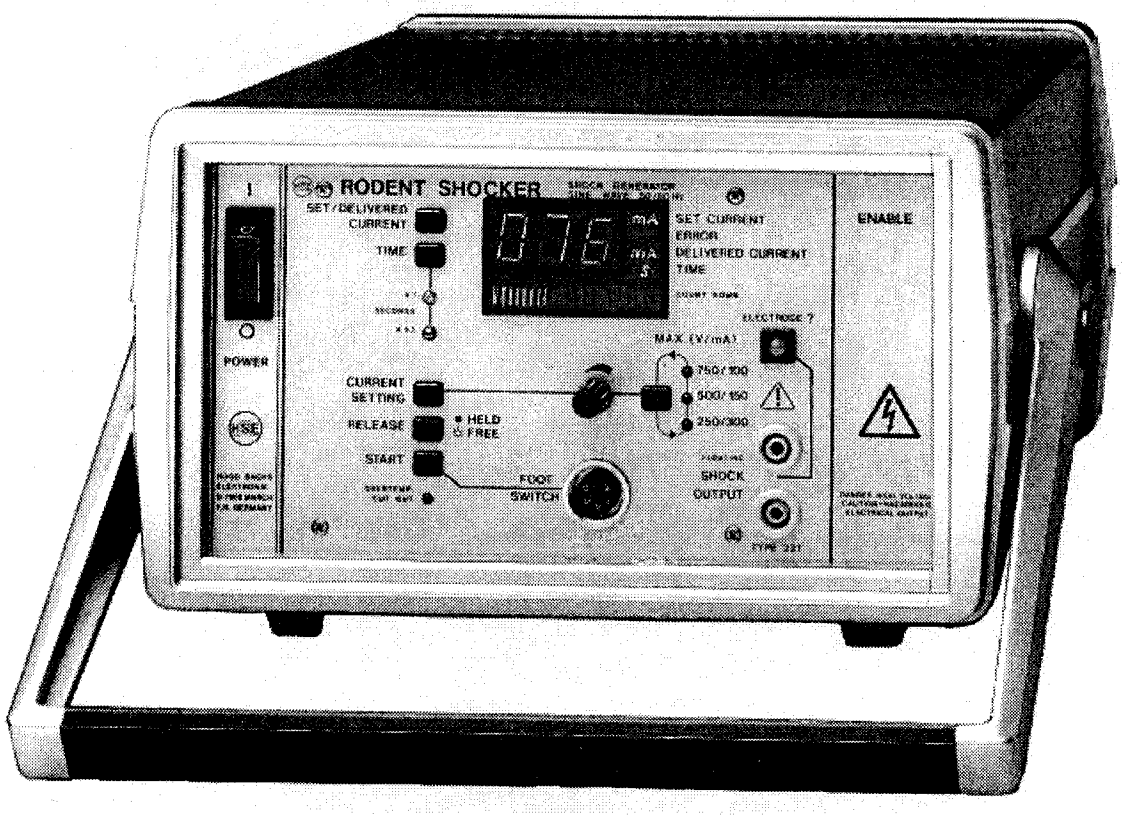
RODENT SHOCKER

SHOCK STIMULATOR
Type 221



This equipement is desingned exclusively for animal experiments and must not be used for clinical applications

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Supplement to the Operating Instructions for the HSE RODENT SHOCKER Type 221

Current version of the Operating Instructions: 170588

For equipment No.: ..... Key No.: ..... Supply date: .....

For equipment after delivery date Janary 1997, with serial numbers from 97031 onwards.

The Rodent Shocker is used to generate shock stimulation. In accordance with its intended use it produces output voltages and currents which are hazardous to humans (max. 750 V, 0,5 A!).

In view of the latent hazard associated with this equipment it is absolutely essential that the Operating Instructions are carefully read before the equipment is started up and used, and that all notes, especially the hazard warnings and all safety precautions, are fully observed.

In order to conform to enhanced demands on safety, all equipments from the above serial number onwards are fitted with an additional safety key switch (marked ENABLE) and also a red flashing warning light. After the equipment has been switched on all the functions are enabled but the output of stimuli (shocks) is blocked. The stimulation output is only enabled after the key has been inserted and the switch has been operated; the red flashing light acts as a warning of the latent hazard.

In order to prevent the use of the equipment by unauthorised personnel which could endanger themselves and other persons, the following procedure is recommended:

- ☞ **Always store the key separately from the equipment !**
- ☞ **Limit access to the key to properly authorised persons!**
- ☞ **The key must be inserted in the switch only while the eqipment is being used !**
- ☞ **After the equipment has been used the key must be removed immediately and stored separately from the equipment (see above)!**

CE Conformity

CE This equipment and accessories conform to the requirements of the Low-Voltage Guideline 73/23/EWG as well as the EMC Directive 89/336/EWG and are accordingly marked with the CE symbol. For conformity with the Standard it is essential that the Instructions for Use are observed when operating the equipment.

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Date: 18.03.97

Operating Manual for the

RODENT SHOCKER

(Version 170588)

CONTENTS

*** HUGO SACHS ELEKTRONIK 7806 MARCH GERMANY ***

1. INTRODUCTION. PRELIMINARY NOTES

The stimulator RODENT SHOCKER Type 221 is specially designed for the electrical triggering of spasms in small experimental animals (mice, rats). Suitable electrodes are used to pass a sinusoidal 50 Hz or 60 Hz current pulse of variable intensity and duration through the brain of the animal.

The current flowing through the brain triggers synchronous excitation of the neurons which leads motorically to clonic and/or tonic convulsions, psychically to loss of consciousness with a loss of sensory and memory capacity. In this way the electrically induced seizure is similar to an epileptic attack and is therefore a standard model for the pharmacological testing of antiepileptic drugs.

Compared with the old shock generator Type 207 the newly developed RODENT SHOCKER offers numerous advantages. The use of a microprocessor not only offers improved indication of the various functions but also ensures enhanced safety in use.

Advantages:

- increased output power, up to 75 Watt (rats)
- choice of output voltage (250-500-750 Volt)
- digital indication of shock current in milliamp
- bargraph display of elapsed time from 0.1 to 9.9 seconds
- permanent monitoring of output circuit
- immediate shut-down on open output or on excessive electrode resistance (with error indication)

2. DESCRIPTION OF EQUIPMENT

The RODENT SHOCKER is contained in a strong aluminium case. The handle can be set to a number of positions by pressing the two knobs at the sides on the handle hinges. Swinging the handle down permits its use as a foot so that the unit can be set at an angle.

2.1 Supply

The back of the equipment carries the supply socket with the fuses. Next to the socket is a metal plate with a recess; behind it is the selector switch for the supply voltage:

(220 V ◄---► 110 V)

Before you connect the equipment to the supply, check that the voltage setting is correct for your supply voltage. If not specified otherwise the equipment is supplied set for 220 Volt.

If you change the supply voltage you must also fit the correct fuse:

for 220 V: T 630 mA or T 0.63 A
for 110 V: T 1.2 A

2.2 Front panel

The front panel carries the controls and the indications (see Section 6 and Fig. 7) for operating the equipment.

At the left top is the mains switch with a built-in signal lamp.

In the centre is the indicator for shock current, time setting, elapsed time and current fault. Operation of a key displays the corresponding function.

The 5-pin socket marked "FOOT SWITCH" is used to the connect up the foot switch supplied with the equipment. The current pulse can then be triggered with the foot so that the hands remain free for handling the animal.

Connector: miniature round plug, 5-pin, male
Binder 09-0013-02-05 and compatible plugs
Amphenol-Tuchel T 3360 001 and compatible plugs

Foot switch connection: contact between pins 1 and 5.

The shock electrodes are connected to the sockets marked "SHOCK OUTPUT". The sockets (for 4 mm banana plugs) are shrouded as protection against touching them. Use only electrodes fitted with suitable shrouded plugs.

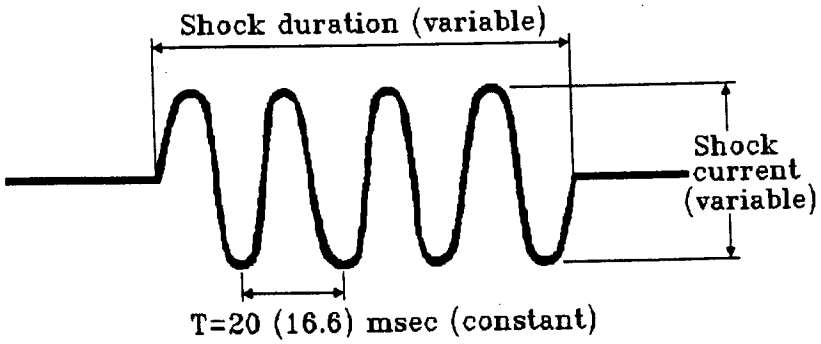


Fig. 1: Form and duration of shock produced. The periodic time T is not variable; it corresponds to the supply frequency. For a frequency of 50 Hz it is T = 20 msec. (USA, 60 Hz, T = 16.6 msec)

2.3 Selection of output parameters: current/voltage/power

The output passes through a special transformer circuit and is fully isolated, i.e. the output sockets have no electrical connection to the case, the ground contact of the mains supply cable or any other part of the circuit. This feature ensures complete safety when working with the equipment.

The output circuit of the RODENT SHOCKER is arranged as a constant current source. This means that the voltage increases automatically with the actual external resistance and the selected current actually flows through the electrodes connected to the unit. This increase is limited by the maximum available voltage.

The maximum output power produced by the RODENT SHOCKER is 75 W. The electrical power P (Watt) is obtained by multiplying the voltage U (Volt) with the current I (Amp):

$$P\ (W) = U\ (V) \times I\ (A)$$

With 75 Watt power output and a current demand of I = 100 mA (0.1A), for example, the maximum voltage available is 750 V, because

$$U\ (V) = P\ (W) / I\ (A) = 75W / 0.1A = 750\ V.$$

The RODENT SHOCKER provides a choice of three different output current ranges. The maximum voltage can then be derived as explained above.

Choice of current-voltage combinations

100 mA / 750 V
150 mA / 500 V
300 mA / 250 V

Figs. 3a to 3c show the output characteristics as a curve plotted against load resistance. The limits of the three ranges are shown in Fig. 2.

The most suitable current-voltage combination for a particular application depends largely on the animal used and on the method of applying the shock.

Shock electrodes with a smaller contact surface generally have a higher contact resistance. When applying shock through ear electrodes there is also a higher resistance than when using eye electrodes. This is turn requires (by Ohm's Law) a higher voltage to ensure that the set shock current level is in fact applied.

In general it is found that working with smaller animals (mice) requires smaller currents but higher voltages than when using larger animals (rats). Further details will be found in Section 5, DETAILS OF EXPERIMENT.

One of the three current-voltage combinationS is set automatically when the equipment is switched on. This combination can be selected on a switch inside the equipment (see Section 4.3, Setting the equipment status).

The combination setting can be changed at any time by holding down the key CURRENT SETTING and at the same time pressing the key MAX(V/mA). Each time the key is depressed the display changes to the next current/voltage combination. When the equipment is switched off the setting selected in this way is lost.

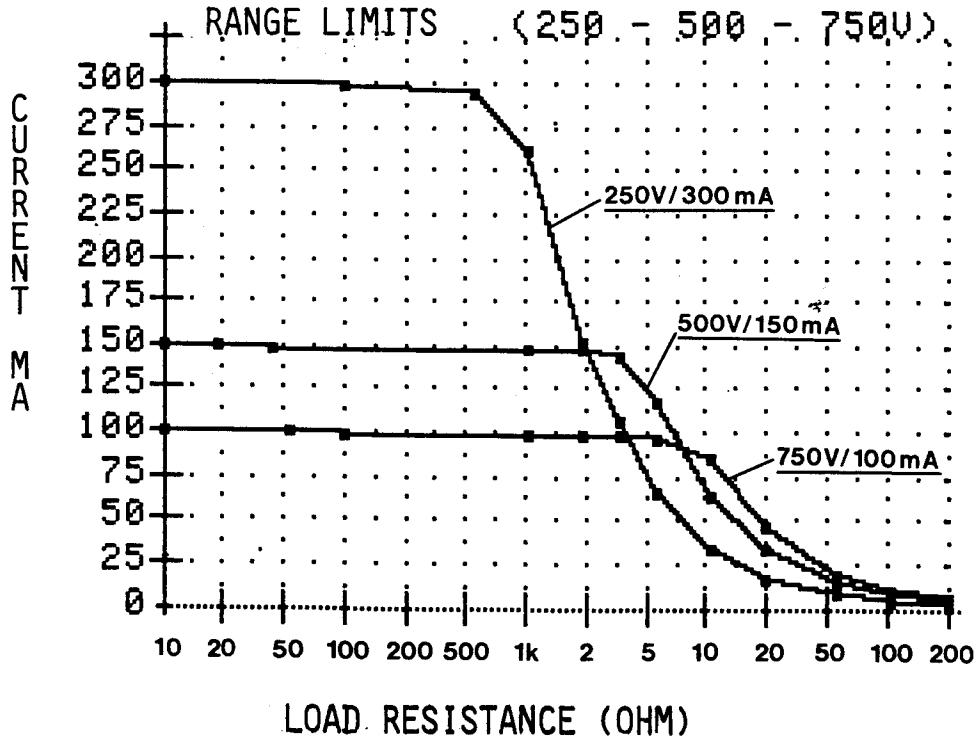


Fig. 2: Limitations of the current-voltage characteristics in the three available combinations for different load resistances.

Fig. 3a

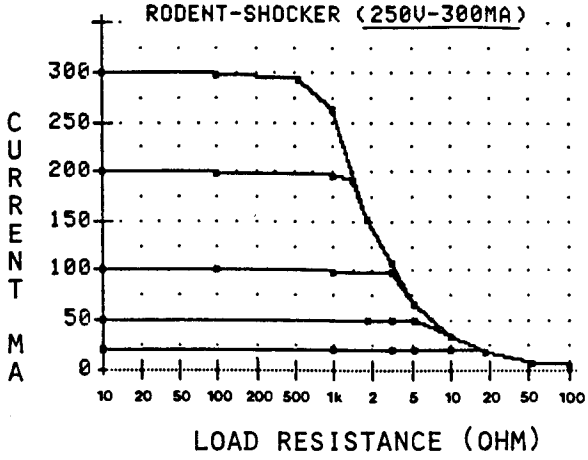


Fig. 3b

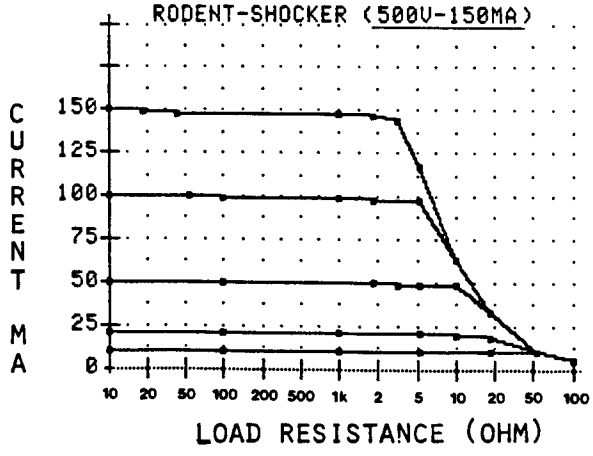
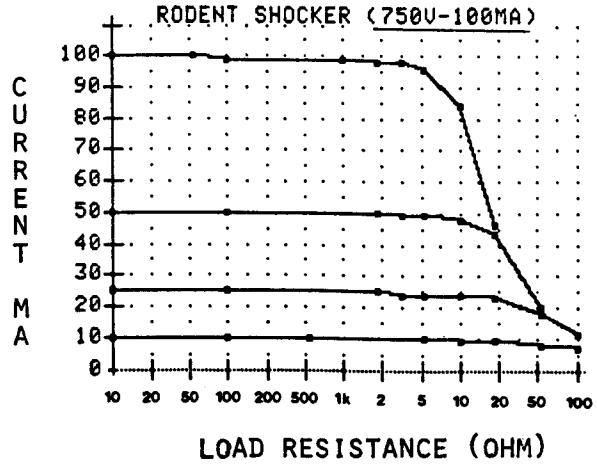


Fig. 3c



Figs. 3a-3c: Output characteristic of the RODENT SHOCKER in the three current/voltage ranges. The shock current is plotted vertically; the total load resistance (electrode and body resistance) is shown horizontally.

2.4 Setting the shock "intensity" (shock current)

The shock current is set by rotating the control knob and is indicated on pressing the CURRENT SETTING key. The adjustment range depends on the current-voltage combination selected (see above).

Note:

If you change the current with the control without pressing the CURRENT SETTING key at the same time, there is a time delay until the new value is indicated.

2.5 Setting the shock duration

The shock duration can be selected in the range from 0.1 to 9.9 seconds in 0.1 sec steps. The adjustment is made by means of a small screwdriver at the two selector switches below the TIME key.

Note:

If you change the time setting you must press the TIME key in order to check the new time settings on the display and to enter the new value into the timer unit.

During the actual shock you can monitor the elapsed time on the bargraph display below the main digital display. If the set shock duration is 1 second or longer, the length of the bargraph represents 100% of the time setting. As the chock continues the bargraph shortens in 10% steps. If the time setting is between 0.1 and 0.9 sec the set time is represented directly as length of the bargraph.

The current time setting can be checked at any time by pressing the TIME key; it is then indicated in digital form.

2.6 Triggering the shock and control of timing

As a safety measure the RODENT SHOCKER is fitted with a trigger block. The shock can only be triggered when the block has been released by intentionally pressing the RELEASE key. The current status of the block is shown by the light signal (LED) in the centre of the key:

Shock trigger blocked:	LED is dark	(o HELD)
Shock trigger unblocked:	LED is flashing	(* FREE)

When triggering of a shock is unblocked the bargraph is visible. When triggering is blocked the bargraph is dark.

The shock can be triggered:

- by pressing the START key, or
- by the foot switch which is plugged into the FOOT SWITCH socket (see Fig. 4).

The shock can be controlled in two ways, either in the trigger mode or in the hold mode.

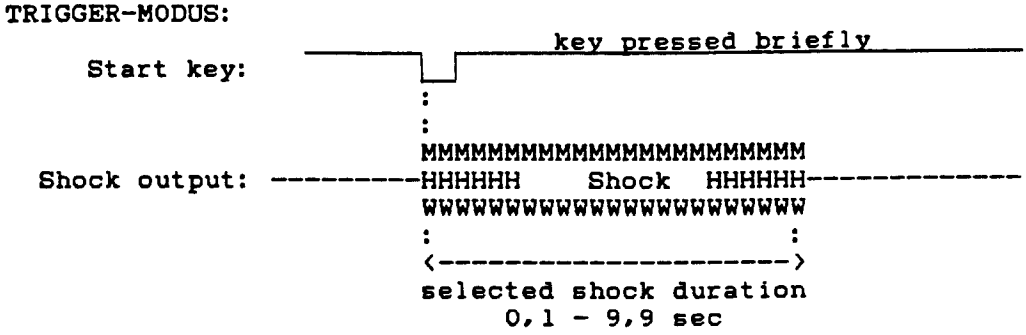
Trigger mode: in the trigger mode the shock is started by briefly pressing the key and then continues for the selected duration (0.1 - 9.9 sec). Once it has been started the shock can be only interrupted by pressing the RELEASE key. The START key has no effect once the shock has been triggered.

Hold mode: with the unit in the hold mode the START key has to be held down until the set time has elapsed, i.e. until the normal end of the shock has been reached. If the key is released while the shock is being produced the shock is interrupted.

In normal use it is advisable to work always in the "hold mode" unless there are special reasons against this. The hold mode provides enhanced safety compared with the trigger mode. If any fault occurs while the shock is being produced, you can react immediately by releasing the key and thereby terminate the shock application. This is not possible in the trigger mode.

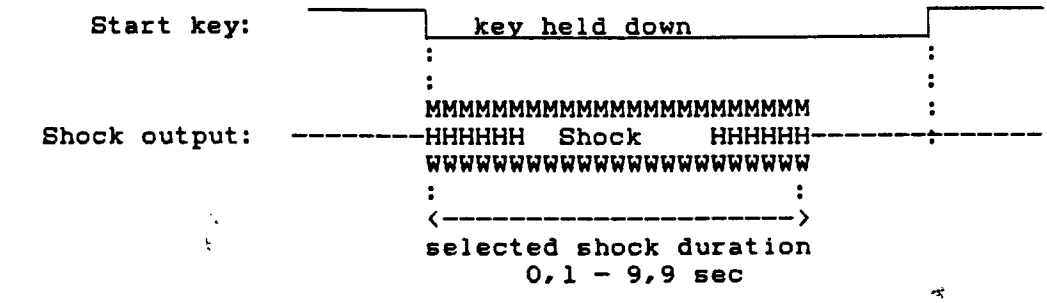
The type of shock control (trigger mode or hold mode) can be selected inside the unit by means of a switch (see Section 4, Starting up, functional tests. function selection). The equipment is normally supplied set for the hold mode.

TRIGGER-MODUS:



HOLD MODE:

a) Normal sequence:



b) Shock termination:

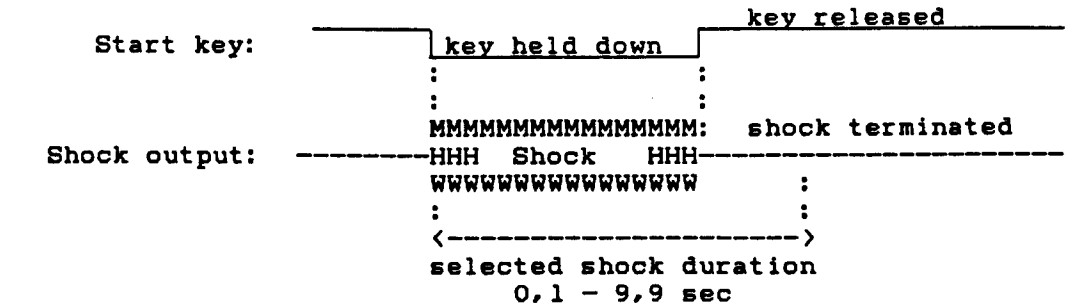


Fig. 4: Triggering a shock in the different trigger modes. Above: in the trigger mode the shock is started by briefly depressing the key and can then no longer be influenced. Below: in the hold mode the shock application can be terminated by releasing the key.

2.7 Monitoring functions, fault recognition, fault indication

The output circuit of the RODENT SHOCKER is being monitored permanently. As soon as a serious fault is detected the output current is interrupted, there is a double beep and a fault message.

The fault report (signal light ERROR flashing) is cancelled by pressing any key except the START key.

The various possible faults and the equipment reaction are summarised below.

* With open-circuit output:

  - electrodes not connected
  - electrodes do not make proper contact
  - electrode resistance too high (above 100 kOhm)
  - break in electrode cable

  the yellow light "ELECTRODE ?" is flashing and triggering of the shock is blocked. If you try to trigger a shock under these conditions, your attention is drawn to this fault by a repeated beep as long as you press the START key.

* If the current is interrupted during the shock, e.g. through unreliable contact in the electrode circuit (bad connection) there is an immediate double beep and the shock is terminated. This is followed by the digital display flashing the final current reading for about 5 seconds. The display then changes back to the selected current SET CURRENT. The fault light ERROR continues to flash and indicates that there has been a fault.

* If the set current decreases by more than 50% during the set shock period, e.g. through an increase in electrode resistance, the ERROR light begins to flash. The shock is not terminated. After the end of the shock the final current reading can be checked by pressing the key SET/DELIVERED CURRENT.

* If the current increases by more than 10 mA during shock application the shock is terminated immediately and there is a double beep. The error light then flashes to indicate the presence of the fault condition.

  This fault occurs when the current setting is increased during the shock application by turning the potentiometer, or if there is some malfunction in the equipment.

In order to reduce the danger as far as possible you should give careful consideration to the use of the equipment and to include the working area and the entire surroundings into the safety assessment.

The working area must be carefully chosen. Carry out a safety check each time before using the equipment. Pay special attention to electrodes, electrode cables and plugs. Have a quick look at the equipment case. If there is any external damage to the case or if there are any signs that liquid may have found its way into the case, you should not use the equipment until after it has been checked by a qualified service engineer.

3.2 Safety precautions

Below are summarised a number of points which you should observe - for your own safety - when using the equipment.

Working area:

The working area must always be kept dry, there must be no water outlet in close proximity (danger of splashing !)

The work bench must not have any metal frame which can be touched, and the work top must have an insulating covering.

Water pipes and heating pipes must not be run in close proximity to the working area.

Use of the equipment:

If you have any suspicion that the equipment is damaged, do not use it until it has been checked thoroughly.

If you notice any damage to the case which suggests that the unit has been dropped, do not use the equipment until it has been checked thoroughly.

It is essential to protect the equipment against humidity !

Never switch on the equipment if any liquid has found its way inside it !

IMPORTANT: Never touch the conducting parts of the electrodes with both hands !

Grasp the electrodes only by the handles provided for this purpose !

Ensure that the handle region of the electrodes is dry and free from electrode jelly or other conducting paste !

Never use any damaged electrodes or electrode cables !

Where possible wear insulating gloves, at least on the hand holding the animal.

Use only electrodes with shrouded plugs !

Never work with moist hands !

4. STARTING UP, FUNCTIONAL TESTS, FUNCTION SELECTION

4.1 Before switching on

Before you connect the equipment to the electrical supply using the mains cable supplied, check that the supply voltage set on the equipment is the same as your supply voltage (220 or 110 V) (see Section 2.1).

Then switch on the equipment with the main switch marked POWER. The mains lamp lights up immediately and the equipment performs a self-test, with all signal indications on the equipment flashing twice. The self-test is completed after about 4 seconds. This is followed by display of the current setting SET CURRENT and indication of the current-voltage combination MAX. (V/mA) which has been set inside the equipment.

If the equipment behaves differently in any way, a fault has to be suspected and the equipment has to be checked by a qualified service engineer.

4.2 Functional tests, getting to know the equipment

If the equipment is OK according to Section 4.1, you should make yourself familiar with the equipment functions. Always remember the hazards explained in Section 3 and always "play safe".

Initially do not connect any electrodes to the shock output,

Check the set shock duration by pressing the TIME key. Use the small screwdriver supplied with the equipment and adjust the shock duration. It should be possible to vary the duration within the range 0.1 to 9.9 seconds in 0.1 second steps. Check this by suitably turning the two switches X0.1 and X1 with the screwdriver while holding down the TIME key. Then set the expected shock duration for your experiments, e.g. 0.5 seconds.

Next check the setting functions for shock current and maximum voltage (MAX. (V/mA).

Press the CURRENT SETTING key and at the same time turn the setting knob. The display shows the changes in the current setting. Next release the key and turn the knob again. You should see that indication of the changed setting is now delayed by 1 - 2 seconds.

The adjustment range of the shock current depends on the current setting of the current-voltage combination MAX. (V/mA). If you change this combination, there is a corresponding change in the current range and therefore in the actual current setting.

Now change the current-voltage combination by holding down the CURRENT SETTING key and briefly pressing the MAX. (V/mA) key. The appropriate signal lamp lights up to indicate that the next combination is selected. At the same time you can note the appropriate change in the current setting on the digital display.

Next carry out a functional test. Observe the hazard notes in Section 3 !

For this test you require a beaker of about 1 litre capacity and a shock electrode, e.g. an HSE eye electrode. "Stand" the electrode in the beaker and pour in saline solution until the conducting parts of the electrode are just immersed. The handle area of the electrode must be kept dry !

Set a current of about 50 mA as described above and release the shock blockage by pressing the RELEASE key. The signal light in the centre of this key which was previously off now begins to flash. The display shows the marks of the bargraph.

Now trigger a shock by pressing the START key (hold the key down until the end of the shock period !) and note the display. The marker near the right edge immediately changes from SET CURRENT to DELIVERED CURRENT, and the bargraph indication becomes shorter in accordance with the time setting and the elapsed time. The current reading should remain the same or vary only slightly during the duration of the shock.

At the beginning and at the end of the shock you can hear a clicking noise which is produced by the control relay.

After the end of the shock period the equipment returns to the initial condition: the marker is again on SET CURRENT, the bargraph marks are again visible and the signal in the RELEASE key which was on continuously for the duration of the shock is now flashing again. The equipment is now ready for triggering the next shock.

Next connect the foot switch supplied to the socket marked FOOT SWITCH and trigger a further shock, this time with the foot switch. Keep the foot switch depressed until the end of the shock period.

The instrument reactions should be the same as described above.

Next you should simulate a circuit interruption while the shock is being produced. First check that the electrode handle is dry, then produce a further shock and remove the electrode from the solution during the shock period.

You can immediately hear a double beep; the bargraph disappears from the display. the digital indication and the two markers ERROR and DELIVERED CURRENT flash for about 5 seconds. The equipment then returns to the same state as before this test except that the two markers ERROR and DELIVERED CURRENT continue to flash to indicate the fault.

Now press the SET/DELIVERED CURRENT key to indicate the current which was flowing at the time the equipment recognised the fault. In the present case the reading should be 000 mA.

After you have carried out all test procedures as described above and repeated them if necessary, you should be sufficiently familiar with the equipment behaviour and there should be no surprises when you are actually using the equipment. However, never forget the high output energy produced by the equipment and remember the hazard notes in Section 3.

4.3 Setting the equipment status (internally)

When the equipment has just been switched on it sets itself to a definite status. This can be changed to suit special requirements, using a 4-way switch inside the equipment (Table 1). The switch is near the top edge of the circuit board located close to the mains switch.

When the equipment is supplied it is set to the functions marked with arrows unless it is otherwise specified.

Table 1: Settings of the selector switch inside the equipment

Switch No.:	1	2	3	4	Function
Settings:	X	X	OFF	OFF	◄-- Hold Mode
	X	X	ON	OFF	Trigger Mode
	ON	OFF	X	OFF	Combination 250V/300mA
	ON	ON	X	OFF	(prohibited !)
	OFF	ON	X	OFF	◄-- combination 500V/150mA
	OFF	OFF	X	OFF	combination 750V/100mA
	X	X	X	ON	test function

Explanation: OFF = switch off (down)
ON = switch on (up)
X = any switch position, no effect
◄-- = standard settings

To change the setting you must open the case. Pull out the mains plug. Then release the two screws at the sides of the front frame. Now you can remove the front frame and also the upper part of the case. Use a small screwdriver or some other tool which is not too pointed (tweezers) to set the switches to the required position according to the table above. Then close the case again before connecting the equipment to the mains supply.

5. DETAILS OF EXPERIMENT

5.1 Experimental technique

The mouse is held with one hand by the fur on its neck (see Fig. 5). An insulating glove should be worn on this hand to protect the operator.

Shock can be induced either by applying moistened electrodes to the eyes, or by application to the ear passage (here the use of commercial EEG /ECG contact jelly is advisable to ensure good current conduction). The shock is triggered with the foot switch. The foot switch must only be actuated when the electrodes are securely applied to the animal (eye or ear) under light pressure.

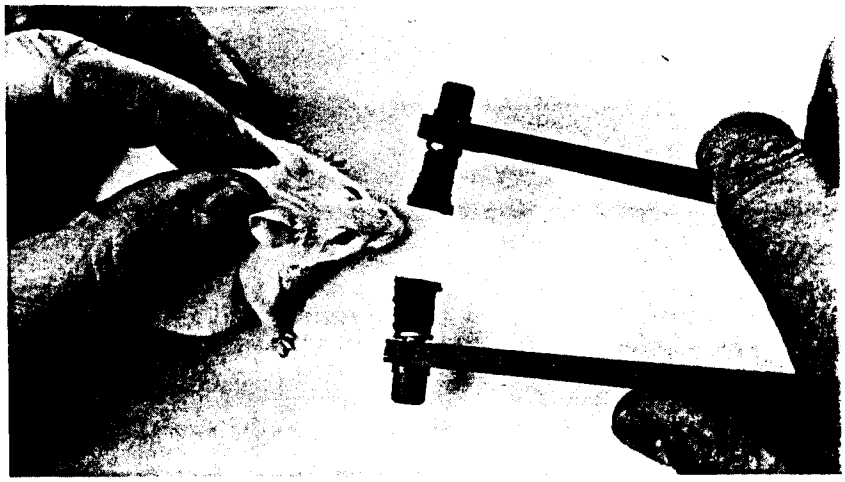


Fig. 5: Use of the eye electrode. (For his own safety it is preferable for the operator to wear gloves.)

Application of 7.5 or 10 mA, depending on the sensitivity of the animal strain, through ear electrodes leads to short-duration clonic convulsion characterised by uncontrolled jumps.

Increasing the current to 20 - 25 mA results immediately in tonic convulsion with interruption of respiration; the hind limbs of the animal are fully extended. This convulsion changes in certain animals to clonic seizure after a few seconds; in other animals it results directly in death. Further increase in current leads to tonic seizure and death in the case of all animals.

Drugs which can be used to treat this condition can prevent mortality, tonic convulsions and eventually also clonic seizure.

5.2 Practical details

(A) Preliminary evaluation of the current which just causes death in all animals of this strain at the time of the experiment. This is the current setting for the experiment.

(B) Application of different doses of the test drug to groups of animals.

(C) After an appropriate time for the test drug to become effective, production of convulsions at the current setting determined in the preliminary experiments.

(D) Protocol to cover the observed symptoms (clonic and tonic convulsions, death).

(E) Preparation of dose-response curve and evaluation of ED50 for all three symptomatic parameters.

Example

Dose-response curves of diphenylhydantoin (Fig. 6)
Groups of 10 mice receive 1 - 5 - 10 - 50 mg/kg orally. After 1 hour convulsions are produced (25 mA through ear electrodes). It is evident that mortality is reduced at lower doses (I), tonic convulsion at higher doses (II), and clonic convulsion at still higher doses (III).

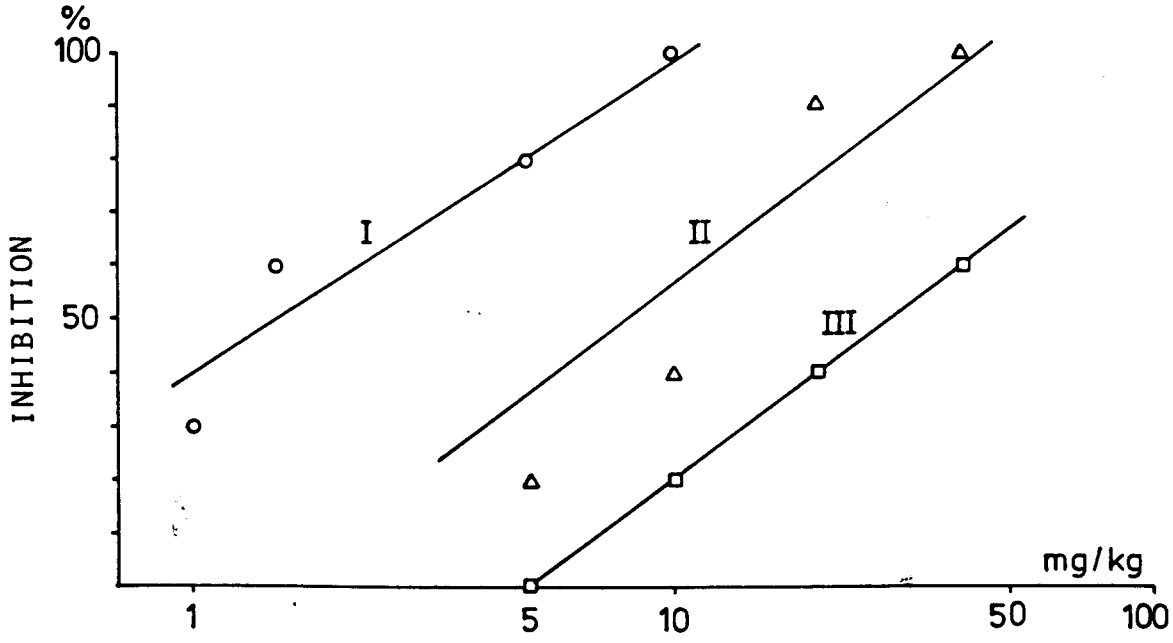
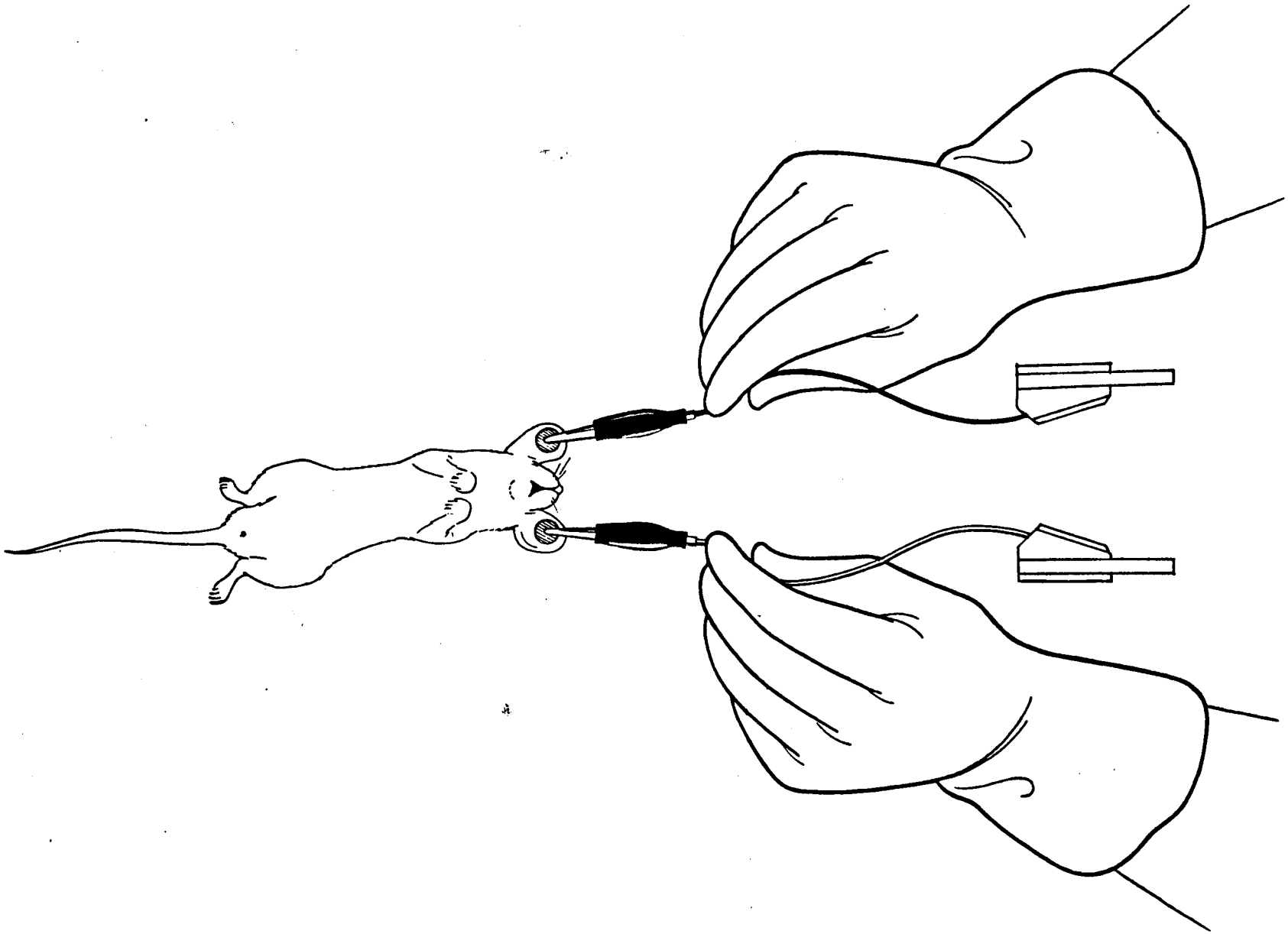


Fig. 6: Dose-response curve for diphenylhydantoin (Example).

5.3 Notes on use - electrodes

Eye electrodes

Eye electrodes are metal electrodes covered with cloth or leather. They must be moistened with saline solution before use and pressed firmly against the eyes to make good contact and ensure passage of the shock current into the body. Care must be taken not to use excessive amounts of saline solution. It is important to prevent excess solution moistening the fur away from the electrodes; the resulting salt bridge on the skin is electrically conducting and would short-circuit the shock current so that only part of the current passes into the body.



Ear electrodes

When ear electrodes are employed either electrode jelly (ECG paste) or saline solution may be used. For safety reasons it is advisable to wear an insulating glove on the hand holding the animal. With saline solution it is again important to avoid forming a liquid bridge between the two applications points. Successful shock is achieved, depending on the animal (mouse or rat), with amplitudes between 25 and 50 mA and shock durations between 0.2 and 0.5 sec.

Successful shock parameters

The following table summaries a number of settings which have proved successful in different laboratories. They are based on experience using the previous shock generator Type 207.

Animal	Application at	Electrodes with	Shock duration	Shock current
mouse	ears	saline solution	0.2 sec	25 mA
mouse	ears	electrode jelly	0.3 sec	33 mA
rat	ears	electrode jelly	0.5 sec	50 mA
mouse	eyes	saline solution	0.3 sec	130 mA
rat	eyes	saline solution	0.3 sec	25 mA

6. MAINTENANCE and CLEANING

The RODENT SHOCKER does not require any special maintenance or attention. It is however recommended to check the equipment and in particular the electrodes (cables, plugs) for external faults and insulation damage each time before use.

If you find or suspect that any liquid has found its way into the case, do not switch it on and use it only after it has been checked thoroughly by a qualified service engineer. In an emergency if no service engineer is available, you must wait at least until the liquid has dried up. Do not use the equipment immediately but first carry out a functional test as described in Section 4.2 (electrodes immersed in saline solution).

Cleaning: pull out the mains supply plug ! Case and front panel can be cleaned by rubbing with a moist cloth. Do not use any organic solvents but only ordinary household detergents. Take care to prevent any liquid passing inside the case.

7. CONTROLS, brief description

The numbers refer to Fig. 7

(1) Mains switch with built-in signal lamp

(2) Key for indicating the shock duration setting

(3) Two 10-position switches for setting the shock duration (0.1 - 9.9 seconds)

(4) Key for current setting

(5) Key with built-in signal lamp for releasing the shock blockage (blocked: light off; clear: light flashing)

(6) Start key for triggering the shock

(7) Overload indication. On overtemperature the output is switched off, the light is on. Allow the equipment to cool down !

(8) Rotary control for setting the shock current (hold down key (4) at the same time !)

(9) Socket (5-pin) for connecting up foot switch (pins 1 and 5)

(10) Key for changing the current-voltage combination (hold down key (4) at the same time)

(11) Shock output; use shrouded banana plugs (4 mm)

(12) Yellow signal lamp. Fault indication "electrode circuit open"

(13) Bargraph. Indication of elapsed time during shock.

(14) Marker and fault indication

(15) Digital display for current and time

(16) Key for indicating current applied

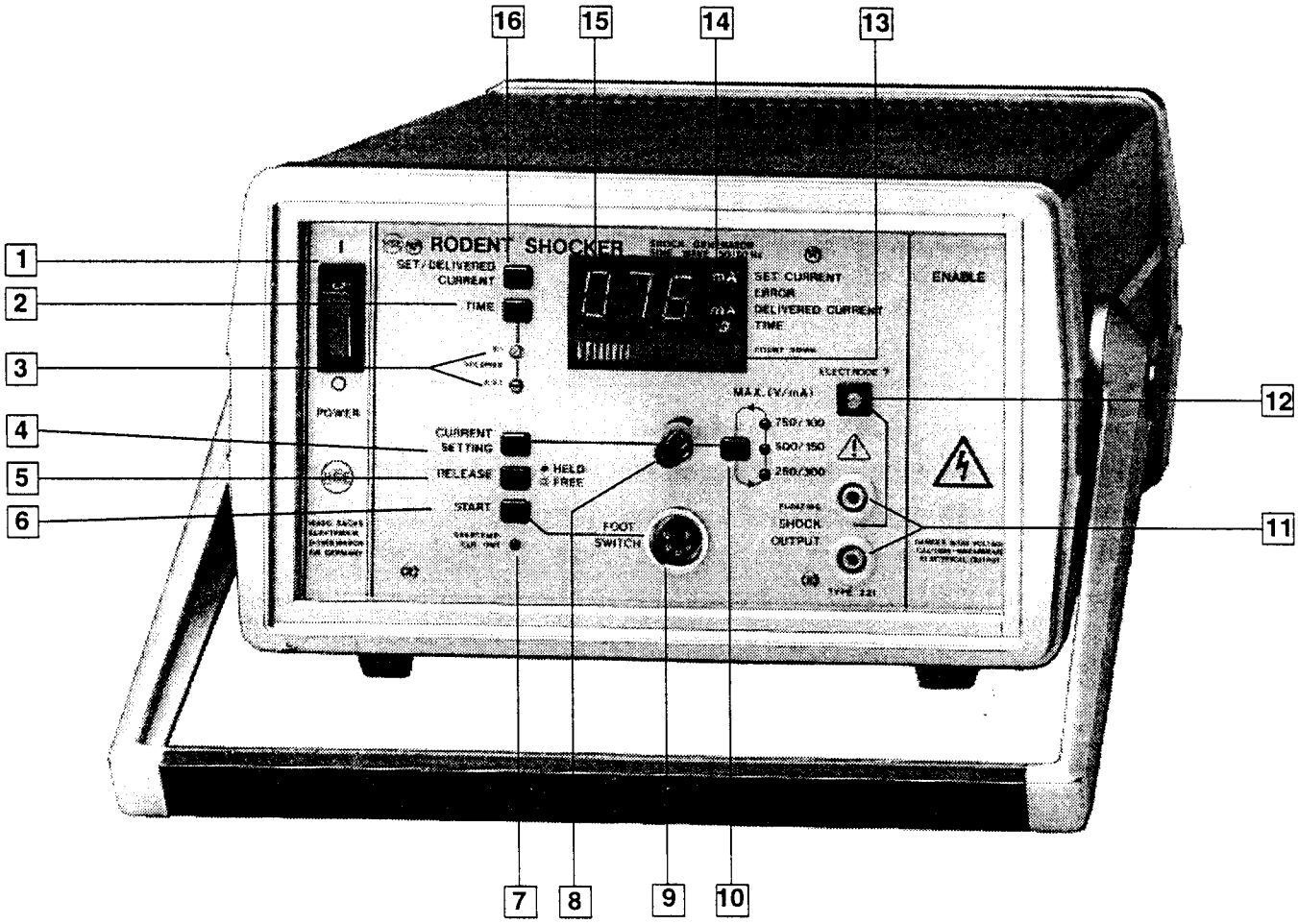


Fig. 7: Front penel of equipment (for brief description of controls see Section 7)

Arch. int. Pharmacodyn. **188**, 284–289 (1970)

Anticonvulsant Properties of Diphenylthiohydantoin (1)

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Abstract—The finding of a similarity in the peripheral neurodepressant properties of diphenylhydantoin and diphenylthiohydantoin (DPTH) suggested anticonvulsant properties for the latter drug. Anticonvulsant properties of DPTH were studied in rats by observing the drug's effects on maximal electroshock seizure (MES) patterns and pentetrazole (PTZ)-induced clonic seizures. The drug protects rats against the tonic hindlimb extensor component of MES, and exerts a small protective effect against PTZ-induced clonic seizures and death. In cats, DPTH does not modify electroencephalographic correlates of PTZ-induced seizure activity. The present study supports the use of tests for peripheral neurodepression in evaluating potential anticonvulsant agents.

Introduction

The early failure to demonstrate an elevation in electroshock seizure threshold in cats with diphenylthiohydantoin (DPTH) (1) discouraged further evaluation of the drug as an anticonvulsant (2). The recent demonstration of similar neuro-depressant actions for diphenylhydantoin (DPH) and DPTH in a peripheral neuroeffector junction (3, 4) and spinal cord (5) warranted the reexamination of the anticonvulsant properties of DPTH. In the present study, therefore, we examined the influence of DPTH on maximal electroshock seizures (MES) and pentetrazole (PTZ)-induced seizures in the rat and PTZ-induced electroencephalographic correlates of seizure activity in the cat.

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Methods

The effects of DPTH on MES pattern and PTZ-induced clonic seizures were studied in 460 Sprague-Dawley male albino rats averaging 48.6 ± 6.4 (S.D.) g in weight. DPTH (5,5-diphenyl-2-thiohydantoin, Chemical Procurement Lab., Inc., College Point, New York) was dissolved in 0.85 % saline by the addition of sodium hydroxide. The drug in doses from 1.6 to 100 mg/kg was injected i.p. in a volume of 1.0 ml/100 g body weight 30 min prior to testing. In preliminary toxicity studies, we observed that after doses from 50 to 200 mg/kg neurological impairment (See Results) was detected within 15 to 20 min. For evaluation of DPTH as an antigonist of MES, a 60 Hz stimulus of 200 msec duration was applied through saline wick corneal electrodes (6). Current intensity was varied in a step-wise manner from 40 to 240 ma. 200 ma regularly produced MES with tonic hind limb extension and was used throughout the study. Observations were made on the behavior of animals after DPTH and on the patterns of seizures following electroshock. A dose-response curve for DPH was also determined by using the same method and found to be in agreement with that reported by Swinyard *et al.* (7). The efficacy of DPTH as an antagonist to PTZ was assessed as follows. Thirty min after DPTH administration, PTZ 100 mg/kg was injected s.c. in a volume of 1.0 ml/100 g body weight. Although Swinyard *et al.* (7) used 70 mg PTZ per kg to be a CD_{97}, in our hands this dose did not produce this high an incidence of convulsive seizures in rats. A dose of 100 mg/kg was used and found to regularly produce clonic convulsions within 15 min. Animals were observed for 2 hr after PTZ administration, and the incidence of clonic seizures and death were noted. Myoclonic jerks and twitchings of facial muscles, limbs or the tail were not considered as convulsions; only those animals exhibiting clonic movements in all limbs and loss of righting were considered to have undergone convulsions. The effect of DPTH alone and DPTH on PTZ-induced electroencephalographic correlates of seizure activity (high voltage, high frequency discharges recorded from biparietal leads) was studied in 14 cats with spinal cord transection at the atlanto-occipital junction. For this study, cats of either sex weighing 2–3 kg were briefly anesthetized with vinylether for tracheostomy and high spinal cord transection. Animals were ventilated mechanically with room air. Arterial blood pO_2, pCO_2 and pH were monitored, and respiratory rate and depth were adjusted to maintain pO_2 above 100 mm Hg, pCO_2 between 16–23 mm Hg and pH between 7.40–7.46. During the testing gallamine triethiodide 5 mg/kg was injected i.v. as required to maintain complete neuromuscular block. DPTH was infused through an indwelling catheter in the iliac vein at the rate of 1 mg/kg/min (0.2 ml/min) until a total dose of 100 mg/kg had been administered. Five min after the start of DPTH infusion, PTZ 5 mg/kg (in 0.5 ml saline) was injected into a cephalic vein, and it was repeated at 5 min intervals until the dose totalled 100 mg/kg. The significance of differences in doses of PTZ

against clonic PTZ seizures, intensity and duration of seizures were diminished and onset delayed, but no actual measurements were made.

The mortality rate after PTZ was diminished by DPTH pretreatment ($p < .05$ for each dose group; Chi-square test) (Table I). While only 1 of 24 control rats (4 %) survived PTZ alone, 62 of 144 rats (43 %) receiving a prior dose of 1.6 to 50 mg/kg DPTH survived the PTZ. After 100 mg/kg DPTH which failed to prevent convulsions, 25 % survived the PTZ.

TABLE I

The effect of DPTH on PTZ-induced clonic convulsions and death in rats. 24 rats were used in each dose group. The control group received i.p. 1.0 ml alkaline saline per 100 g body-weight

Doses of DPTH (mg/kg)	Number of animals protected against clonic convulsions	Number of animals survived
0 (control)	0	1
1.6	3	12
3.125	3	9
6.25	2	10
12.5	5	7
25	4	14
50	4	10
100	0	6

The administration of DPTH to rats in doses from 1.6 to 25 mg/kg produced no apparent signs of neurological impairment. As doses of DPTH increased from 50 to 200 mg/kg there were increasingly severe signs of neurological

TABLE II

The effect of DPTH on PTZ-induced electroencephalographic seizure discharges in cats

	PTZ doses (mean ± S.E. in mg/kg) required for:		
	1st seizure	2nd seizure	recurrent seizures
Control (5 cats)	31 ± 5.3	52 ± 6.4	60 ± 8.2
DPTH treated (5 cats)	24 ± 4.8 (1)	50 ± 5.0 (1)	75 ± 3.5 (1)

(1) Not significantly different from control values.

required to elicit the first, second and recurrent seizures in control and DPTH treated groups were evaluated using Student's "*t*" test. Control observations were made on electroencephalographic patterns in 2 cats which received infusion of DPTH only.

Results

DPTH in adequate doses prevents the hind. limb tonic extensor phase of MES pattern. Fig. 1 shows the dose-response relationship for DPTH modification of MES. The ED_{50} for DPTH is approximately 25 mg/kg.

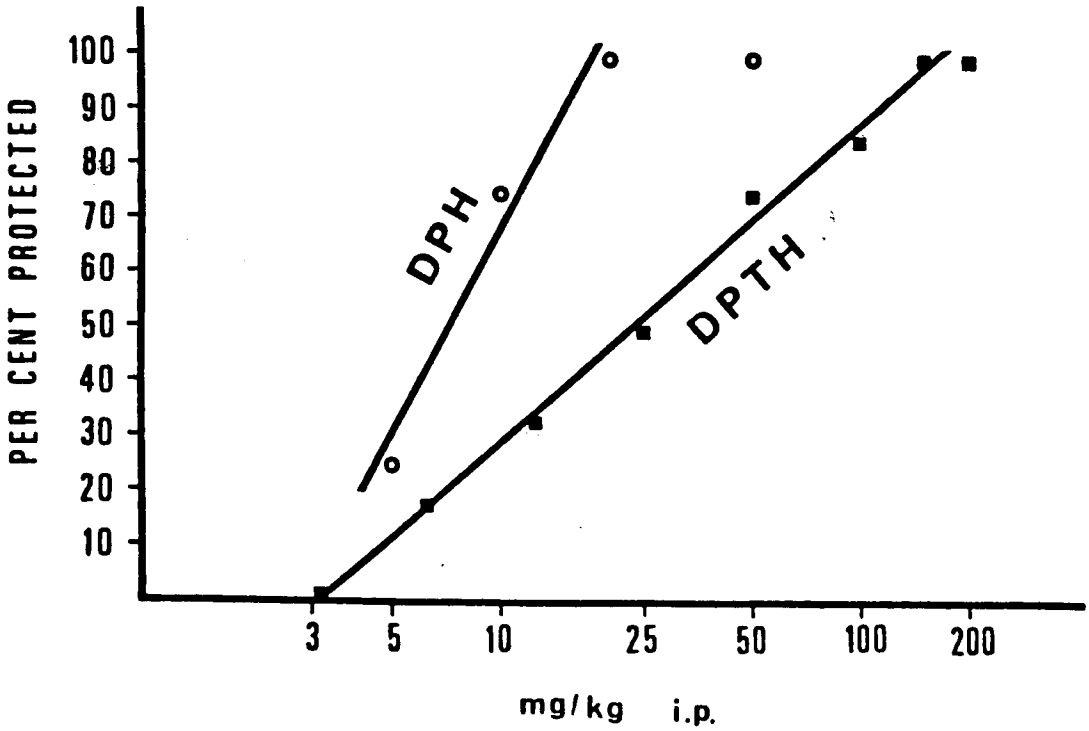

FIG. 1

Protection against maximal electroshock seizure (MES) patterns as a function of dose of diphenylthiohydantoin (DPTH) and diphenylhydantoin (DPH) is shown graphically. Each point represents the results obtained in a group of 24 rats for DPTH and of 12 rats for DPH.

The following anti-PTZ effects of DPTH were observed in rats. In the 3 groups of 24 rats treated with 12.5 to 50 mg/kg DPTH, a protective effect against PTZ-induced clonic seizures was observed ($p < .05$ for each group, Chi-square test); however, a dose-response relationship for this effect of DPTH could not be established (Table I). The 100 mg/kg dose of DPTH gave no protection to 24 rats against PTZ-induced seizures; it was difficult to dissociate PTZ-induced tremor and convulsant activity from that induced by DPTH (*vide infra*). It was our impression that in animals not protected by DPTH

deficit starting with somnolence, abnormal spread of hind limbs and ataxia, proceeding to body and limb twitches, loss of righting and generalized tremor. These lasted several hours after the 200 mg/kg dose of DPTH.

In 2 cats, the i.v. infusion of DPTH alone in doses up to 100 mg/kg in 100 min did not produce any alteration of electroencephalographic patterns. As can be seen from Table II, DPTH did not influence the dose of PTZ necessary to evoke electroencephalographic evidence of seizure activity by any of the criteria employed. As a positive control for the method used, trimethadione, a known antagonist of PTZ was also examined. In 2 cats, administration of 5 mg/kg/min trimethadione by i.v. infusion up to a total dose of 500 mg/kg was followed by complete absence of PTZ-induced electroencephalographic evidence of seizure activity.

Discussion

In the present study, DPTH protected rats against MES. The failure of earlier workers (1) to demonstrate anticonvulsant activity for DPTH on the one hand, and our demonstration of anti-seizure activity on the other lies in the difference in methods. Elevation of electroshock seizure threshold by a drug represents only one facet of the anticonvulsant potential of the drug. The use of multiple assay procedures has been emphasized by several workers (6-9). By using the MES modification test, anticonvulsant properties for DPTH can be demonstrated.

Like DPH, DPTH has been reported to have similar peripheral neurodepressant properties; both drugs suppress posttetanic potentiation in the cat soleus neuromuscular junction (4). As reported for DPH (10), DPTH reduces posttetanic potentiation in cat spinal monosynaptic pathways (5). With DPTH, however, this CNS effect occurs rapidly and is considerably shorter in duration, parallelling its entry into the CNS and redistribution in body tissues (11). DPH and DPTH both depress respiratory and cardiovascular function and antagonize cardiac rhythm disturbances induced by digitalis (12); they have also been reported to relax uterine smooth muscle (13). The finding of anticonvulsant activity for DPTH is therefore not surprising.

In a study of anticonvulsant properties of a series of other substituted thiohydantoins, Gesler *et al.* (2) found them to be more effective against PTZ-induced seizures than against those induced by maximal electroshock. In the present study, DPTH, like DPH, was effective in modifying maximal electroshock seizure patterns in the rat. DPTH was also effective in reducing the incidence of PTZ-induced clonic seizures and death in rats. This effect, although statistically significant, was of limited value and unimpressive.

Thus, it appears that hydantoins and their thio derivatives offer a spectrum of anticonvulsant activity. Diphenylhydantoin is effective against MES but not against PTZ-induced seizures (14). Diphenylthiohydantoin is somewhat

less potent against MES but has some activity against PTZ-induced seizures. Finally, 3-allyl-5-isobutyl-2-thiohydantoin (albutoin) is less effective against MES than against PTZ-induced seizures (2).

In view of the similarities between DPTH and DPH in both central and peripheral nerve tissues as well as in the cardiovascular system and the recent report of clinical evaluation of albutoin (15), this more rapidly acting derivative of DPH appears to warrant further investigation.

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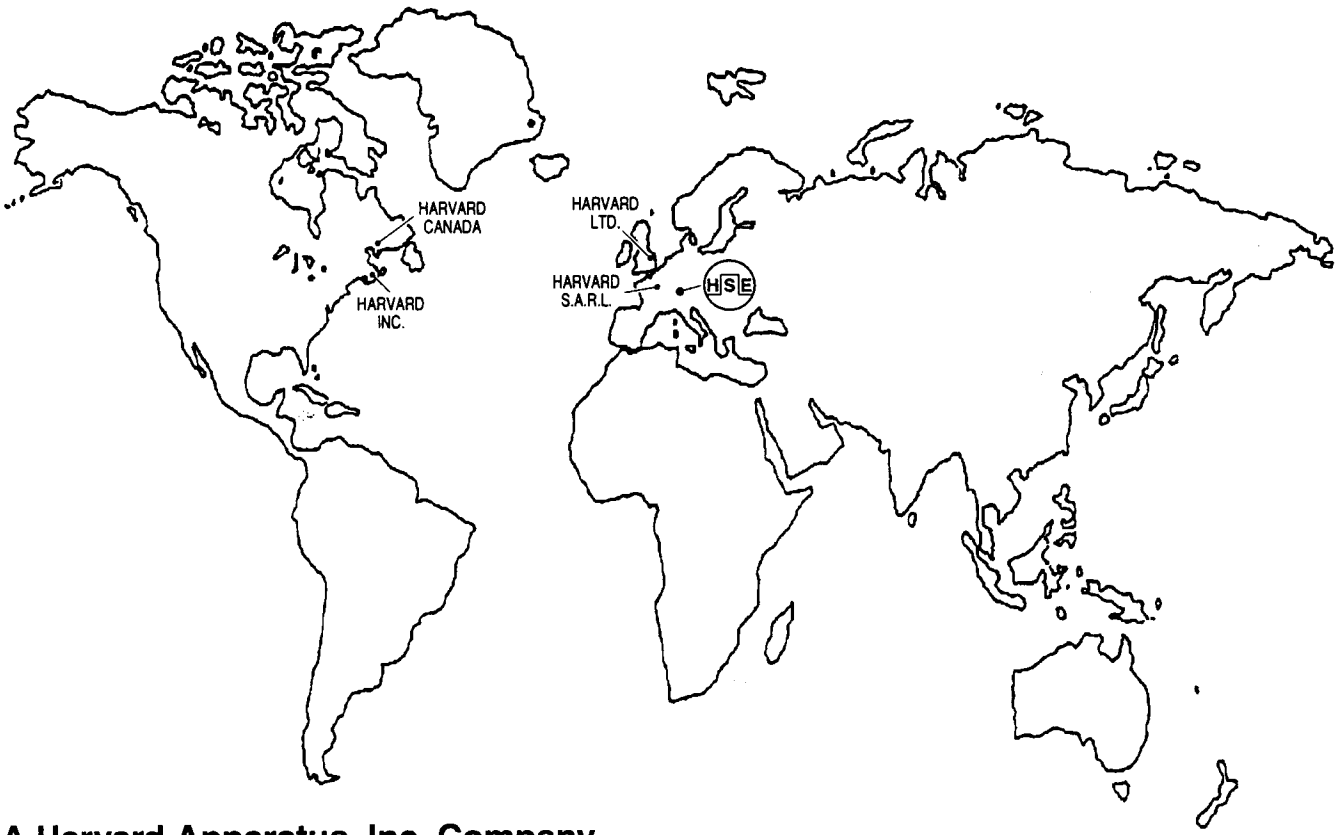

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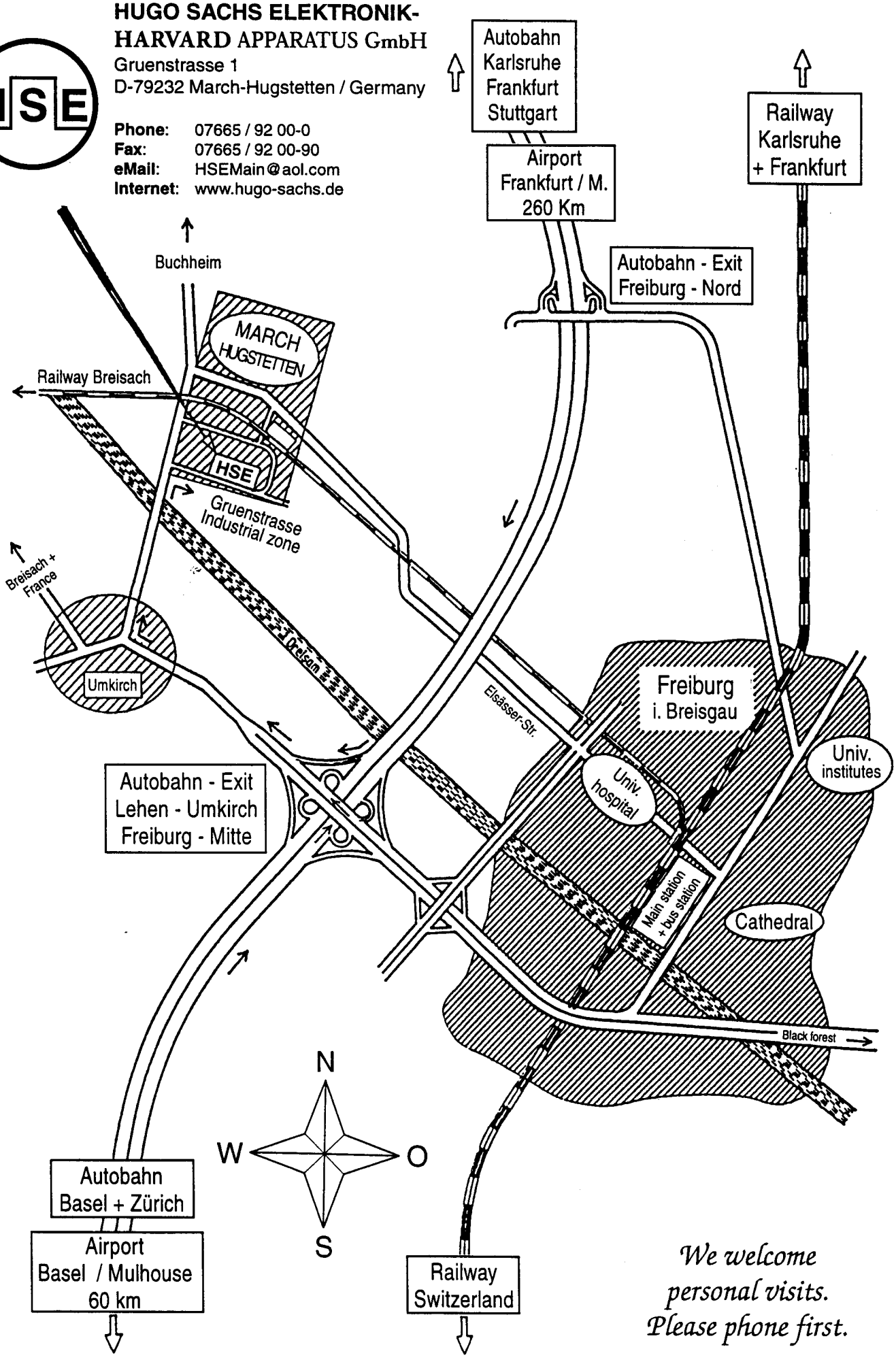

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