THE DIFFERENTIAL EFFECT OF QUINIDINE AND PYRILAMINE ON THE MYOCARDIAL ACTION POTENTIAL AT VARIOUS RATES OF STIMULATION

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It has already been reported that quinidine and quinidine-like agents produce a pronounced reduction in the maximum rate of depolarization of the myocardial action potential as recorded with intracellular microelectrodes (Weidmann, 1955b; Johnson, 1956). However, we have since observed that even in the presence of relatively large doses of quinidine there is no reduction in the maximum rate of depolarization if the rate of stimulation is sufficiently slow. This is of interest in view of the hypothesis put forward by Weidmann for the mechanism by which quinidine reduces the maximum rate of depolarization of action potentials derived from Purkinje tissue. His suggestion is that quinidine acts on the mechanism responsible for transporting sodium ions through the fiber membrane during depolarization. We have examined the dependence of the effects of quinidine and pyrilamine on the frequency of stimulation of guinea pig ventricular fibers with a view of gaining further information on the manner by which these drugs interfere with the transport of sodium ions during depolarization.

METHODS. Preparations of guinea pig right ventricle were obtained in the manner previously described and the same microelectrode recording equipment was used (Johnson, 1956). The usual method of examining the effects of drugs on the membrane resting and action potentials is to make a number of penetrations into different fibers, to record the data which is required at each preparation, and to take the mean of the values so obtained before administration of the drug, during its administration, and after washout. In view of the relatively large variation of membrane properties seen in this type of experiment, it was decided that fine changes in membrane behaviour could better be ascertained if the same fiber were kept under observation throughout each experiment. In spite of the mechanical activity of the muscle it was possible to keep the microelectrode in one fiber for periods up to four and one-half hours. Several such experimental runs were made on each preparation. The results are from seven preparations, only one drug being tested on each. If accidental withdrawal of the microelectrode occurred, comparisons were made between results obtained from one fiber before, and another after administration of the drug. Some results were obtained from the same fiber during the administration and after partial recovery from the drug. As the quinidine-like agents tend to depress the mechanical activity of the preparation, withdrawal of the microelectrode during such circumstances is far less common than in actively beating tissue.

When it was considered that the microelectrode was securely lodged in a fiber, the threshold for stimulation at the external electrodes was found. Measurements were then made of the amplitude of the action potential and of the maximum rate of depolarization (maximum rate of rise of the action potential) in the arbitrary units provided by the oscilloscope grille. These measurements were carried out over a wide range of stimulation frequencies (usually 0 1 to 10/sec.), and the increases in threshold for stimulation at the external electrodes at high frequencies were noted. Whenever a change in action potential amplitude occurred, we determined whether this was due to an alteration of the diastolic resting

potential or of membrane reversal (overshoot). Quinidine sulphate (10 microgm./ml.) or pyrilamine maleate (7.5 microgm./ml.) was then introduced into the bath by changing over from the inflow of normal Krebs-Henseleit to Krebs-Henseleit solution containing the drugs in the above concentrations. After five to ten minutes the above experimental procedure was repeated and finally the drug was removed by washing out the bath with fresh Krebs-Henseleit solution.

RESULTS. *The effect of frequency of stimulation on the membrane resting and low action potentials of the normal fiber.* As the rate of stimulation was increased from low values (0.1/sec.) there was no discernible change in the amplitude or maximum rate of rise of the action potential until a frequency of 2 to 4/sec. was reached. Above this frequency the maximum rate of rise of the action potential progressively declined as the frequency of stimulation was increased such that at 10/sec. the value was 10 to 50 per cent less than below 2 to 4/sec. This was consistently observed.

It is known that a reduction in the membrane diastolic resting potential will cause a decrease in the maximum rate of depolarization of Purkinje fibers (Weidmann, 1955a). We were able to show that there was no significant change in the diastolic resting potential with a sudden increase in rate of stimulation (from 0.4 to 4/sec.) sufficient to cause a substantial fall in the maximum rate of rise of the action potential. Any change which might have occurred was within the short term spontaneous drift of the amplifier, contact potentials and membrane noise (± 1.5 mV).

The amplitude of the action potential also declined with increase in frequency of stimulation, the decline commencing at approximately the same frequency (2 to 4/sec.) as that of the maximum rate of depolarization. At a frequency of 10/sec. the amplitude of the action potential was 10 to 25 per cent less than at frequencies below 2 to 4/sec. As no concurrent reduction in diastolic resting potential was observed, these changes in action potential amplitude represent alterations in the degree of overshoot.

All these changes, including the increases in stimulation threshold with increasing frequency of stimulation, are illustrated in fig. 1, 2 and 3 which are the results of typical experiments displayed in graphical form.

The effect of quinidine sulphate and pyrilamine maleate. The addition of 10 microgm./ml. of quinidine sulphate to the bathing solution had the following effects. At low rates of stimulation (0.1/sec.) the maximum rate of rise of the action potential and its amplitude approached closely to values obtained prior to the administration of the drug. However, the maximum rate of rise declined steeply and the threshold for stimulation increased at a much lower frequency of stimulation than in the absence of the drug. No difference was observed between the declines in action potential amplitude with increasing rate of stimulation in the normal and in the quinidine-treated preparation. These changes are illustrated in fig. 1 and 2. In fig. 1, data from a normal fiber and one affected by quinidine are presented graphically. The action potential amplitude and maximum rate of depolarization are almost identical in the two fibers at a stimulation rate of 0.1/sec. The maximum rate of depolarization of the fiber affected by quinidine is reduced drastically at rates of stimulation at and above 0.5/sec.

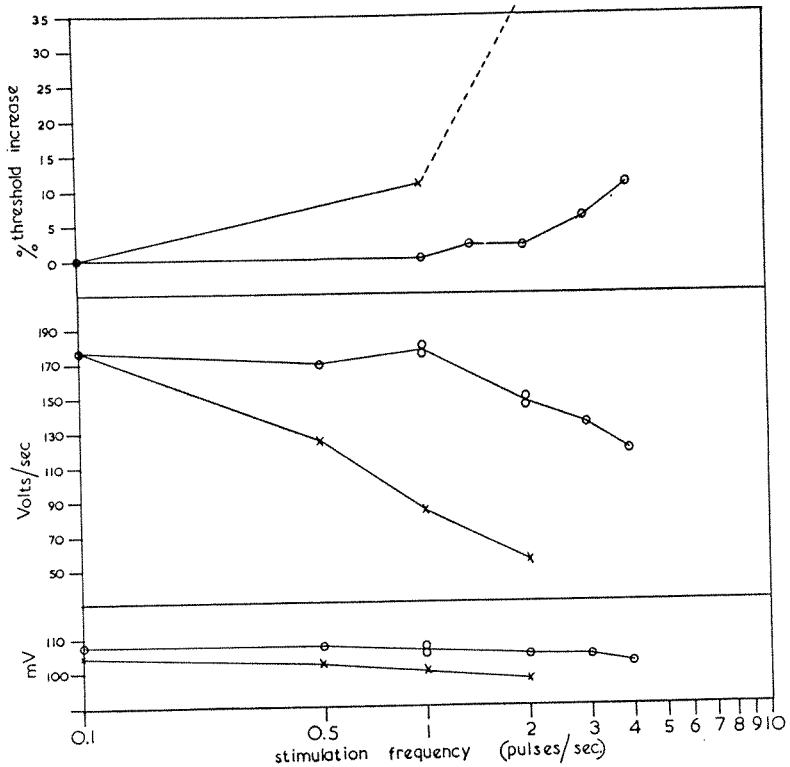

FIG. 1. The percentage increase in stimulation threshold, the amplitude (mV) and maximum rate of rise (Volts/sec.) of action potentials from guinea pig ventricular fibers plotted against frequency of stimulation.

×——× in the presence of 10 microgm./ml. of quinidine sulphate; O——O after washout and partial recovery from the drug. All the data obtained from one fiber.

compared with no change until 3/sec. in the normal fiber. Quinidine produced no significant alteration from the normal in the decline in action potential amplitude at high frequencies of stimulation. Data in fig. 2 were obtained from one fiber in the presence of and following the removal of quinidine sulphate (10 microgm./ml.). This clearly shows that at a frequency of stimulation of 0.1/sec. the maximum rate of depolarization of the fiber in contact with quinidine remains unchanged after the drug is removed; at frequencies higher than 0.1/sec., the maximum rate of depolarization is considerably lower in the presence of quinidine. Pyrilamine maleate had essentially the same effects as quinidine on the preparation. The results displayed in fig. 3 were obtained from one fiber before and during the administration of 7.5 microgm./ml. of pyrilamine maleate.

When the tissue was exposed to either drug no change in the diastolic membrane potential could be detected as the frequency of stimulation was increased. If the frequency of stimulation was suddenly increased or decreased, the maximum rate of depolarization did not change immediately but there was a time lag of approximately 5 sec. during which the new value was

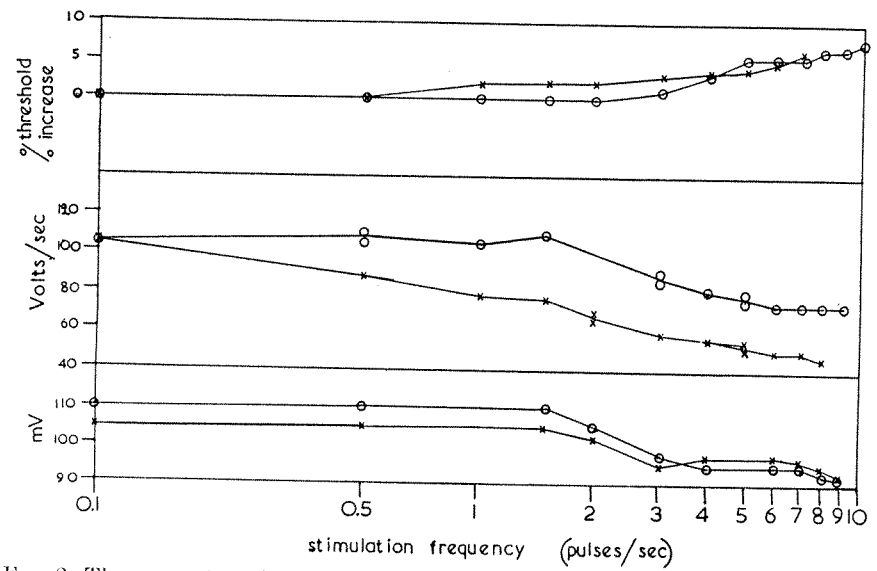

FIG. 2. The percentage increase in stimulation threshold, the amplitude (mV) and maximum rate of rise (Volts/sec.) of action potentials from guinea pig ventricular fibers plotted against frequency of stimulation.

O——O data from one fiber before the administration of quinidine and ×——× data from another fiber in the presence of 10 microgm./ml. of quinidine sulphate.

gradually assumed. It was difficult to detect such a time lag in the untreated tissue as it was not always possible to produce a sufficiently large reduction in maximum rate of depolarization to enable the phenomenon to be clearly seen. The time lag is illustrated in fig. 4 where successive action potentials and their first differentials from a fiber affected by quinidine are recorded on the one exposure following a sudden increase in frequency of stimulation from 0.4 to 4/sec. The maximum rate of depolarization gradually falls with each successive action potential at the high rate of stimulation until it becomes stable at a lower level after several beats.

The lower frequency at which the stimulation threshold began to increase, as compared with the normal fiber, is almost certainly due to an increase in the time for the return of resting excitability after excitation. Observations made throughout the present work indicate that the resting excitability may not have completely returned by the end of the repolarization phase of the action potential. By recording as close to the stimulating electrodes as possible in order to minimize the conduction time of the propagated action potential from the stimulating electrodes to the recording electrode, and as there were no serious differences in the duration of the action potential throughout the tissue (confirmed by experiment). we were able to show that even when stimuli were falling some 100 msec. after the completion of the repolarization phase, an increase in stimulation threshold was observed. In one extreme case, in the presence of 50 microgm./ml. of quinidine sulphate the tissue was absolutely refractory to stimulation at a rate of 1/40 sec. yet the action potential at frequencies slightly below this was completed within

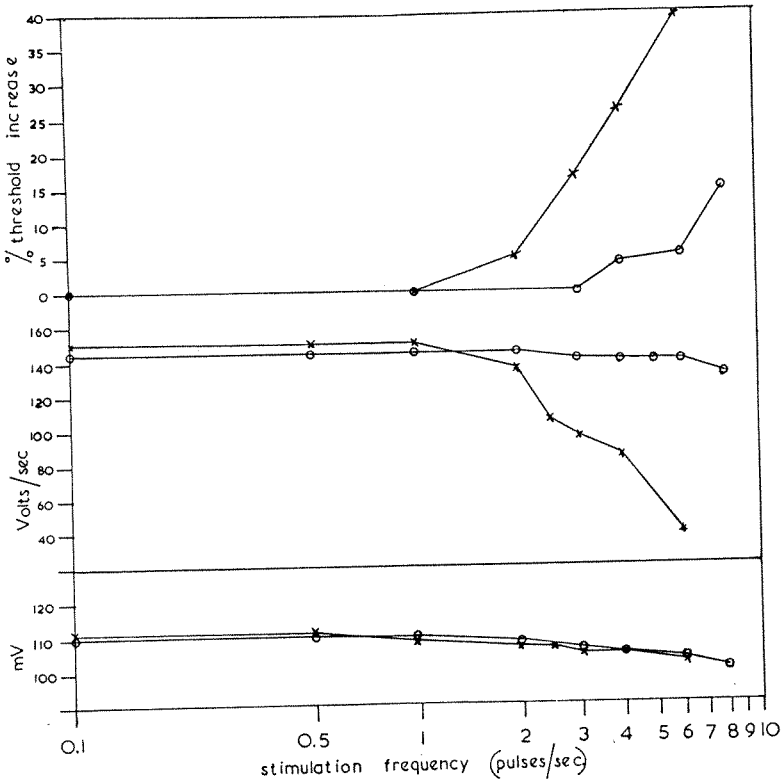

FIG. 3. The percentage increase in stimulation threshold, the amplitude (mV) and maximum rate of rise (Volts/sec.) of action potentials from guinea pig ventricular fibers plotted against frequency of stimulation.

○——○ before the administration of pyrilamine; ×——× in the presence of 7.5 microgm./ml. of pyrilamine maleate. All the data were recorded from one fiber.

approximately 500 msec. Furthermore, at frequencies above 1/40 sec. the tissue would not respond to any of the stimuli, showing that the subthreshold responses produced by these stimuli were sufficient to so exhaust the excitatory mechanism as to render the tissue completely inexcitable.

DISCUSSION. There is evidence that the depolarization phase of the membrane action potential is associated with a selective increase in the permeability of the membrane to sodium ions and a consequent net flux of sodium ions into the fiber down an electroconcentration gradient. Further evidence exists that the passage of sodium ions into the fiber is not a simple diffusion process but is associated with a "sodium carrying" mechanism consisting of "sodium carrying units", (Weidmann, 1955a). According to Hodgkin and Huxley (1952) these sodium carrying units exist in three states: the *resting* state where they may be converted into *active* units when the fiber is depolarized, the *active* state where they are engaged in transporting sodium ions into the fiber and finally the *inactive* state where they are neither carrying nor are capable of carrying sodium ions. Weidmann (1955b) has suggested that these three states may be connected as

(1) Resting ⇌ Active → Inactive. (with ? above and below)

A satisfactory explanation of the effects of quinidine and pyrilamine described in the present work must account for a) the frequency selective nature of the decline in maximum rate of depolarization that the drugs produce, b) the fact that "normal" values of maximum rate of depolarization can be obtained in the presence of the drugs if the tissue is driven at a sufficiently low rate, c) the time lag experienced before the maximum rate of depolarization assumes its new value upon a sudden increase or decrease in rate of stimulation, and d) the failure to observe a paralleled decline in action potential amplitude with the fall in maximum rate of depolarization in the presence of the drugs. The maximal rate

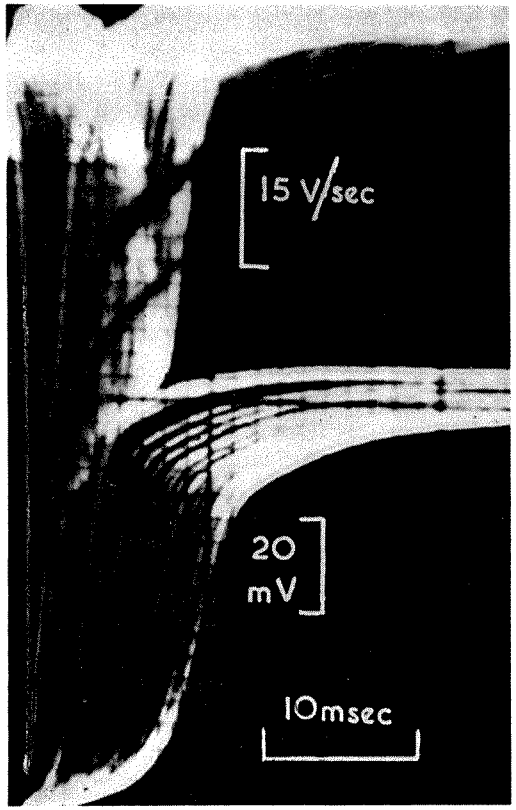

FIG. 4. Record showing successive action potentials and their first derivatives from one fiber exposed to quinidine sulphate immediately following a sudden change in stimulation frequency from 0.4 to 4/sec.

The maximum rate of rise progressively declines and reaches its final value after several beats. Upper trace: first differentials of the action potentials. Lower trace: action potentials. The upper trace is displaced to the left in order to avoid interference with the lower trace.

of depolarization can be considered as proportional to the sodium current or rate of entry of sodium ions during depolarization (Hodgkin and Katz, 1949). There are a number of possible ways by which quinidine and pyrilamine could reduce the rate of entry of sodium ions during depolarization. However, any mechanism which involves a reduction in the number of resting units available at the start of the action potential, either by a direct action or one involving a reduction in the rate of reactivation of inactive to resting units, and any mechanism which interferes with the movement of the carrier through the membrane would require that there be a reduction in the peak value of sodium permeability as well as the rate of entry of sodium ions during depolarization. Unless the sodium permeability normally rose to a value sufficient to swamp the permeability of the membrane to other ions (K^+ and Cl^-), a reduction in peak value of the sodium permeability would be reflected in a reduction in the magnitude of overshoot. It seems unlikely that the sodium permeability rises to a value sufficient to swamp the permeabilities of the membrane to other ions; if this were the case the potential attained at the peak of depolarization should approach that of the sodium equilibrium potential for a sodium concentration cell and would be relatively insensitive to further increases in sodium permeability. Neither of these conditions appears to be satisfied as the degree of overshoot, in Purkinje tissue, for example, falls some 20 mV short of that required for a membrane exclusively permeable to sodium ions (Draper and Weidmann, 1951) and, more convincingly, the overshoot can be increased in Purkinje fibers if the membrane is hyperpolarized prior to the initiation of an action potential. Unless guinea pig ventricular fibers, unlike Purkinje fibers, become exclusively permeable to sodium ions during depolarization, the failure to observe any concurrent reduction in action potential amplitude with the reduction in maximum rate of depolarization in the fiber exposed to quinidine or pyrilamine indicates that under the conditions existing in the present work the drugs reduce the rate of entry of sodium ions without affecting the peak level to which sodium permeability rises during depolarization. The drugs appear therefore to slow the rate of production of active units (slow the rate of rise of sodium permeability during depolarization) without affecting the final number in the active state (peak sodium permeability). In such circumstances a reduction in maximum rate of depolarization would be observed without a decline in action potential amplitude. The drugs do not appear to have a direct effect on the rate of production of active units; provided that the tissue is driven at a sufficiently low frequency the maximal rate of depolarization returns to a value close to that of the fiber unaffected by the drugs. Obviously some subsidiary mechanism is affected as is further suggested by the existence of a time lag before the maximum rate of depolarization assumes its new value after a sudden change in rate of stimulation. Comment on the possible nature of this mechanism is not at present justified.

The increase in stimulation threshold at the higher rates of stimulation shows that the stimuli are falling in the relative refractory period of the tissue beneath the stimulating electrode. As this increase in threshold often commenced at the same frequency as the decline in maximum rate of depolarization, it might be

suggested that there is a direct causal relationship between the two. One piece of evidence appears to be against this view. The change in maximum rate of depolarization which results from a sudden increase in stimulation rate is effected over several beats; the first action potential at the new rate has a slightly lower value than the previous beat, and every succeeding action potential for a few seconds shows a progressively lower maximum rate of rise. This is contrary to what one would expect were it directly connected with the decrease in refractory period which is known to accompany an increase in stimulation rate. The first action potential at the higher rate should show the lowest rate of depolarization as presumably the refractory period is longest at this moment and the stimulus would have fallen further into the relative refractory period than subsequent stimuli.

Weidmann (1955b) has shown that quinidine reduces the maximum rate of rise and overshoot of action potentials derived from Purkinje tissue driven at a constant rate. He found that "normal" values of the maximum rate of rise could be obtained in the presence of quinidine if the fiber were hyperpolarized prior to the initiation of an action potential. He concluded that the effect of hyperpolarization was to increase the number of resting units available at the start of the action potential and that quinidine affected the sodium transport mechanism by shifting the reactions shown in (1) to the right. However, our findings show that quinidine can cause a decline in the maximum rate of rise without producing a concurrent decrease in overshoot. This finding is incompatible with such a hypothesis which requires a concurrent reduction in the amplitude of the action potential as well as a decline in maximum rate of rise. One explanation of this difference may be that in Weidmann's experiments the tissue was exposed to the action of quinidine for one hour prior to the taking of measurements whereas the equivalent time in the present experiments was five to ten minutes. Perhaps the results of Weidmann refer to later effects of quinidine.

SUMMARY

The maximum rate of depolarization and amplitude of action potentials from guinea pig ventricular fibers together with the threshold for stimulation have been measured over a range of stimulation frequencies (0.1 to 10/sec.) in the presence of quinidine sulphate or pyrilamine maleate.

In the normal fiber, a progressive reduction in amplitude and maximum rate of depolarization of the action potential, and increase in stimulation threshold (external electrodes) commence at approximately 2 beats/sec. while no change in membrane resting potential could be detected at this or higher rates.

In the presence of quinidine sulphate (10 microgm./ml.) or pyrilamine maleate (7.5 microgm./ml.) a) the progressive decline in the maximum rate of depolarization and increase in stimulation threshold which occur commence at a much lower rate of stimulation and were more pronounced than in the normal fiber, b) at sufficiently low rates of stimulation the threshold for excitability and the maximum rate of depolarization were unchanged, c) compared with the normal fiber there was no significant change in the decline in action potential amplitude

with increasing frequency of stimulation and d) when the rate of stimulation was suddenly increased or decreased there was a time lag occupying several beats during which the maximum rate of depolarization progressively changed to a new stable value.

These findings are discussed in relation to some possible ways by which the drugs could affect the transport of sodium ions into the fiber during depolarization.

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TOLERANCE TO SOME EFFECTS OF BARBITURATES

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The present study was undertaken to describe certain changes which occur during the course of continuous administration of secobarbital and pentobarbital in doses of 0.4 gram per day. Of particular interest was the development of tolerance which may be defined operationally as follows: 1) the dosage of a drug must be increased to maintain the initial degree of effect, or 2) the initial degree of effect is observed to decrease when the same dose is administered repeatedly. The latter defines tolerance as it is used in the present study. When such definitions are used, no inferences need be made concerning the nature of the mechanisms underlying its development. Although it is generally agreed that tolerance to barbiturates does occur (Seevers, 1931; Fraser and Isbell, 1954), attempts toward quantitative demonstrations of this phenomena in man have met with little success. Isbell and associates (1950) found that it was very difficult to determine whether tolerance developed during the course of experimental addiction to barbiturates, because of the marked fluctuation in the effects of the drugs from day to day. They concluded, however, that tolerance must occur since patients withdrawn from barbiturates show much greater intoxication when replaced on the same dosages which they had attained gradually during addiction. Idestrom (1954), however, succeeded in demonstrating tolerance to the effects of phenobarbital on flicker-fusion and obtained suggestive evidence that cross tolerance to phenobarbital and amobarbital may exist.

Intoxication, with its attendant impairment of performance, may be classified as an undesirable effect of barbiturates, whereas sedation and hypnosis, under appropriate circumstances, are desirable effects. The major purpose of the present study was to obtain quantitative data on the rate and degree of development of tolerance to both classes of effects of barbiturates.

METHODS AND MATERIALS. *Subjects.* Eighteen physically healthy subjects ranging in age from 25 to 57 years volunteered for the experiment. All were male postaddicts who were serving sentences for violations of the Harrison Narcotic Act and who were completely recovered from the debilitating effects of opiate withdrawal.

Clinical observations. Patients were housed in a closed ward and were observed continually by specially trained attendants for a preliminary period of 30 days without drugs, for 90 days during administration of barbiturates, and for 10 days after barbiturates were abruptly discontinued. The observations were as follows: 1) rectal temperature, systolic and diastolic blood pressures, pulse and respiratory rates were taken three times daily after resting in bed for 20 to 30 minutes, 2) body weight and caloric intake were measured daily, 3) hours of sleep were recorded at one-half hour intervals throughout each day, and 4) degree of intoxication was rated every eight hours on a four-point scale as follows: 0, no evidence of intoxication; 1+, mild intoxication; 2+, moderate intoxication; and 3+, marked intoxication.