REVIEW ARTICLE

BIOLOGICAL ASSESSMENT OF TRANQUILLISERS. PART I*

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No review on tranquillising drugs would be complete without definitions of the novel jargon to be used. We shall almost entirely avoid the use of some titles recently coined, including psychosedative, hypnosedative, neuroleptic, neuroplegic and ataraxic. We think that at present these postulated types of drug are insufficiently distinguished from each other to allow their description other than by the general terms psychotropic drug and tranquillising drug. We define a psychotropic drug as one affecting the mind in some manner, and we define a tranquillising drug as a non-hypnotic drug that has a sedative or calming effect, including an action of this kind in psychoses or psychoneuroses. A tranquillising drug or tranquilliser falls last in the following series of central depressants: anaesthetic, hypnotic, sedative, tranquilliser. There is overlap between adjacent members of this series, but none between members not adjacent.

Most tranquillising drugs have only recently been introduced and the methods available for their assessment, though numerous, are as yet imprecise. This situation is widely recognised and frequently attacked. Thus the following statement appeared[1] in July, 1957: "Dr. D. R. Laurence expressed the astonishment of a pharmacologist at the flimsy evidence which launched new drugs on the tranquilliser market and apparently persuaded clinicians to prescribe them for their patients". No pharmacologist who has worked in this difficult field would deny that this statement can to some extent be justified, but many would consider it overemphatic. The major problem of replicating in the laboratory the actions of potential tranquillisers in human psychoneuroses and psychoses, remains almost completely unsolved, but very many experimental techniques have been proposed for the evaluation of tranquillisers and do, we believe, provide a secure foundation on which more specific methods will be elaborated and on which clinical investigations may be based. Our object is to survey these known techniques and to give our personal opinions of their usefulness.

We shall concentrate mainly on methods suitable for the pharmacological evaluation of tranquillisers. These drugs can certainly be sought by examining the ability of novel compounds to affect enzymes concerned in the physiological disposition of presumed transmitters such as noradrenaline or 5-hydroxytryptamine (which we shall call serotonin throughout) or by examining their ability to block the arousal reaction of Magoun,

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but we prefer to regard such biochemical and neurophysiological techniques as essentially investigative at present and shall consider them only briefly.

The methods we shall survey are grouped under the following rather arbitrary headings:

(1) Methods of measuring general sedative action.

(2) Behavioural methods (not involving conditioning).

(3) Neurophysiological techniques.

(4) Antagonism to psychotomimetic drugs.

(5) Conditioning methods.

We have attempted to cover the literature available to us before April, 1958, and apologise to authors whose papers have been missed.

1. Methods of Measuring Sedation

Many methods of measuring sedation have been proposed and some of them have been proved valuable by continuous use since long before the introduction of tranquillising drugs. Their value, of course, depends on the extent to which one expects or wishes sedation to be a property of the drug one is looking for. Thus most methods in this section will detect reserpine or chlorpromazine, both powerful sedatives, but many will miss benactyzine, which is not a general sedative.

A. *Potentiated Narcosis*

The potentiated narcosis test measures the influence of experimental drugs on the duration of sleep induced by a standard hypnotic. This technique was first introduced as a test for sedative action by Winter in 1948[2], though there had been many earlier investigations on the combined actions of two central depressant drugs, including several on the effect of alcohol on barbiturate sleep. When the combined effect of two drugs is to be determined there are many possible procedures[3]: the most informative are relatively complex. However, Winter[2] found that if the potential sedatives were given to groups of mice at a definite interval before a fixed dose of the hypnotic, comparisons of the geometric means of the sleeping times provided a simple but adequate measure of sedative action. This is the method generally used, but Winter's criterion of recovery from sleep, the ability of mice to walk normally with their eyes open, has been abandoned in favour of the sharper end point of recovery of the righting reflex. The hypnotic most frequently used is hexobarbitone. We find that precision is much increased by carrying out the test at a constant temperature between 34 and 36°.

Tranquillisers that prolong sleep after barbiturates or other hypnotics include the phenothiazines, chlorpromazine[4–6], promethazine[4,5], promazine[7], and chlorpiprozine (perphenazine)[8], the rauwolfia alkaloids Rauwiloid[9], reserpine[10,11], and rescinnamine[10] and a mixed group: benactyzine[12], hydroxyzine[13], methylpentynol[14], methylpentynol carbamate[15] and meprobamate[16]. This is probably the only test that will accept every type of drug

for which tranquillising actions have been claimed. Unfortunately a very large variety of other substances are accepted too. Such central depressants as sedatives, hypnotics and anaesthetics are obviously effective. But so are adrenaline, analgesics[17], antihistamines[2], histamine[18], serotonin[19,20], lysergic acid diethylamide[21], iproniazid[22], thiamine[23], cholesterol[24], glucose and its metabolic products[25], sucrose[26], glycerin[26], inorganic nitrates and nitrites[27], iodides[26], sodium chloride[28], various solutions[29], and water[28]. The activity of this wide range of substances is doubtless due to the existence of several possible ways in which drugs may prolong barbiturate action. Hypnotics, sedatives and tranquillisers may give true addition of effect, even if a tranquilliser is of a type which by itself does not cause marked sedation. Thus, tranquillisers diminishing awareness or blocking the arousal reaction should be active. Some other substances may make the brain more susceptible to the action of barbiturates. Brain tissue respiration is inhibited by barbiturates[30], and it has been suggested that iodides, which are known to decrease the oxygen uptake of tissues, might thus potentiate barbiturate narcosis. Phenothiazines[31–33] and serotonin[20] also depress brain metabolism *in vitro*, and might partly act in a similar way. On the other hand, it has been claimed that the relatively feeble depression of brain metabolism caused by barbiturates may not be associated with their hypnotic effects[34]. Also, a drug given in toxic dose might be expected to prolong apparent sleep: some so-called depressants of brain metabolism might well be systemically toxic in the doses necessary to depress brain metabolism.

The hypnotic would act more effectively if its access to the brain were facilitated, and one way in which this might occur is through increased permeability of the brain capillaries. Histamine and possibly serotonin could act in this way. Nitrates cause dilatation of capillaries and might also facilitate the passage of hypnotics into the brain. It has been suggested that substances with a high osmotic pressure when injected intraperitoneally draw water from the tissues and blood and so raise the hypnotic concentration[26].

The hypnotic will also act longer if its absorption is delayed and prolonged, e.g., by vasoconstriction such as adrenaline or serotonin might cause, or if its metabolism or excretion is blocked. Iproniazid[22], SKF525A[35–37] and Lilly 18,947[38] all block the metabolism of barbiturates and so potentiate their action. Fouts and Brodie[22] put forward a method of rejecting such "false" potentiators. In their view the true potentiator will reinduce sleep if administered during awakening. Drugs that act by interfering with the metabolism of the hypnotic have no effect under these conditions. This reverse test can be used for screening purposes though we find that high doses even of potent drugs, e.g., 20 mg./kg. of, chlorpromazine i.p., may have to be used.

The advantages of the potentiated narcosis test are that it is easy to use as a routine procedure, and that potentially useful tranquillisers are unlikely to be missed. However, its gross lack of specificity means that it must be supplemented with other more selective tests. In our experience 20 to 30 per cent of randomly selected compounds are able to prolong hexobarbitone

sleep when given (by pretreatment) in the relatively modest dose of 100 mg./kg., and their subsequent examination is a formidable task.

B. *Hypothermia and Reduced Metabolic Rate*

The sedative effects of chlorpromazine and reserpine as measured by potentiation of barbiturate sleep or reduction of activity have been found to be proportional to the accompanying fall in body temperature[39] and it has consequently been suggested that sedation is caused by interference with the mechanism of temperature regulation[40]. We do not support this view, though we agree that hypothermia may contribute to sedation measured by some non-specific methods, including potentiated narcosis.

Tranquillisers have also been tested for their action on the metabolic rate. The oxygen consumption of the whole animal may be measured, e.g., by the methods of Maclagan and Sheahan[41], or Capraro[42]. Chlorpromazine[4], pecazine (mepazine)[43], reserpine[44] and other central depressants[44] reduce oxygen consumption as does serotonin[44,45]. The respiration of isolated brain slices is depressed by chlorpromazine[46,47] and pecazine[47], but not by reserpine even when the tissues are made more sensitive by electrical stimulation[47]. However, the oxygen uptake of the whole brain is reported not to be affected by chlorpromazine[48].

We do not consider these non-specific methods of estimating tranquillising activity very useful as screening methods, though they are useful in enlarging knowledge of the drug's type and site of action and of its side effects.

C. *"Fall-time" Methods*

The "fall-time" tests assess the agility of control and treated animals, usually mice. The methods fall into two groups, those using inclined planes or fixed horizontal rods, and those using rotating rods and cylinders. The angle of the slope or the rate of revolution of the cylinder is adjusted so that normal mice remain on the apparatus and mice dosed with the type of drug to be studied fall off. The results are expressed in terms of the time they stay on, or as the percentage falling off.

Of the first group, the earliest was Thompson's[49] sloping wire-mesh, designed for the assay of insulin. This method has also been used for the assay of curare[50], and, more recently, for investigating the combined effects of alcohol and tranquillisers in rats[51]. Some workers replaced the wire-mesh by a smooth metal plate and used it for measuring sedation[52,53]: the controls ran down the plate and sedated mice slid down. Sedation can also be studied by putting mice on a narrow horizontal rod and observing how long they stay on.

The second group of tests forces the animal to move if it is to stay on the apparatus. The first apparatus of this type was the hollow rotating cylinder inclined at an angle, designed by Young and Lewis[54] for the assay of insulin, and later used for the assay of curare[55], and the measurement of sedation[56–58]. Horizontal rotating rods have also been used for evaluating sedatives[59–61].

The classes of substances that are active in these tests are those like curare[50,55] which cause paralysis, convulsants such as strychnine[57] and insulin[49,54], hypnotics such as pentobarbitone[57], alcohol[51,57] and methylpentynol carbamate[62], and tranquillisers such as the phenothiazines promethazine[52], chlorpromazine[51,52,63,64] and pecazine[52], reserpine[57,58,63,64] and deserpidine[58], and also meprobamate[63,64], but not benactyzine[63,64]. The effect measured is clearly neurotoxicity since it is not, at least hypothetically, an essential concomitant of tranquillisation, and since its counterpart in man must be loss of ability to perform adroit movements, including those of automobile-driving. The tests might be most valuable if used to discard rather than select potential tranquillisers.

D. *Reduction of Spontaneous Activity*

One of the most obvious signs of sedation in animals is a reduction in their so-called spontaneous activity. Methods of measuring activity have been in use since the end of the last century. Pedometers were used on dogs as early as 1896[65] and they have since been used for sheep and pigs[66]. But for small laboratory animals other, more convenient, methods were devised, and these methods fall into three main groups involving three different types of activity cage, those which revolve, those which move up and down ("jiggle" cages), and those which are fixed.

The revolving drum activity cage that rotates about a horizontal axis as the animal runs in it was described by Stewart in 1898[67]. The revolutions of such a drum may be registered kymographically[67–70], or by means of a mechanical[69–74] or electrical[75] revolution counter. Methods of estimating the reliability of revolving drums and of calibrating them have been suggested by Shirley[76] and Lacey[77]. The experimental animal can be forced to take all its exercise in the drum by allowing it no external living cage[67,68,72,74,78–80] or only a very small one[69,71,73]. Voluntary running activity may be recorded by allowing the animal a larger living cage so that it may enter the drum at will[70,75]. A variation of the revolving drum is the horizontal turntable described by Farris and Engvall[81], but records of activity from this apparatus will vary according to whether the rat has been running round the periphery of the turntable or nearer the centre.

The revolving wheel records only the running activity of an animal and not small movements. The second type of activity cage enables total activity to be measured. Szymanski[82] in 1914 devised a cage supported by an air tambour so that movements of the animal caused pressure changes which were recorded on a kymograph by means of another tambour. It was originally designed for salamanders and mice but has also been used for rats[83] and monkeys[84,85]. Spring-mounted activity cages were also suggested by Szymanski[86] and these have been more widely used. Movements may be recorded kymographically by means of an attached lever[87–93], or by a pneumatic system[94–97], but kymographic recording makes quantitative treatment of results difficult. Quantitative records have been obtained using a Harvard work adder[98,99] or even a device of sealskin[100] to convert the vertical cage movements into the rotation

of a wheel which inscribes a cumulative record on the drum[98] or works a revolution counter[99,100]. A numerical measure of activity can also be obtained by using electrical contacts to operate either a lever making vertical marks on a kymograph[101] or, most conveniently, a pulse counter[102,103]. If the cage is suspended by a strip metal spring instead of a coiled spring the strip itself can provide one of the contacts[103]. Photoelectric recording, using a flag attached to the cage to break a light beam, has also been tried[104].

Szymanski[82] introduced another type of activity cage constructed like a lever balance, with one arm supporting the cage and the other arm recording the movements of the animal towards and away from the pivot. The recording arm writes directly on a kymograph[82] or operates a work adder[105]. Other cages have been designed in which movement of the animal causes tilting of the cage in any direction[106] or in one plane[107,108], the recording being mechanical or electrical.

Waterman[109] attempted to improve the "jiggle" cage by an arrangement that reduces cage movements to a minimum compatible with mechanical recording. Work adders and microswitches respond to very slight cage movements[110]. Even less movement of the cage is necessary when the vibrations are transmitted to a gramophone pickup[111,112], or to the diaphragm of a permanent magnet loudspeaker[113]. The output of either is amplified and a numerical count[113] or an ink recording[111–113] obtained. Another electrical means of recording very slight cage movement is provided by the change in resistance of carbon granules by which the cage is supported[114].

The third main group of methods measures the activity of the animal more directly. An attached thread or chain has been used to measure the activity of fish[82], mice[115,116] and monkeys[117]. Direct observation of the number of squares an animal enters on a squared floor has also been used[118]. An animal moving on smoked paper will record its own activity[119] and the results can be made quantitative by measuring light reflected from the paper[120]. A mouse may be placed on dry sand on a gauze so that as the mouse moves sand comes through the gauze and is collected and measured[121]. The activity of the animal within a cage can be used to produce changes in capacitance between a vertical metal antenna in the centre of the cage and the cage itself[122]. A similar method employs metal foil squares in the insulated roof of the cage instead of the antenna: movements of the animal induce changes in capacitance between roof and floor, this capacitance forming part of that of a tuned circuit[123].

The photoelectric method of recording activity was first introduced by Siegel[124], who used a rectangular cage across which a light beam shone on to a photoelectric cell. When the animal interrupted the light beam an impulse counter was activated. Winter and Flataker[125] used a similar method but reflected the light beam twice off the sides of the cage. Dews[126] used a single direct light beam and found that results showed less variation when mice were tested in groups of five. Modifications include the use of circular cages[61] and of several separate light beams[127]. Infra-red rays can be used to record activity in total darkness[124].

These devices are very numerous and their design has involved much time and inventive ingenuity but differences between them are probably relatively unimportant when the gross effects of drugs are studied. The most important distinction is that the wheel, tilting cage and photo-beam methods measure mainly running and walking activity, whereas the "jiggle" cage also measures small cleaning, and other localised movements, and tremors. The best methods are probably those which cause the least possible disturbance to the animal, allowing it to move freely on a stable flooring. Another disadvantage of the moving cages is that they are difficult to calibrate. Our own preference is for the light-beam type.

Spontaneous activity of rats and mice is depressed by chlorpromazine[105,128], reserpine[129], meprobamate[105] and by small doses of azacyclonol[130,131]. Benactyzine[111] and large doses of azacyclonol[130] increase activity. There are some species differences because benactyzine reduces activity in the monkey[132], whereas azacyclonol does not affect monkey activity, and has mainly a stimulant effect on cats and dogs[131]. The results may also differ according to the conditions of the test. Brown[133] has used differences in action on the spontaneous activity of grouped and single mice to distinguish hypnotics from tranquillisers, and it has also been shown that phenobarbitone depresses the nocturnal activity of rats but has little effect on diurnal activity[134].

Such differences merit more attention than they have received. It is possible that more careful study of animal movement under a variety of different conditions by precise photo-beam methods would much increase the specificity of such methods towards different drugs. It might also considerably increase their convenience. Thus, when spontaneous daytime activity of mice is studied it is often the brief exploratory activity displayed by these nocturnal rodents when they are placed in a new cage. After 15 to 30 minutes such activity rapidly declines and the control animals subsequently appear quite tranquil. Nocturnal activity, though intense and prolonged, is less conveniently recorded, and because it occurs in bursts, more variable.

For these reasons and because it has other theoretical advantages, many authors study the effects of tranquillisers on hyperactivity rather than on spontaneous diurnal or nocturnal activity. Hyperactivity can be induced in monkeys by frontal lobe lesions, and this type has been shown to be reduced by chlorpromazine and reserpine[127]. More usually it is induced by stimulant drugs such as amphetamine, pipradrol, methyl phenidate, or caffeine.

E. *Antagonism of Drug-induced Hyperactivity*

Chlorpromazine, rauwolfia alkaloids and azacyclonol antagonise amphetamine[9,128,130,131], pipradrol[131,135] and methyl phenidate[63,64,136,137], though in monkeys the combination of reserpine and methyl phenidate causes alternation of depression with violent biting and jumping activity[136]. Small doses of meprobamate antagonise methyl phenidate stimulation but larger doses and benactyzine enhance the effects[63,64]. Other stimulants such as caffeine, cocaine[56] and mescaline[63,64] have been used. The

effects of most stimulants decline quickly, but four injections of β:β-iminodipropionitrile in mice produce a hyperactive state which lasts for months[138,139]. Mice so treated show circling activity similar to that described as a genetical abnormality[140,141]. Other chemical agents also produce a similar "waltzing syndrome"[142–144] but the mice treated with iminodipropionitrile (IDPN mice, "souris tournantes") have been investigated most thoroughly and used for testing sedatives and tranquillisers. Chlorpromazine[138] and reserpine[138,139] both reduce the activity of IDPN mice. Thuillier and Nakajima[145] divide psychotropic drugs on the basis of their action on IDPN mice into four classes; neuroleptics, tranquillising sedatives, hypnotics, and autonomic excitants. Delay coined the term neuroleptic for drugs that have powerful sedative actions but are not narcotic, that antagonise aggressiveness, agitation and psychotic states, that act predominantly on subcortical regions and that have important autonomic effects[146]. The neuroleptics, which include chlorpromazine, reserpine and hydroxyzine, are said to stop the agitation and circling of IDPN mice and to normalise their response to noxious stimuli, whereas the tranquillising sedatives, which include benactyzine, mephenesin, meprobamate, methylpentynol and analgesics, reduce the hyperactivity and circling but produce ataxia and ataxic responses to noxious stimuli[145]. Hypnotics, including barbiturates, arrest circling activity only at narcotic doses, and autonomic excitants, including methamphetamine and lysergic acid diethylamide, diminish activity and produce trembling but no ataxia[145]. This is an interesting method: it is to be hoped that further study may confirm its usefulness.

F. *Anticonvulsant Tests*

Central depressants, including tranquillisers, may antagonise convulsions produced by passing an electric current through the brain, or by injecting convulsant drugs such as leptazol.

Drugs (other than specific anti-epileptic drugs) effective against electroshock include barbiturates[105,147], alcohol[147] and stimulants such as mescaline and dexamphetamine[147]. Of the tranquillisers, meprobamate[105,148] and hydroxyzine[13] and some phenothiazines[149] are effective, chlorpromazine being variously reported to be effective[105] or to have only slight activity[149]. Azacyclonol has little or no activity[130], whereas reserpine[150,151] and benactyzine[132] enhance the susceptibility to convulsions, though again reports differ, and reserpine is said to have no effect and benactyzine even a very slight protective action[63]. We find that reserpine enhances the susceptibility of rats and that chlorpromazine is relatively ineffective, though large doses lower the threshold.

Similar results are reported against leptazol-induced convulsions. Barbiturates[63,64,105,152] and meprobamate[63,64,105,148] have protective actions, azacyclonol has only slight activity[63], chlorpromazine has no effect[63,105,144], and reserpine is ineffective[63] or enhances susceptibility[150]. Hydroxyzine[13] and benactyzine[63] are ineffective.

It has been suggested that tranquillisers should be tested against but should not antagonise strychnine-convulsions[153]. Phenothiazines[63,154],

reserpine[63,150,153], benactyzine[63] and azacyclonol[63] have no effect on strychnine convulsions, and hydroxyzine potentiates them[13], but meprobamate[64,148], like phenobarbitone[63,152], does have an antagonistic effect. Other convulsants that have been used include picrotoxin, nicotine, cocaine and amphetamine.

We consider that these anticonvulsant methods may be helpful in defining the pattern of a drug's central actions; moreover, the frequent association of psychoneuroses or psychoses with epilepsy may sometimes allow them to have direct clinical application. They are, nevertheless, useless as primary screening methods for novel tranquillisers.

G. *Amphetamine Toxicity*

In 1940 Gunn and Gurd[155] noticed that the symptoms of excitement caused by injection of amphetamine or related compounds in mice, were much more pronounced if the mice were kept together in one cage, rather than singly. Chance[156] reported that the increased stimulation that occurs with grouped mice led to a marked increase in the toxicity of stimulants. The toxicity of amphetamine was increased nearly ten times by keeping the injected mice in groups of ten instead of in individual cages. Protection against a lethal dose of amphetamine has been used as a test for tranquillisers[4,15], but the effect of tranquillisers on amphetamine toxicity to grouped mice particularly, has only been investigated more recently by Lasagna and McCann[157] and by Burn and Hobbs[158]. Pentobarbitone did not affect toxicity for grouped or individual mice, but phenobarbitone raised the LD50 for grouped mice to that of individual mice, but only at doses that produced prolonged sedation and ataxia[157]. Chlorpromazine and reserpine protected grouped mice at doses that hardly affected the toxicity of amphetamine for individual mice[157,158] and had no prolonged after-effects[157]. Promazine had a similar action but was less potent[157]. Meprobamate, methylpentynol and benactyzine were inactive[158]. Burn and Hobbs[158] conclude that the difference between drug effects on grouped and single mice shows that the test is more than an anti-amphetamine test: they claim that it is a test against fright and therefore a valid test for tranquillising agents. We think that the specificity of the method needs further study, and that its advantage over the methods described in sections D and E has not been fully demonstrated.

H. *Audiogenic Seizures*

Donaldson in 1924[159] was the first to describe running seizures in rats precipitated by the sound of jingling keys. Since then there has been an enormous amount of work on these seizures. Reviews of the literature have been published by Finger[160] up to 1947 and by Bevan[161] from 1947 to 1954. For some time there was a controversy as to whether such seizures were a reflex response to auditory stimulation, or were caused indirectly by conflict between the need to escape and the inability to do so. The evidence for each view has been brought together by Munn[162] who concludes that it is rare to find seizures produced by conflict alone without auditory stimulation. But audiogenic seizures are not simply reflex

behaviour, since providing a shelter[163] or allowing the animal to make a well established instrumental response[164] often has a protective effect.

The seizure in the rat consists of an initial startle response followed by violent running and jumping activity which usually leads to tonic and clonic convulsions followed by coma, but which may pass straight into the comatose state[165] sometimes described as "catatonia"[166] or "catalepsy"[167]. Much work has been done in an attempt to define the essential characteristics of the seizure-inducing stimulus. Sounds are usually of high frequency, but high intensity is a more important factor. Interrupted tones are less effective than steady tones[168], but a short priming sound before the test stimulation enhances susceptibility[169]. In spite of all this work, the essential stimulus characteristics have not been clearly defined, and most investigators, like those last mentioned[169], use an electric doorbell as the sound source.

Not all rats or mice are susceptible to audiogenic seizures. The percentage in any colony depends on the genetics of the strain. By selective breeding it is possible to produce strains with very differing susceptibility, but though a correspondence between susceptibility and emotionality as otherwise measured has been reported, it would appear to be due only to chance combination[170]. Rats can be made more or less susceptible by change in diet or by administration of drugs. The dietary factors are very varied. Deficiencies of magnesium[171,172], amino acids[173] or even a reduced food intake[173] increases susceptibility, whereas excess thiamine decreases seizure-incidence and injection of L-glutamic acid reduces severity[174]. It is interesting that hydration protects against audiogenic seizure although it increases susceptibility to electrical and leptazol-induced convulsions[175].

But although seizure incidence is influenced by so many factors, the use of a single strain of animals fed on a standard diet allows the incidence to be used as a criterion of drug activity. The clinical anticonvulsants bromide[176,177], phenytoin[178–181], phenobarbitone[176,181,182] and troxidone[181] are effective against audiogenic seizures, the last three more effective than against comparable electrically-induced convulsions[181].

Alcohol is very effective in preventing audiogenic seizures in rats[183,184] at non-ataxic doses[183]. Reserpine protects[56,167,185] and so do chlorpromazine[167,185], pecazine[167] and meprobamate[167,185]. Benactyzine gives a maximum of 50 per cent protection, higher doses enhancing the convulsions[167].

The audiogenic seizure is probably one of the most useful techniques available for assessing tranquillisers. This view appears justified both by the nature of the seizure and by the reported activities of known drugs. Its chief fault, in this context, is its susceptibility to non-tranquillising anticonvulsants, which must be separately eliminated.

I. *Stress and Adrenocortical Function*

Audiogenic seizures and other behavioural responses to alarming stimuli are stressful responses and stress is known to activate the adrenal cortex[186] by causing release of corticotrophic hormone from the pituitary [187,188]. Mason and Brady[189], in an experiment on the conditioned

emotional suppression of lever-pressing in monkeys (see section Q) showed that disruption of lever-pressing was associated with high plasma concentrations of 17-hydroxycorticosteroids. Daily intramuscular doses of reserpine protected against the disturbance of behaviour and at the same time the amount of plasma steroid remained normal However, the behavioural and endocrine responses are not absolutely linked because on withdrawal of reserpine the conditioned anxiety response reappeared within a week whereas the steroid concentrations took about three weeks to rise again. Reserpine given intravenously, like chlorpromazine and azacyclonol, itself produces moderate rises in the amount of circulating corticosteroids[190] though pentobarbitone reduces the amount. The effects of chlorpromazine[191] and reserpine[192] at least are mediated through the pituitary.

Besides influencing the amount of circulating corticosteroids, experimental stress causes depletion of adrenal ascorbic acid[193]. Usually the ascorbic acid concentration is expressed in weight per hundred grams of gland, but Olling[194], investigating reported sex differences in sensitivity to the ascorbic acid depletion test, concluded that a better index would be the total quantity of ascorbic acid in the gland, to allow for differences in adrenal weight. Although rauwolfia alkaloids reduce adrenal ascorbic acid[195], both reserpine and chlorpromazine given to rats before submitting them to stress, prevent the depletion of ascorbic acid that would otherwise have occurred[196]. We have been unable to demonstrate a comparable action of meprobamate, using aversive conditioning as a stressful procedure.

The anti-stress activity of a series of barbiturates measured by the ascorbic acid method appears only at doses that produce deep sleep[197]. Most non-barbiturate hypnotics can also depress the stress reaction and some of these, including alcohol, do so at non-hypnotic doses[197].

Instead of using experimental stress, drugs can be tested for their action against known pituitary-adrenal activating agents. Various substances can cause depletion of adrenal ascorbic acid, including adrenaline, histamine and morphine[198]. Chlorpromazine and reserpine block this effect of adrenaline, and, to a lesser extent, that of morphine, whereas pentobarbitone blocks the depletion due to aspirin and morphine but not that due to adrenaline or histamine[199]. The difficulty of interpreting much of this work arises from the uncertainty whether effects of such drugs as chlorpromazine and reserpine arise from central or peripheral actions: this is unfortunate because the assessment of stress is in principle one of the best approaches to tranquilliser assessment, and there is much to be said for attempts to make this assessment of stress one of objective measurement rather than observation. Such alternatives to adrenal cortical studies as evaluation of defaecation, micturition and muscle tension suffer from similar disadvantages because of side-effects of tranquillisers, and emotional elimination in any case is not a good measure of fearfulness[200,201]. Kreezer[202] has listed methods of measuring emotionality, including the "startle" response to disturbing stimuli which Tripod has used for testing tranquillisers[63,64] and which was earlier used for testing sedatives[87]. These methods deserve further study.

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