smoking filtered and denicotinized cigarettes. This pattern could be an abnormal attention response, the author commented. Hauser *et al.* (84) studied EEG changes on cigarette-smoking in healthy young adults. An increase in the dominant alpha frequency of 1 or 2 cycles/second occurred in over 80% of the initial group, but 6 records were considered equivocal because of poorly developed alpha rhythm or marked alpha inhibition. Excluding these 6, then, 16% of smokers and 30%

of non-smokers showed no change in the dominant alpha frequency, while 84% of smokers and 70% of non-smokers did show a change. In over one-third of the subjects, the alpha frequency shifted during the initial inhalation of cigarette-smoke. All shifts occurred during the first cigarette, and once the shift occurred, it persisted without fluctuation in relation to inhalation. Opening the eyes for prolonged periods caused a temporary return to the basal state. As long as the subjects remained in the easy-chair with their eyes closed, the shift persisted (up to fifty-one minutes). Moving about for a short period would cause a return to the basal state. If the subject then smoked another cigarette, the shift would occur again. Five subjects who had shifted 1 or 2 cycles/second simulated smoking by means of a glass cigarette stuffed with cotton, and two specially prepared nicotine-free cigarettes; 4 showed some increase in alpha frequency with the glass cigarette, and all showed an increase with the low-nicotine cigarette similar to that noted with the regular cigarette. There was no evidence from data on blood pressure and pulse-rate that the effect was directly mediated by cardiovascular changes, nor did data from measurements of hyperventilation suggest that it was mediated by a lowering of blood carbon dioxide. The fact that the increase in dominant alpha frequency occurred in healthy young adults, smokers and non-smokers alike, in this series, demonstrated to the authors that smoking in some way affects the central nervous system. In their opinion, the shift would seem to represent a psycho-physiologic response related more to the act of smoking than to physiologic or metabolic effects from substances present in the cigarette smoke. Bickford (8) analyzed EEG tracings obtained in the above study, and considered that the shift in alpha rhythm was related to some concomitant action of smoking, perhaps a shift in attention, and not to the effect of any inhaled agents from the cigarette itself.

C. Tremor

Tremor is one of the more characteristic effects of nicotine in both man and lower animals (111, pp. 82 ff.). Small but effective doses of nicotine given by rapid intravenous injection to mice produced, as their first effect, tremor (162). Intravenous injections of nicotine up to 0.6 mg/kg induced only tremor in mice, whereas with higher doses tremor was rapidly followed by clonic convulsions or even by seizures of the tonic-extensor type; hence, for a study of a pure tremor effect, it has been stated that the dose of nicotine in mice should not exceed 0.5 to 0.6 mg/kg (11). Nicotine dosage for production of pure tremor undoubtedly depends on experimental conditions, *e.g.*, strain of mouse, rate of injection, *etc.* In the unanesthetized rabbit, even a small (0.15 mg/kg) dose of nicotine produced fibrillary twitchings, while 0.35 caused clear-cut muscle twitching in 100% of the animals used (21). The nicotine tremor effect in other mammals has not been so thoroughly quantitated. In man, even approximately "smoking" doses of nicotine have produced muscle tremor (111, p. 471), and persons suffering from acute or fatal nicotine poisoning have in some cases displayed tremor (111, p. 473). Although nicotine has a demonstrable effect directly on skeletal muscle, nicotine tremor is undoubtedly a result of central nervous system stimulation.

Nicotine tremor has served profitably as an experimental condition for testing potential anti-Parkinsonian drugs. These tests, however, tell us little about the pharmacological action of nicotine; in them, nicotine is used solely as a pharmacological tool. For this reason, only a brief account of the effects of other drugs on nicotine tremor will be given here.

The following drugs or compounds have been reported to protect against, or to antagonize, tremor induced by nicotine in unanesthetized mammals (rabbits, unless otherwise specified), to varying degrees when administered in sufficient dosage [for bibliographic references, details, and especially qualifications the reader is referred to Larson *et al.* (111, pp. 85–88) except for authors indicated in parentheses below]:

Adiphenine, aminoketones [especially 2-piperidinoethyl phenyl ketone], amphetamine, Atremon [chromone-2-carboxylic acid] (rat), benactyzine, bicyclic derivatives of pyrrolidine (mouse), caramiphen, chlorcyclizine, decamethonium, diethazine, diethylaminoethyl ester of diphenylpropyl acetic acid, diethyl propanediol (mouse and rat), dimenhydrinate, diphenhydramine, diphenylhydantoin, ether, ethopropazine, hexamethonium, hydroxyzine, methantheline, methdilazine (117), 4-oxydiphenylethane derivatives, 4-oxystilbene derivatives, pentobarbital, phenindamine, phenothiazine derivatives [especially β-diethylaminoethyl-phenothiazine-10-carboxylate] (40), piridocaine, promethazine, pyrilamine, tetraethylammonium, trihexyphenidyl, tubocurarine, 92 G.T. [β-diethylaminoethyl-2-phenyl-2-(hydrocyclopentyl) ethanoate hydrochloride], 883 F [diethylaminomethyl-3-benzodioxane], 933 F [piperidine-methyl-3-benzodioxane], 2559 F [tri(triethylammoniummethoxy)-1,6,3-benzene tri-iodide], 3015 R.P. [dimethylaminoethyl-N-dibenzo-*p*-thiazine].

Nicotine tremor in the unanesthetized rabbit was said to have been potentiated to some extent by the following drugs: amphetamine, epinephrine, methamphetamine, *l*-norepinephrine, phenylephrine, and phenylpropanolamine.

The following drugs and compounds have been found ineffective against nicotine-induced tremor, at least in the concentrations studied: metaraminol, nordefrine, magnesium, thiazinamium methyl sulfate, (N-ethyl, N,β-chloroethyl)aminomethylbenzodioxane, pavatrine, pempidine and its N-ethyl homologue, sparteine, and 3580 R.P. [diethylaminoethyl-N-dibenzo-*p*-thiazine ethyl iodine].

In addition to the above substances, the following drugs and compounds, which had been found to be active against nicotine tremor by some workers, have been reported by others to be inactive: atropine, Dibenamine, hexobarbital, mephenesin, paraldehyde, pentamethonium, phenobarbital, procaine, scopolamine, and 3015 R.P. [dimethylaminoethyl-N-dibenzo-*p*-thiazine].

It is probably safe to say that most, if not all, of such discrepancies are due to the different dosages employed by the different workers.

Regarding the mechanism of nicotine tremor, Langley and Dickinson (110) concluded that the effect of the drug on the skeletal muscles in mammals appeared to be entirely due to a stimulation of the central nervous system. Cahen (20) concluded from his work that, in addition to a central action, nicotine

exerted a separate and distinct peripheral effect on striated muscle. The central effect, in terms of nicotine contracture, was located by Stoyanovskii (163) in the spinal cord, while Bovet *et al.* (12) argued for a mesencephalic origin of these tremors. It is apparent from the list of antagonistic drugs given above that nicotine tremor may be blocked at several points, for example, centrally by barbiturates or peripherally by neuromuscular blocking agents.

D. Convulsions

If the dose of nicotine is sufficient, tremor is usually followed by convulsions. Fatal doses of nicotine generally cause convulsions in all mammals, but the convulsive dose of nicotine is generally understood to be that dose which induces convulsions from which the animal recovers. A table of convulsive doses of nicotine in laboratory animals has been given by Larson *et al.* (111, p. 50), which shows rather less consistency among the results of the several investigators than might be expected. The numbers of animals reported in most series cannot be considered sufficient. An exception is the report of Stone *et al.* (162), who, in a series of over 350 mice, found that intravenous injection of 0.84 mg nicotine/kg caused a 100% "clonic response," but 87% mortality. A "safer" intravenous convulsive dose in this species may be taken as 0.4 to 0.45 mg/kg (76, 100, 103). In rats, the average intravenous convulsive dose ($C.D._{50}$) of 60 animals of one strain was 0.234 mg nicotine tartrate/kg, and of 40 animals of a second strain, 0.31 mg/kg (78). The intravenous convulsive dose in rabbits varied between 0.5 and 2 mg/kg; in cats, the reported dose was 1 mg/kg, and in dogs, the intravenous convulsive dose may be taken as 0.6 to 2 mg/kg (111, p. 50). Blum and Zacks (10) analyzed the relationship between nicotine-induced convulsions and mortality in male albino rats about 120 days old, and found a zero correlation for practically the entire dosage range used, apparently because nicotine may cause convulsions without implicating vital functions in a deleterious fashion.

The character of the convulsive phenomena produced in mice by intravenously injected nicotine varies considerably with the dose of nicotine. Doses up to 0.6 mg/kg induced tremor only, whereas with higher (*e.g.*, 0.84 mg/kg) doses, the tremor was followed rapidly by clonic convulsions, and then by seizures of the tonic-extensor type (11, 162). Results with other species do not seem to have been so well quantitated.

Following administration of a convulsive or subconvulsive dose of nicotine, a subsequent convulsive dose may fail to cause convulsions (143, 150). A large number of drugs have been shown to have a greater or lesser inhibitory effect on nicotine convulsions in several species. In general, these drugs may be classified as having anesthetic, atropine-like, adrenolytic, ganglioplegic, or antihistaminic effects. Since the antagonistic effect depends on the species used, the doses of nicotine and nicotine-antagonists employed, and on the general conditions under which the experiments have been performed, the following lists should be regarded primarily as an index, and details sought in Larson *et al.* (111, pp. 47–49) or in the original papers. The following drugs have been reported to show a more or less antagonistic action against nicotine convulsions, at least under certain experimental conditions: acetylcholine, adiphenine (23, 111) and quaternary

derivatives (23), alcohol, aminoketones [especially 2-diethylaminoethyl phenyl ketone], amphetamine, atropine (76, 100, 111), azacyclonol, barbital, belladonna alkaloids (103), benactyzine, benzotropine methanesulfonate (100), 1-bicycloheptenyl-1-phenyl-3-piperidino-propene (76), 1-bicycloheptyl-1-phenyl-piperidino-propanol (76), 1-bicyclo-oxy-heptyl-1-phenyl-3-piperidino-propanol (76), butylscopolamine, caramiphen (76, 100, 111), chloral, chloralose, chlorisondamine, chlorphenoxamine (100), chlorpromazine, curare, cycrimine (100), diethazine, dihydroergotamine, diphenylhydantoin, diphenhydramine (100, 111), ethopropazine (76, 100, 111), heptamethonium, hexamethonium, hexobarbital, Hexonate [the salt of hexamethylene bis (trimethylammonium) and nicotinic acid], Histol [*p*-chloro-α-methyl-β-dimethyl-aminoethyl benzhydryl ether hydrochloride], hydroxyzine, hyoscyamine (103), Keithon [β-diethyl ester of chlorphenoxamine] (100), mecamylamine, mecamylamine congeners (50), mepazine (100), meperidine, mephenesin, meprobamate, methadone, methamphetamine, 1-methyl-2(1′-methylpyrrolidyl) propyl-2-pyrindole, methyl phenidate, 1-methyl-2-trimethylamino-propyl-2,9-pyrindole, morphine, orphenadrine (100), oxy-cyclohexylphenyl-acetic acid-diethylaminoethyl ester (76), pempidine, pempidine congeners (50), pentamethonium, pentobarbital, pentolinium, phenobarbital, pipradiol, procaine, procyclidine (100), promethazine, scopolamine, SKF 525A (β-diethylamino diphenylene propyl acetate), thiamine, thiamine propyl disulfide, tribromethanol, trihexyphenidyl (76, 100, 111), trimethadione, tubocurarine, urethane, 4.560 R.P. [chloro-3(dimethylamino-3′-propyl)-10-phenothiazine], 2559 F [tri-(triethylammoniummethoxy)-1,2,3-benzene tri-iodide], 26539 [N-ethyl homologue of 1:2:6:6-pentamethyl-piperidine].

In mice, a number of the above agents were more effective against the tonic than the clonic phase of nicotine convulsions (27, 162). Atropine, caramiphen, diphenhydramine, diphenylhydantoin, ethopromazine, mephenesin, pentobarbital, phenobarbital, trihexyphenidyl, and trimethadione, if active at all, were effective only against the tonic convulsive phase and mortality, and did not prevent the clonic response of nicotine-injected mice (162). Atropine and scopolamine, barbital, benactyzine, diphenylhydantoin, diphenhydramine, meperidine, mephenesin, meprobamate, methadone, morphine, and promethazine, all more readily abolished the tonic-extensor component of the nicotine seizure, whereas the initial clonic seizure was more efficiently abolished by promazine and chlorpromazine (26, 27).

The following drugs were found to be without effect in controlling nicotine convulsions under the conditions of the experiments: azacyclonol, chlorisondamine, hexamethonium, isethionic acid, meprobamate, 1-methyl-2-triethylaminopropyl-2,9-pyrindole, 1-methyl-2-trimethylaminoamyl-2,9-pyrindole, pentamethonium, reserpine (27, 142), riboflavin, tetraethylammonium, tetramethonium, trimethonium, and yohimbine. It was only following moderate doses of reserpine that the motor response to nicotine remained unchanged; after high doses, the response to nicotine, habitually exclusively clonic in the unanesthetized rabbit, became clonico-tonic (142). This modification of nicotine convulsions was considered to be due to a central action of reserpine.

A small number of drugs reportedly can potentiate or facilitate nicotine convul-

sions: amphetamine and methamphetamine, epinephrine and norepinephrine, isonicotinic acid hydrazide and 1-isonicotinyl-2-isopropyl hydrazide, benactyzine and hydroxyzine, and histamine.

With few exceptions, those workers who have investigated the antagonistic action of the above drugs against nicotine-induced convulsions have used as their criterion the weakening or suppression of visible seizures. In the same way, earlier (and some more recent) workers have assumed that the appearance of gross convulsions following local applications of nicotine to various areas or regions of the CNS axis (136, 148, 184), or their disappearance following nerve or CNS section at various levels, afforded a more or less valid indication of the convulsant effect of the drug (111, pp. 45–47). Although the appearance or suppression of gross, visible convulsions is a crude [and often misleading (24)] criterion of site and mechanism of action, yet all evidence points to the conclusion that the site of nicotine convulsions is the central nervous system, and not the peripheral (nervous or muscular) apparatus.

A more refined technique for the investigation of the site and character of nicotine convulsions is offered by the encephalograph, and recent use of this instrument has yielded interesting, if not conclusive, results. Some account of these experiments has been given above under *Brain Potentials*, and they will now be amplified as they relate more specifically to nicotine convulsions and antinicotinic, anticonvulsant drugs. It will be recalled that Longo and his associates found that nicotine produced a typical EEG picture of "grand mal" convulsions in both the unanesthetized rabbit (118) and the "isolated brain" preparation (175). Von Berger and Longo (175) noted that the convulsive syndrome from nicotine was clearly different from that produced by other convulsants, such as strychnine and pentylenetetrazol, and that its antagonists lay in a category of agents with characteristic antinicotinic properties, at both the peripheral and central levels. Ethopropazine, trihexyphenidyl, caramiphen, and diphenhydramine, which had been shown to be antagonistic to nicotine-induced tremor, likewise proved to be antagonistic to the appearance of the convulsive waves produced by nicotine; the protective action lasted about 1 hour, after which a dose of nicotine reproduced the attack in full (118). The site of the antinicotinic effect of these latter drugs was considered by Longo *et al.* (119) to be at the level of the reticular system, and probably involved a cholinergic mechanism, such as blocking acetylcholine activity centrally.

Silvestrini (156) also reported that nicotine fully reproduced the picture of an attack of "grand mal," the EEG tracing showing the convulsive pattern. In doses of 15 to 20 and 3 to 5 mg/kg, respectively, benactyzine and hydroxyzine completely inhibited the convulsive activity but lesser doses caused a potentiation instead of inhibition. Morocutti and Sergio (127) recorded cortical electrical activity, with results similar to those reported by Longo and his associates, and further found that the discharge of "spikes" in the second phase (convulsive attack) was antagonized by amphetamine, methamphetamine, and methylphenidate.

In the awake, non-narcotized rabbit studied by Stumpf (164), an EEG dis-

charge pattern, similar to that seen in convulsions, followed the intravenous administration of nicotine. The convulsive discharge pattern was prevented by chlorpromazine and hexobarbital, but not by scopolamine. This worker regarded the action of nicotine on the EEG pattern as relatively specific, and as apparently due to alterations in hippocampal excitation elicited by the drug (47).

Silvestrini (156) considered that the convulsive effect of nicotine was due to a general state of excitation, probably including the cortical neurones. Longo *et al.* (119) implicated the reticular substance; Stumpf (164) and Dunlop *et al.* (47), the hippocampus. Sacra and McColl (151) believed that nicotine produced its central convulsant action at the level of the diencephalon. Therefore, upper levels of the central nervous system have been broadly implicated as sites of the convulsive action of nicotine.

E. Nicotine paralysis

Early workers noted that in animals poisoned with nicotine, convulsions are followed by paralysis, resembling that produced by curare (149). Moore and Row (126) concluded from their experiments on frogs that motor paralysis was mainly due to paralysis of the intramuscular parts of motor nerves, although their experiments did not show that central action was completely absent. Von Hof and Schneider (177) stated that in the intact frog nicotine showed a distinctly central point of attack.

Yabuno (184) injected 0.01 ml of undiluted nicotine into various parts of the brain of cats, controlling the injections by means of Clarke's stereotaxic instrument, and afterwards checking the location of the nicotine so injected by histological means. Coma (limbs flaccid, corneal reflex abolished or only slight, reactions to painful stimuli nearly absent) was achieved by injections into the central gray matter at the level between the oculomotor and the trochlear nuclei inclusively, the substantia nigra, and the medial thalamic nucleus. Disturbances in consciousness were also observed in animals injected in the dorsal part of the lateral thalamic nucleus or in the hypothalamus, and in animals injected at obscure sites (probably in the ventricular system); but these parts of the brain were not authenticated. Puncture and nicotine injection in the mesencephalic reticular formation, the superior colliculus, the ventral part of the lateral thalamic nucleus, and the hypothalamus did not produce coma or semi-coma (tonus of muscles almost normal; corneal reflex and other reflexes to mechanical stimuli definitely sluggish). Also, cats injected in the medial portion of the hypothalamus reacted normally to mechanical stimuli, although they appeared to be in a state of sleep. Histologic examination showed that the morphologic change in the area injected with nicotine corresponded to that of destruction. However, the author did not decide whether the disturbance in consciousness was due to the destructive effect of nicotine (or to any equally corrosive liquid) or to its initial stimulating effect (if one can conceive, in this type of experiment, that nicotine was able to exert any characteristic pharmacologic action at all). It was assumed that in the case of nicotinization of the central gray matter of the midbrain the stimulation might be responsible for coma, while in the case of the substantia

nigra or the medial thalamic nucleus, destruction was the probable cause of coma. Sakata (152) employed a technique similar to that of Yabuno on unanesthetized cats, 0.01 ml of pure nicotine being injected at the desired site. Coma was produced by injection at such sites as the mesencephalic central gray, mediodorsal portion of the posterior hypothalamus, anterior cingulate cortex, trigonum olfactorii, *etc.* There was no particular change in the EEG pattern which was common to all cases and occurred in parallel to behavioral coma. The author noted the absence during coma, of generalized spindle or high-voltage, slow activities which are known to accompany sleep or usual coma. Burst activities were also observed. The main mechanism underlying the elicitation of coma and of EEG changes seemed to be the stimulant action of nicotine on the local neuronal assembly. Desynchronization of surface EEG during coma seemed likely to be due to excitation of the ascending reticular activating system or of the thalamic diffuse projection system, or both, which is caused in turn by excitation of the loci nicotinized. It was postulated that unresponsiveness occurred as a result of severe disturbance of normal functioning of the reticular system or the diffuse projection system, or both, due to abnormal or ictal excitation and post-ictal exhaustion. Inasmuch as in these studies all signs of the coma syndrome were expressed externally *via* efferent pathways, it was not decided whether, in the coma here reported, the higher cerebral functions were impaired by ascending effects from the lower cerebral loci nicotinized, although this possibility was held to be likely.

F. Nicotine catalepsy

Nicotine causes cataleptic rigidity in the limbs of the frog (3); this effect occurs following destruction of the spinal cord, or in the denervated limb after unilateral section of the brachial plexus (58).

In contrast to normal animals, rats or mice placed in the lateral position following subcutaneous injection of effective doses of nicotine remained there for several seconds; this was diagnosed as catalepsy (133). Gutierrez-Noriega (75) studied the catalepsy produced in dogs by intravenous injection of nicotine. The greatest percentage of animals with catalepsy, and also the intensity of catalepsy, occurred at a dose-level of 3 mg nicotine tartrate/kg; with doses above (4 and 5 mg/kg) and below (1 and 2 mg/kg) this, the percentage showing catalepsy progressively declined, and intensity was also less on either side of the optimal dosage. Catatonia produced in the dog by nicotine was found to be less specific than that produced by bulbocapnine (7); in mice, nicotine had no clear-cut effect on the cataleptic state produced by bulbocapnine (161).

The whole matter of the site of action of nicotine in producing this phenomenon requires further study.

VI. MEDULLARY FUNCTIONS

A. Vomiting

Small doses of nicotine are emetic in cats and dogs, but large doses fail to cause vomiting, and are, in fact, anti-emetic (*cat*, 70; *dog*, 75). Intramuscular injection

of 1.5 mg nicotine bitartrate/kg was uniformly effective in evoking emesis in both cats and dogs within fifteen minutes (105, 106). By subcutaneous injection, the average vomiting doses were, respectively, 0.58 and 0.6 mg nicotine/kg (17). In dogs, the optimal intravenous dose was 2 mg nicotine tartrate/kg (75); a dose of 0.1 or 0.2 mg/kg never caused vomiting when given intravenously, but intracisternal injection of this dose always resulted in strong vomiting (176). The oral emetic dose of nicotine in dogs was said to be about 50 times more than the minimum intravenous emetic dose (52).

Nausea and vomiting are by far the most common symptoms and signs of acute nicotine poisoning in man (111, p. 473); even the administration of relatively small amounts of nicotine by mouth, or subcutaneous or intravenous injection, causes nausea and vomiting, particularly in those subjects not accustomed to the use of tobacco (98, 112, 125, 159). Effective doses fall in the range of 1.5 to 12 mg subcutaneously and 5 to 16 mg by mouth; by comparison, the amount of nicotine derived from the ordinary smoking of a cigarette is about 2 mg.

In cats, large and repeated doses of nicotine resulted in the disappearance of the emetic effect (70); in dogs, repeated injections of nicotine gave no indication of the development of tachyphylaxis for the emetic action (18, 19). These divergent results may be due not to species differences, but rather to differences in dosage, the relatively small doses used by Busse and Lendle (18, 19) probably exerting only a central effect, and the relatively large doses employed by Gold and Brown (70) causing, in addition, partial motor paralysis and thus concealing the central effects. In man, some degree of tolerance to the emetic effect of nicotine develops in habitual smokers, since they do not show the well-known nausea and vomiting exhibited by the novice smoker. This tolerance, however, must be relatively slight, since an increase in "dosage" of cigarettes or cigars or even abnormally rapid consumption of tobacco, may lead to nausea and vomiting even in habituated smokers.

Barbital did not abolish the emetic action of nicotine in cats (70), but this may have been due to insufficient dosage; Cheymol and Quinquaud (33) found that intravenous injection of the barbiturate preparation, Somnifène, blocked nicotine emesis in dogs in dosage of 0.3 to 0.4 ml/kg, while 0.2 ml/kg was not effective. Chloralose suppressed the emetic action of nicotine in dogs (31). Chlorpromazine (105, 106, 107) and fluphenazine, perphenazine, and trifluprom azine offered no significant protection against the emetic effects of nicotine (107). The emesis-inhibiting effect of relatively small doses of azamethonium was attributed by Busse and Lendle (18, 19) to a central nervous antagonism.

Reports on the efficacy of atropine in inhibiting nicotine emesis are contradictory. According to some writers, atropine showed an inhibitory effect (32, 51); according to others, atropine was ineffective (82, 105, 106). Nicotine emesis was inhibited in dogs by N-butylscopolammonium bromide, an atropine-like compound (18).

With respect to sympatholytic drugs, ergotamine tartrate lessened or abolished the emetic action of nicotine (30, 81); yohimbine suppressed the nicotine emetic effect (29); Dibenamine was ineffective (18).

Ganglionic blocking agents, such as tetraethylammonium (18, 105, 106) and hexamethonium (105, 106), were effective in blocking nicotine emesis, as was azamethonium (18, 19).

Nicotine did not abolish the emetic action of apomorphine in cats, indicating to Hatcher and Weiss (83) that it did not markedly depress the vomiting center. Although Eggleston and Hatcher (52) had concluded that the emetic action of nicotine was central, Hatcher and Weiss (82) modified this by proposing that nicotine increased the excitability of the vomiting center, and afferent impulses coming to it from the heart then induced vomiting. Hatcher (80) stated that "very small amounts of nicotine increase the reflex excitability of the vomiting center to which they are applied directly, but they cause emesis by their peripheral (cardiac ?) action after their intravenous injection"; Hatcher and French (81) felt it probable that large doses of nicotine also acted peripherally to induce vomiting. More recently, the site of emetic action of nicotine (and lobeline) has been reconsidered by Laffan and Borison (105, 106) in the light of the current concept that centrally acting emetic agents do not act directly on the vomiting center. Two basic experimental approaches to the problem of localization were utilized, namely, ablation of the emetic chemoreceptor trigger zone (CT zone) in the area postrema of the medulla oblongata, and selected interruption of trunk afferents without impairment of the motor function of vomiting. Strongest support for the CT zone as the emetic receptor site comes from experiments on dogs. In these animals, ablation of the medullary emetic CT zone sufficed for protection against the emetic effect of nicotine and lobeline. In cats, however, the alkaloids were found to act at a peripheral locus as well as at the CT zone. Thus, in order to prevent the vomiting response in cats, it became necessary to perform the combined procedures of spinal deafferentation, mid-cervical vagotomy, and CT zone ablation; this combination was accomplished either by interrupting peripheral nerves directly, along with ablating the CT zone, or by making a broad medullary lesion which appeared to destroy concomitantly both the CT zone and centripetal emetic pathways.

B. Respiration

The classical sequence of respiratory events following administration of nicotine to dogs, cats, and rabbits (on which species the most detailed investigations have been carried out) may be described as an initial brief arrest of respiration, followed in turn by stimulation (increased rate and, generally, depth), a secondary, prolonged period of apnea, depression (slower and more shallow, irregular, or Cheyne-Stokes breathing), and eventually, with lethal doses, by cessation of respiratory movements and ultimate central paralysis. Respiratory failure in nicotine poisoning has been demonstrated unequivocally by Thomas and Franke (167, 168), Franke and Thomas (62), Franke (59), Gold and Brown (70), and Gold and Modell (71) to be due to peripheral curare-like paralysis of the respiratory muscles, during which the respiratory center continues to function, as revealed in cross-circulation experiments (59) and study of phrenic-nerve potentials (71). With the other events of the respiratory picture following nico-

tine, however, the respiratory center appears to be involved, either directly or *via* nicotine action on the afferent branches of the respiratory reflexes. It is unnecessary in this review to do more than list these reflexes from nicotine-sensitive chemoreceptors, which are known or suspected to lie in the carotid body (reviewed by Heymans, 89), aorta (35, 131), auricles (45), lungs (41, 42), spleen (28), and even ear (124). Non-chemoreceptor reflexes also may be involved in the respiratory effects of nicotine, for example, from pulmonary deflation receptors *via* vagal afferent fibers (137), from the tongue *via* the sublingual nerve (170), and from the ear *via* the great auricular nerve (113). The inhibitory reflex from the nose excited by inhalation of tobacco-smoke (63, 79, 99) is not a specific nicotine effect; however, the apnea and respiratory stimulation and depression observed in animals following inhalation of tobacco-smoke is probably largely due to the nicotine content of the smoke, since such changes do not appear on inhalation of smoke from a plant other than tobacco (44).

Following the injection of nicotine into anesthetized dogs, respiration showed an initial short expiratory arrest, then a brief acceleration, followed by a second expiratory apnea lasting as long as two minutes (43, 46, 85, 155); this second apnea had been originally named "nicotine apnea" by de Almeida (43), but many succeeding writers have used the term indistinguishably for both periods of respiratory arrest, so that it is now desirable to speak of "primary" and "secondary" nicotine apnea. In anesthetized cats given nicotine intravenously, an immediate prolonged expiration occurred (46, 71, 155); this species was said not to show a secondary nicotine apnea (85), but Domaye (46) reported that a secondary expiratory apnea was also demonstrable in cats anesthetized with urethane. The appearance of secondary nicotine apnea is more consistent in anesthetized than in non-anesthetized animals (43). In rabbits also brief respiratory arrest has been observed immediately following intravenous injection of nicotine (130); this primary nicotine apnea is inspiratory, however, although the secondary apnea, when it appeared, was expiratory (46).

Domaye (46) has made a detailed analysis of both primary and secondary nicotine apnea. In dogs, cats, and rabbits, the primary apnea produced by nicotine failed to appear after bilateral vagotomy at the cervical level. When the same dose of nicotine was injected into the left ventricle, the primary apnea did not occur, leading to the conclusion that it is chiefly, if not entirely, a reflex, probably from the lung, with the afferent path running *via* the vagus nerve. Experiments by Takasaki *et al.* (165a, b) appeared to localize the receptors for nicotine apnea in the lungs, and specifically between the pulmonary-artery bifurcation and the pulmonary vein. Many other writers have reported that primary nicotine apnea is abolished by vagotomy (41, 43, 72, 73, 86, 182). Bilateral vagotomy just *below* the hilus of the lung, however, did not affect nicotine apnea, although bilateral vagotomy *above* the hilus did abolish this effect (165b). The central component of primary nicotine apnea has been variously interpreted. J. F. Heymans and C. Heymans (96) concluded from cross-circulation experiments that the condition was due to a paralysis of the respiratory center. Takasaki *et al.* (165a,b), however, who also used the Heymans'

technique, concluded that nicotine apnea was not due to a direct effect of nicotine on the respiratory center, but rather, as indicated above, to an effect on receptors distributed in the area of the pulmonary artery. Since application of nicotine to the floor of the fourth ventricle of anesthetized dogs invariably produced an instantaneous cessation of respiration with the chest in the expiratory position, Nicholson and Sobin (132) concluded that nicotine apnea was probably the result of stimulation of some expiratory mechanism, either direct excitation of an expiratory "center" or the facilitation of afferent impulses tending to bring about expiration. The idea that nicotine apnea was due to stimulation and not depression was supported according to Nicholson and Sobin, by the simultaneous sharp decrease in pulse rate, probably the result of stimulation of the cardio-inhibitory center. The exceedingly brief latent period of the nicotine apnea was said to make it improbable that any extramedullary factors were involved. The complete cessation of phrenic nerve-potential discharges during nicotine apnea led Gold and Modell (71) and Domaye (46) to conclude that the apnea represented complete expiratory arrest (*i.e.*, expiratory apnea); but Nagasaki (130), who also recorded action-currents of the phrenic nerve following intravenous injection of nicotine in rabbits, found that the brief respiratory arrest immediately following the injection was a "hyperpnoeic apnea" due to extreme stimulation of the respiratory center; the diaphragm, receiving continuous impulses from the center, fell into a tonic contractive state. (In explanation, it will be recalled that this apnea in the rabbit is inspiratory, but in the dog and cat, expiratory.)

After bilateral vagotomy and denervation of the carotid-sinus area, both the primary nicotine apnea and respiratory stimulation by nicotine disappeared, leaving only the secondary nicotine apnea unaffected (46). When, in addition, the spinal cord was transected just below the origin of the phrenic nerves, and both phrenic nerves severed, the animals being maintained under artificial respiration, nicotine still caused the secondary apnea, as revealed by the phrenic action-current. This was taken by Domaye (46) to suggest a central origin of the secondary apnea. Secondary nicotine apnea was unaffected by vagal stimulation (43) and not prevented by vagal section (43, 86). It would appear, however, that the mechanism of secondary nicotine apnea is still not thoroughly worked out.

There seems little doubt that nicotine may also exert its classical action—stimulation, followed by depression—directly on the respiratory "center" (or "centers"), although, because of lack of data, it is difficult, if not impossible, at this time to integrate most of the respiratory pharmacology of nicotine with current concepts in respiratory physiology. Application of nicotine to the vagal nucleus in the medulla of cats (77) or to the floor of the fourth ventricle (70) caused pronounced respiratory stimulation; but Comroe (36) has pointed out that injection of drugs into the fourth ventricle is not equivalent to localized injections into the medulla, and that it is probable that systemic absorption takes place more readily than penetration through the 5 mm of nervous tissue separating the respiratory center from the surface of the brain stem. Using the Horsley-Clarke stereotaxic instrument, this last worker injected 1 to 5 % nicotine

directly into the region of the respiratory center in anesthetized cats, and reported that weak stimulation of respiration occurred with only 4 of 45 injections.

Another type of experiment demonstrating a direct stimulation of the respiratory center is that in which the respiratory effects of small and large doses of the drug are tested in animals with the carotid sinus nerves intact or severed. Small doses of nicotine produce marked stimulation of respiration in animals with intact sinus nerves, but not following bilateral section of the nerves; with large doses, however, the stimulating effect was approximately equal, whether or not the sinus nerves were intact, and it seems reasonable to conclude that doses of this size act directly on the respiratory center (1, 88, 183). Stimulation of the respiratory center seemed especially marked in the decerebrate animal (154, 183).

Central paralysis of the respiratory center (or centers) as a late result of nicotine has been demonstrated in cross-circulation experiments by Franke (59), J. F. Heymans and C. Heymans (96), and Houssay and Hug (97). But the peripheral curare-like paralysis of the respiratory muscles invariably occurs before central respiratory paralysis or failure (168).

To sum up the respiratory pattern of nicotine: low doses cause reflex hyperpnea *via* the carotid and aortic bodies; higher doses result in direct stimulation of the respiratory center; lethal doses effect peripheral curare-like paralysis of the respiratory muscles; and extremely high doses result in paralysis of the respiratory center.

C. Vasomotor functions

The term "center," as currently used, implies a functional rather than an anatomical unit—one in which impulses from a variety of sources are integrated. Reference is commonly made to functional divisions of the vasomotor center, *i.e.*, the vasoconstrictor, vasodilator, venomotor, cardio-accelerator, and cardio-inhibitory centers, but these subdivisions appear to have no anatomical counterpart. They are terms of convenience in the description of aspects of its motor effects (H. E. Hoff in Fulton, 64).

Cross-circulation experiments in dogs made it clear that nicotine may stimulate the cardio-inhibitory center directly (95, 96, 97), although this direct central mechanism is far less sensitive than the carotid body reflex mechanism by which nicotine also provokes cardiac slowing (90, 91, 92). These same cross-circulation experiments indicated that nicotine has an intense and very prolonged excitant action on the vagal center; and since, in the intact animal, cardiac inhibition from nicotine is fleeting, the suggestion was made by J. F. Heymans and C. Heymans (96) that the effects of prolonged excitation of the vagal center are rapidly suppressed by a peripheral vagal paralysis. At the stage of peripheral vagal paralysis, the center is still excitable by a second dose of nicotine. While the peripheral vagal slowing is abolished by atropine (Truhart, 169, among many others), central atropinization has no effect on the bradycardia due to nicotine stimulation of the vagal center (95, 96). Doses of nicotine sufficient to block the peripheral autonomic synapses and myoneural junctions did not block the transmission of the carotid sinus baroceptive reflexes in the vagal cardio-inhibitory

center, or suppress the reactions of the center to the direct stimulating effect of acute ischemia (94). Injection of much higher doses of nicotine may block the response of the cardio-inhibitory center to further injections of nicotine, and decrease or suppress the response of the center to the stimulation by acute ischemia; but synaptic conduction in the center to reflex carotid sinus or aortic baroceptive stimulation is still present. Very high doses of nicotine ultimately block the response of the vagal center to reflex and direct stimulation; such doses are very large, and enormously higher than the amount necessary to block ganglionic transmission and excitability. These observations indicate that nicotine does not interfere with the essential processes of synaptic conduction and excitability in the cardio-inhibitory vagal center. Further evidence of an effect of nicotine on the cardio-inhibitory center is afforded by the observation that nicotine bradycardia is prevented by central nervous depressants (72, 73, 141) and deep narcosis (14).

The action of nicotine directly on the cardio-accelerator (sympathetic) center is by no means as clear-cut as in the case of the cardio-inhibitory (parasympathetic) center. Indeed, early opinions (*e.g.*, 34, 169) that nicotine may stimulate the accelerator (sympathetic) apparatus centrally were based not so much on genuine evidence as on more or less incomplete processes of deduction. Minimally effective doses appear to cause tachycardia reflexly through stimulation of chemoreceptors in the aortic and carotid bodies, while larger doses also stimulate the sympathetic ganglia and the adrenal medulla (72, 73). From the data available, it is impossible to estimate the comparative sensitivity to nicotine of the cardio-inhibitory and cardio-accelerator centers; but taking the parasympathetic and sympathetic systems as a whole, the accelerator apparatus appears to be more sensitive to nicotine than the inhibitory system, which is perhaps the explanation why smoking generally leads to some increase in heart rate in man (111, p. 147).

As with the cardio-accelerator center, there is very little, if any firm evidence concerning an action of nicotine directly on the vasodilator center in the medulla. The initial hypotension preceding the characteristic pressor effect of nicotine was attributed by Velich (174) to stimulation of the vasodilator center, and by von Brücke and Kaindl (176) to central stimulation of the depressor mechanism. Ranson *et al.* (140), however, considered that the vasodilator center was not directly involved in nicotine hypotension.

The vasodilator center may be stimulated reflexly by nicotine action on depressor afferent fibers under conditions that appear to be free of concomitant change in heart rate. Thus, nicotine elicits a depressor reflex from somewhere in the vascular bed supplied by the left circumflex coronary artery in the dog (180, 181) and from afferent fibers in the hind limb of the cat (15). A depressor reflex excited by nicotine and involving chemoreceptors in the rabbit ear also has been described (113, 124).

Nicotine hypertension may be produced by direct stimulation of the medullary vasoconstrictor center. However, experiments like those in which nicotine has been injected into the cerebrospinal fluid bathing the medulla (174, 176), producing marked rises in blood pressure more promptly than appeared to be

explicable on the basis of diffusion into the peripheral circulation, furnish at best only suggestive evidence of a direct effect of the drug on the vasoconstrictor center.

In any event, the sensitivity to nicotine of the vasoconstrictor center by direct stimulation by the drug is far less than to reflex stimulation. For example, in dogs, a 1.0 mg dose of nicotine was necessary to produce a vasomotor action of central origin, compared to 0.0001 mg injected into the arterial supply to the carotid body (93). Nicotine also stimulates the vasoconstrictor center *via* pressor afferent fibers other than those from the carotid sinus area: *via* receptors in the auricles (45), the aortic body (72, 73), and perhaps in the spleen (28); a pressor reflex *via* the sublingual nerve (170) and, no doubt, others from other afferent nerves (see H. E. Hoff, in Fulton, 64, *op. cit.*, p. 767). "Whatever their source and route to the vasomotor centers, the impulses appear to work their effects by stimulation of the 'pressor' and inhibition of the 'depressor' centers" (H. E. Hoff, *loc. cit.*).

VII. CEREBELLAR FUNCTIONS

Stefantsov *et al.* (160) found that application of a tampon soaked in 0.5 to 10% nicotine solution to the cortex of the cerebellum, or injection of 1 to 2.5 mg nicotine 1 to 2 mm deep into the exposed cerebellum of cats, resulted as a rule in immediate complete disappearance of contraction of the nictitating membrane following cervical sympathetic stimulation. When disappearance of contraction was immediate, recovery occurred in 8 to 10 minutes. The authors concluded that the cerebellum is concerned not only with control of somatic functions but also takes part in autonomic regulation. These results are difficult to interpret; while it has been shown that electrical stimulation of cerebellar structures causes parasympathetic activation, and inhibition of sympathetic discharge to the pupil and nictitating membrane, these effects are undoubtedly produced at a central, rather than a peripheral level (Moruzzi, 127a, pp. 87–93). In view of the large amount of nicotine employed, it seems possible that sufficient may have been absorbed systemically to produce ganglionic blockade.

Spillane (158) has reported that intravenous injection of 2 to 3 mg of nicotine acid tartrate noticeably increased the ataxia in patients with spinocerebellar ataxia who complained of the adverse effects of tobacco-smoking on their disability. The author suggested that this effect might be brought about by some action of nicotine on the central nervous system involving disturbance of synaptic transmission of nerve impulses. A recent reviewer has remarked that we possess practically no reliable facts about the effect of nicotine on the cerebellum, the brain stem, and the spinal cord in man (173, p. 158).

VIII. SPINAL FUNCTIONS

A. General observations

It has often been reported that the action of nicotine on the spinal cord consists of a stage of stimulation, followed by a stage of paralysis (111, p. 71).

But Franke and Denvir (61) concluded from their work that the spinal depressant action of nicotine, if it occurs at all, had been greatly overestimated in the literature. The apparently contradictory reports in the literature were thought to be probably largely due to the conditions under which the experiments were carried out: general anesthesia was usually used, and the species of animal employed and the dose of nicotine administered varied widely (60).

Bülbring and Burn (16) devised a system whereby one circulation of perfusing blood supplied the spinal cord, and a second supplied the muscles. In dogs so prepared, with the spinal cord divided in the lower thoracic region to avoid the influence of the higher centers, injection of 0.2 mg nicotine acid tartrate into the cord circulation, following prior injection of physostigmine or neostigmine, caused a discharge of impulses from the spinal cord, which were recorded as contractions of the quadriceps or tibialis anterior muscles. Nicotine did not cause spontaneous contractions unless either of these anticholinesterase agents had been given previously.

Application of 2% nicotine to the ventral surface of the spinal cord of the dogfish (*Scylliorhinus canicula* L. Gill) had no effect on the chronaxie (measurement made on the ventral surface), but initial application of the drug to the dorsal surface caused a 30 to 75% decrease in the chronaxie from its original value (144). After the fourth application, however, the chronaxie was elevated to about 200% of its original value (measurements made on the dorsal surface). When nicotine in a concentration of 3 drops per 100 ml of saline solution was brought into contact with the exposed spinal cord of the frog in the region of the emergence of the lumbar nerves, the chronaxie of the centripetal (afferent sensory) nerve underwent no appreciable change, but the excitability of the cord was modified, the voltages being elevated and the curve raised a little on the abscissa axis (122). This was the reverse of the effect obtained with morphine.

Nicotine does not appear to have the blocking effect on synaptic transmission in the spinal cord that it shows on sympathetic ganglia and neuromuscular junctions (171, 172). According to Takagi and Oomura (165), the reflex activity of the isolated spinal cord of the bullfrog (*Rana catesbeiana*) was facilitated in a bath of 0.01% nicotine, but depressed and abolished by 0.1%. In the cat under artificial respiration, as little as 2 mg nicotine intravenously produced a slight depression of reflex activity and increasing dosage up to 150 mg/kg [lethal dose = *ca.* 2 mg/kg] caused further depression but failed to block completely reflex activity. However, topical application of 1% nicotine to the cord did produce block. Intracellular recording from both frog and cat motor neurones showed no change in the synaptic potential after application of nicotine, but the spike appeared after a shorter synaptic delay, and one or more additional spikes appeared. When the synaptic delay became sufficiently short, however, all spikes suddenly disappeared, leaving the still unchanged synaptic potential. Occasionally, the synaptic delay was again increased just before the spike potentials disappeared. Nicotine first increased, and then decreased, excitability of the frog motor neurone. Takagi and Oomura concluded from these studies that high concentrations of nicotine block synaptic transmission in the central nervous

system, acting on the cell body, but not on the synaptic potential. Gualtierotti (74) concluded from experiments on "spinal" frogs that the observed depressant action of nicotine was due to an induced local blocking of neurones; Peters *et al.* (138), from their experiments on the salamander (*Triturus viridescens*), suggested that nicotine acts intermittently on anterior horn cells of the spinal cord and associated motor units.

B. Spinal reflexes

In frogs, reflex activity is enhanced by small doses of nicotine, or as an initial effect of larger ones. The galvanic reflex is weakened or completely suppressed by nicotine when it is still impossible to recognize motor paralysis (177). In frogs poisoned with nicotine, a progressive decrease in reflex activity occurs: the corneal reflex disappears first, followed by reflexes from stimulating the skin of the head, forelegs, body and, finally, hind legs; eventually, all reflexes disappear (110). Using suitable techniques (*e.g.*, the Claude Bernard preparation), however, spinal reflexes may be obtained in frogs in which peripheral paralysis (neuromuscular block) has been produced by nicotine (61, 126, 177). A more detailed account of the effect of nicotine on various reflex activities in several sorts of frog preparations is given by Larson *et al.* (111, p. 72 ff.).

In mammals (cats, dogs), nicotine depresses or abolishes the patellar tendon reflex (knee-jerk) (16, 39, 53, 60, 102, 154, 166, 171, 172, 179). In studies by Schweitzer and Wright (154), increase in the knee-jerk was not apparent at any stage of the response or with any size of dose, but the reflex declined at a rate which varied with the dose. Decerebrate animals proved more resistant to the inhibitory action of nicotine than animals under chloralose, probably because the anesthetic facilitated the depressant action of nicotine. Further experiments by Schweitzer and Wright led them to conclude that nicotine inhibited the patellar reflex by a direct action on the spinal cord; this was confirmed by Werner *et al.* (179), who also found that electrical stimulation of the region of the reticular formation interrupted the inhibition of the patellar reflex by nicotine for the duration of the stimulus. According to Ginzel *et al.* (69), the abolition of the inhibiting effect of nicotine on the patellar reflex through electrical stimulation of the facilitatory area of the reticular formation of the diencephalon could be observed only when the doses of nicotine used were not much higher than those which just caused a complete elimination of the patellar tendon reflex. The inhibitory effect of nicotine on this reflex was not prevented by hexamethonium, diethazine, mephenesin, or guaiacol glyceryl ether (Myocaine) (69) or by atropine (16); it was, however, counteracted by a large dose of acetylcholine (16), by strychnine (69), and by caramiphen, guaiacol glyceryl ether (166), and Parathion (O,O-diethyl O-[4-nitrophenyl] phosphorothioate, E 605) (53). The nicotine inhibition was also counteracted by passage of a 5- to 10-milliampere direct current through the quadriceps muscle in such a way that the anode was applied near the tendon and the cathode in the inguinal region (69).

A number of investigators have compared the effect of nicotine on the monosynaptic patellar reflex with its effect on polysynaptic reflexes (homolateral

flexor, contralateral extensor reflexes). Regardless of the experimental techniques employed (for which the reader is referred to the original articles), all results agree in demonstrating that nicotine diminishes or abolishes the patellar reflex, while the flexor reflex is relatively unaffected or even enhanced by the drug (16, 39, 53, 60, 102, 153, 166, 171, 172, 179).

According to van Harreveld and Feigen (171, 172), in cats under artificial respiration, with reflex action potentials being led off from the ventral roots of L-7 or S-1 while stimulating the homolateral dorsal root at the same segment level, injection of 5 mg nicotine/kg into the carotid artery suppressed the knee-jerk by central action, while the flexor reflex was suppressed by larger (*ca.* 20 mg/kg) doses of nicotine due to the peripheral curare-like action of this drug. The monosynaptic spike of the reflex action potential was depressed or abolished by nicotine in small doses, but reappeared after the administration of large (100 to 200 mg/kg) amounts of the drug. The multisynaptic activity was much less affected by nicotine; the changes were qualitatively similar to those of the knee-jerk, however. In preparations in which the peripheral curare-like effect of nicotine was prevented by establishing a separate circulation in the hind leg, it could be shown that the monosynaptic activity observed after large doses of nicotine was the equivalent of a myotatic reflex activity (knee-jerk) in the unpoisoned state. The depressing effect of nicotine in small doses on the knee-jerk was thought by these workers to be due probably to a side-action. On the other hand, Taugner and Culp (166) assumed that nicotine in threshold doses led, above all, to excitation of interneurones; and they suggested that the paralysis of the patellar reflex was possibly brought about in part by excitation of interneurones of higher order which exerted an inhibitory reaction on this reflex. This latter explanation was accepted by Erdmann and Schaefer (53).

Curtis *et al.* (39) reported pharmacological studies on spinal reflexes in cats under light pentobarbital anesthesia, with all lumbar dorsal roots either cut or crushed. Following injection of 0.025 mg nicotine essentially into the lumbar arteries, the testing monosynaptic reflex in L-7 and S-1 segments was almost invariably transiently depressed; polysynaptic reflexes were simultaneously depressed; monosynaptic reflexes of both flexor and extensor motor neurones were also depressed. This depression was thought to be due to the transient excitation of a special group of interneurones, the Renshaw cells, lying in the ventromedial region of the ventral horn of the spinal cord, which are known to be cholinergically activated from the motor axon collaterals, and which exert a powerful inhibitory action on motor neurones (see also 37, 38, 48, 49).

According to Koll and Schütz (102), there is much evidence which proves the presence of cholinergically activated elements in the central nervous system, the action of which is increased by the influence of anticholinesterase drugs. The rise of reflex discharges under the influence of these substances is interpreted as a consequence of increase of acetylcholine at cholinergic synapses and the action of acetylcholine at these synapses has been called a "nicotinic" one. These authors therefore felt it would be of interest to investigate whether nicotine itself has a similar effect on these cholinergic elements; for this purpose, experi-

ments were performed on several reflex systems of low-spinal cats. Reflex discharges were elicited by electrical stimulation of (a) the afferent α-fibers of the gastrocnemius nerve and of the nerve to the flexor-hamstring group; (b) the afferent, higher threshold A-fibers of the same nerves; (c) the afferent α-γ-fibers of the sural and superficial peroneal nerves; (d) the afferent post-γ-fibers, and (e) the afferent C-fibers of the latter. Records were taken from the homolateral L-7 or S-1 ventral root. In another series of experiments, the mechanically elicited knee-tendon jerk, and the homolateral flexor reflex (elicited by electrical stimulation of the superficial peroneal nerve), were recorded myographically. In dose-ranges of 0.04 to 4 mg nicotine bitartrate/kg, intravenously, only depression of all investigated reflex-discharges occurred; increases were never observed. Monosynaptic reflex-discharges were much more diminished than polysynaptic ones. Also, the polysynaptic nociceptive reflex-discharges, elicited by stimulation of afferent post-γ- and C-fibers, were only depressed, and the knee-tendon jerk and homolateral flexor reflex were never augmented by the action of nicotine, but were regularly diminished. The authors concluded from these results that it does not seem possible to speak in general terms of a "nicotinic" action of acetylcholine in the central nervous system. They considered that discrepancies between the depression of monosynaptic reflexes by nicotine and acetylcholine, and their augmentation by anticholinesterase drugs, need further investigation, as does the much weaker depressive action of nicotine on polysynaptic reflexes.

IX. SUMMARY

In general, small doses of nicotine have a stimulating action on the central nervous system whereas large doses depress. However, studies of the central nervous actions of nicotine have not yet fully established confident correlations of the action of this drug with central levels. For this reason it has not been possible to consider its actions on the basis of a strictly neuroanatomical outline.

Mammalian studies also indicate that conditioned reflexes may be inhibited by nicotine. It does appear that nicotine abolishes the relationship between the strength of the conditioned stimulus and the response, for example, salivation. Increasing the dose of nicotine results in progressive inhibition, culminating in total loss of conditioned food reflexes. While the mechanism of action of nicotine upon conditioned reflexes has been speculated upon in the literature, there is presently no certainty as to whether a specific site or mode of action is involved or whether one is dealing here with a general depressant effect upon animal behavior as a whole. In some experiments, nicotine caused marked inhibition of conditioned reflexes with increase of secretory responses to the unconditioned stimulus of feeding. This has been held to prove that certain doses of nicotine have a stimulating influence on the centers of unconditioned reflexes. Thus, it has been said that the effect of large doses of nicotine is to inhibit cerebral cortical function and not cause paralysis of the "center" of salivation. Although suggestive of the need for further investigation and the line which such investigations might take, these studies do not help greatly in localizing the neurophysiological mechanisms involved.

With respect to learning experiments in animals, a somewhat similar unsatisfactory situation exists. Nicotine appears to reduce the performance of white mice in a maze, with regard to both running time and errors in acute and chronic experiments. However, these effects on performance are probably not due to any specific interference with the learning process itself but may be explained on the basis of non-specific effects upon the general health of the animal. There does not yet seem to be any conclusive evidence, either in animals or in man, of a specific effect of nicotine upon learning.

As has been pointed out, a feature of the reported experiments on the effect of nicotine upon sensorimotor functions is the very high concentrations of nicotine solutions used in topical applications to the brain surface or local injections within the brain. These concentrations were so high that they could never be reached by systemic administration of even lethal doses; moreover, the concentrations were often sufficiently high to produce local non-specific tissue necrosis at the site of application. Therefore, it is doubtful that these experiments on sensorimotor functions gave results that offer indication of the actual pharmacological action of nicotine.

It has been found that visual action potentials in the optic lobes evoked by flashing lights in the eyes in frogs are abolished within a few minutes by local application of nicotine to the exposed optic lobes. These studies and the others that have been reviewed indicate the breadth of possible fields of study of the effects of nicotine upon brain potentials. Turning to electroencephalographic changes in mammals, nicotine administration causes typical "grand mal" seizure patterns if sufficiently large doses are given. Smaller doses appear to cause only prolonged desynchronization. With somewhat larger doses, there may be tonic and clonic contractions as well as muscular fibrillation and the appearance in the EEG tracing of convulsive patterns. Very likely the desynchronizing effect of nicotine is due to excitation of the reticular formation. The convulsive effect has been ascribed to a general state of excitation, probably including cortical neurones. With even larger doses, EEG changes characteristic of an arousal reaction are obtained. With regard to the electroencephalographic changes in the human subject associated with cigarette-smoking, namely an increase in the dominant alpha rhythm, it appears that this is the result of psycho-physiologic changes relative to the act of smoking rather than to physiologic or metabolic effects from specific substances present in cigarette-smoke.

Tremor, one of the highly characteristic effects of nicotine both in man and in lower animals, is undoubtedly a result of central nervous stimulation, although nicotine does have a demonstrable effect directly upon skeletal muscle. Tremor is usually followed by convulsions in animals if the dose of nicotine is sufficient. The site of the convulsive action of nicotine has been variously localized. For example, it has been argued that the antinicotinic effect of ethopromazine and other drugs is at the level of the reticular substance and probably involves a cholinergic mechanism, such as blocking acetylcholine actions centrally. It has been held also that the convulsive action of nicotine is due to alteration in hippocampal excitability, or that it acts at the level of the diencephalon, or that

the convulsive effect is due to a general state of excitation probably including cortical levels. The upper levels of the central nervous system are probably broadly implicated as sites of the convulsive action of nicotine.

With regard to the paralysis which is seen to follow the convulsions in experimental nicotine poisoning, mechanisms similar to those involved in convulsions seem to be present. The site of action of nicotine in causing catalepsy has not been identified with certainty and further study of this matter is required.

As to medullary functions, it has been stated that we possess practically no reliable facts about the effects of nicotine on the brain stem (or the cerebellum or spinal cord) in man. Nausea and vomiting are the most common symptoms and signs of acute nicotine poisoning in man, and the localization of the emetic action of nicotine has been the subject of considerable study. Small doses of nicotine are emetic while large doses fail to cause vomiting and in fact may even be anti-emetic. As to the site of the emetic action, investigations in animals in general indicate that central as well as peripheral factors are involved. Small doses of nicotine applied topically to the vomiting center increase its reflex excitability. However, it may be that nicotine does not act directly upon the vomiting center in exerting its emetic action. Ablation of the emetic chemoreceptor trigger zone (CT zone) in the area postrema of the medulla oblongata in dogs suffices for the protection of the animal against the emetic effect of nicotine. In cats, however, nicotine appears to act upon some peripheral locus as well as at the CT zone, since to prevent vomiting due to nicotine in this species it is necessary to carry out spinal deafferentation and mid-cervical vagotomy as well as CT zone ablation. It would therefore appear that both central medullary and afferent nervous mechanisms are involved.

With small and moderate doses of nicotine, it appears to have been established that the effects on respiration are reflexly mediated, and that lethal doses effect peripheral curare-like paralysis of the respiratory muscles. Similarly, the bradycardia produced by nicotine is of reflex origin, comparatively very large doses being required to stimulate the cardio-inhibitory center directly. There is virtually no evidence for a direct central origin of the cardio-accelerator effect of nicotine. Minimally effective doses appear to cause tachycardia reflexly through stimulation of chemoreceptors in such structures as the aortic and carotid bodies, while larger doses stimulate the sympathetic ganglia and the adrenal medulla. This mechanism also holds for the hypertensive effects of nicotine, although large doses may also produce vasomotor action of central origin.

The actions of nicotine upon cerebellar function are poorly understood. The report that topical application of nicotine to the exposed cerebellum causes disappearance of contraction of the nictitating membrane in response to cervical sympathetic stimulation warrants further investigation, since it provides another indication of the possible jurisdiction of the cerebellum over autonomic regulation. Further studies of the effects of nicotine upon cerebral cortical and cerebellar visceral control mechanisms are also indicated.

As to the effect of nicotine upon spinal functions, some studies indicate a stage of stimulation followed by a stage of inhibition or paralysis. However, it has been

stated that the spinal depressant action of nicotine has been considerably overestimated in the literature. It has been held that nicotine does not have the blocking effect on synaptic transmission in the spinal cord that it shows on sympathetic ganglia and neuromuscular junctions. Other studies show that nicotine diminishes or abolishes the patellar reflex in animals while the flexor reflex is relatively unaffected. Monosynaptic activity appears to be depressed while multisynaptic activity is much less affected by nicotine, and may even be facilitated. It may indeed be that nicotine excites internuncial neurones, for example, the Renshaw cells. Analysis of the literature on spinal functions has exposed many unresolved discrepancies.

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