A NEW METHOD FOR THE BIOLOGICAL ASSAY OF CURARIZING AGENTS

BY

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SUMMARY

A method for the bio-assay of curarizing agents is described. Suxamethonium is injected intravenously into anaesthetized and intubated rats. The response is measured as the time of respiratory depression. Doses ranging from 0.311 to 1.05 μg/g give rise to linear responses when the time of respiratory depression is plotted against log dose. The assays are performed within this range. High and low doses, both of the standard and unknown, are injected into two animals each. The index of precision is 0.14. Precision, specificity, cheapness and the possibility of a wider use (cholinesterase determination) are emphasized.

The assay of curarizing agents has been performed so far in intact animals using an all-or-none response (mouse drop-off test), or in isolated neuromuscular preparations, which give better quantitative data (continuous distribution) (Riesser and Neuschloss, 1921; Norton and de Beer, 1954; Bulbring, 1946). It seemed, therefore, desirable to devise a method that employing the intact animal might at the same time give rise to an adequate dose response curve expressed in a continuous variable.

The method here described measures the effect of the curarizing agent, in this case suxamethonium, by the respiratory activity of the rat following the intravenous injection of the substance. It is simple and accurate, and the apparatus required may be constructed in the laboratory at a very low cost. In addition it can also be applied to determine indirectly the activity of cholinesterase employing suxamethonium as the substrate.

METHOD

The rat is anaesthetized with ether and immediately weighed so as to establish the μg/g dose of suxamethonium to be employed. The larynx is then visualized by a direct laryngoscopy which is carried out by means of an otoscope of appropriate diameter.

The vocal cords are touched with a surface anaesthetic and a polyethylene tube (3 mm dia.) is passed 1.5 cm into the trachea (these figures apply to rats weighing about 250 g). After the endotracheal tube is in place it is connected to a small Y-shaped glass tube. The other end of this Y-tube is connected to a T-shaped glass tube the long branch of which is inserted through a cork into a glass flask which is filled with water up to a height of 11 cm. The free extremity of this T-tube is then connected to a similar system as shown in figure 1. The free end of this T-tube receives oxygen from a cylinder provided with a regulating valve. The rat, which at this moment is in a very light plane of anaesthesia, is breathing spontaneously and to be sure that the tube is within the trachea, and to have a rough idea of the amplitude of the respiratory movements, the rubber tube at a must be occluded and at the same time the fingertip of the operator must close the resting free end of the Y-tube (b).

In these conditions (a and b closed), during the inspiratory phase the subatmospheric intrapulmonary pressure will make the water ascend in the T-tube of flask No. 1, the movement being proportional to the amplitude of the respiratory movements, the rubber tube at a must be occluded and at the same time the fingertip of the operator must close the resting free end of the Y-tube (b). In the expiratory phase the water in the T-tube will descend.

A reference point is then marked on the T-tube (flask No. 1) half way up to the upper limit attained by water during normal respiration. When this point is reached after apnoea from suxamethonium injection has subsided and respiration is becoming again normal the assay is considered to have ended.
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FIG. 1
The apparatus assembled ready for the assay

a = rubber tubing;
b = free end of Y-tube;
c = escape holes in corks.

Occlusion of a and b simultaneously for 10–15 seconds every 20 seconds indicates, by the height of the water level in the descending limb of the T-tube in flask No. 1, the amplitude of spontaneous respiratory movement before and during the bio-assay.

The time elapsing from the injection of suxamethonium until the moment in which the water level in the manometer reaches this previously specified point (half the normal excursion) is accurately recorded with a stopwatch and taken as the response to a given dose.

Flask No. 2 is only used as a security valve so as to avoid an excessive pressure during the inflation of the lungs and also when recording the respiratory movements, so that when the tube is occluded at a oxygen will escape through the hole c in the cork. Flask No. 1 has also a hole in the cork (fig. 1).

When suxamethonium is injected into a vein of the tail apnoea or respiratory depression ensues, according to the dose injected; control of ventilation must be started then, regulating the frequency and amplitude of inflation of the lungs with the fingertip at the free end (b) of the Y-tube.

Every 20 seconds the controlled ventilation must be stopped for about 10 or 15 seconds (occluding in a and b simultaneously) so that the level of spontaneous respiration may be observed, and when it reaches the expected level (half the normal respiration range) the experiment is over.

The time, in seconds, elapsing after the injection is called "time of respiratory depression" and is taken as the response to the dose used.

At this moment the animal is capable of breathing by itself without visible deterioration but is still connected to the oxygen supply until it struggles or tries to pull out the tube with its forelegs.

It is convenient not to use the same animal for two or three days after each experiment. The same animal can thus be used as often as required.

RESULTS
A dose response curve for suxamethonium was obtained by the method described. For this purpose nine albino rats weighing from 250 to 300 grams were injected intravenously with four doses of suxamethonium increasing by a factor of 1.5, i.e. 0.311, 0.446, 0.700, and 1.05 μg/g as can be seen in figure 2. Not all the rats were injected with all four doses.

As is shown in figure 2, each successive dose of suxamethonium induces an increase in respiratory depression of about 200 seconds.

A linear regression was obtained plotting the time of respiratory depression against the log dose. $F_{\text{step}} = 175.68 \ (P < 0.01)$; the precision index ($\lambda$) was 0.12, $r = 0.89$.

In view of the results obtained, this dose range was considered adequate and nine bio-assays were performed as follows: two doses of the unknown and two doses of the standard were injected into
two animals each, the results being statistically analyzed as four-point assays (Bliss, 1952). An example is shown in figure 3.

The mean results were:
Unknown = 110.8 per cent of the standard; result obtained = 116.4 per cent; mean precision index (\(\lambda\)) = 0.1445.

The individual results for each bio-assay are summarized in Table I. The potency ratio is calculated as follows:

\[
\frac{\bar{y}_s - \bar{y}_u}{b_a} = M
\]

where \(\bar{y}_s\) is mean response of standard, \(\bar{y}_u\) is mean response of unknown and \(b_a\) is mean slope. Antilog \(M\) is the potency ratio.

### Table I

<table>
<thead>
<tr>
<th>Assay</th>
<th>Potency ratio</th>
<th>Observed index ((\lambda))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.225</td>
<td>1.318</td>
</tr>
<tr>
<td>2</td>
<td>0.900</td>
<td>0.744</td>
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<tr>
<td>3</td>
<td>1.000</td>
<td>0.722</td>
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<tr>
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<td>1.100</td>
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<td>5</td>
<td>1.280</td>
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<td>6</td>
<td>0.835</td>
<td>0.888</td>
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<tr>
<td>7</td>
<td>1.380</td>
<td>1.671</td>
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<tr>
<td>8</td>
<td>1.260</td>
<td>1.250</td>
</tr>
<tr>
<td>9</td>
<td>1.000</td>
<td>1.003</td>
</tr>
<tr>
<td>Mean</td>
<td>1.108</td>
<td>1.164</td>
</tr>
</tbody>
</table>

### DISCUSSION

The possibilities offered by the present method of obtaining quantitative data employing the entire animal, together with the specificity and precision, make its results superior to the mouse drop-off test, in which the all-or-none response does not permit a satisfactory statistical analysis. A much more satisfactory method than the mouse drop-off test is the frog rectus abdominis bio-assay (Riesser and Neuschloss, 1921) modified by Norton and de Beer (1954). This is extremely sensitive but the present method has a better index of precision (\(\lambda\)) and has the advantage of using the entire animal so that its specificity is greater. The same can be said for the rat diaphragm preparation of Bulbring (1946).

### ACKNOWLEDGMENT

I am much obliged to Dr. Sol L. Rabasa from the Instituto de Investigaciones Medicas de Rosario for his valuable assistance and advice.

### REFERENCES


UNE NOUVELLE MÉTHODE D’APPRECIATION
BIOLOGIQUE DE SUBSTANCES
cURARISANTES

SOMMAIRE
Description d’une méthode de "bioassay" (=analyse biologique) des substances curarisantes. On injecte par voie i.v. du suxaméthonium à des rats anesthésiés et cathétérisés. Le résultat est mesuré par la durée de dépression respiratoire. Des doses de 0,311 à 1,05 µg/g provoquent des réactions linéaires lorsqu’on met en rapport la durée de dépression respiratoire avec la dose logarithmique. Les dosages ne sont faits que dans les limites susnommées. Des doses élevées et des doses faibles, tant du standard connu que d’une inconnue, sont injectées à chaque test à deux animaux. L’indice de précision est de 0,14. L’auteur fait remarquer la précision, la spécificité, les frais insignifiants et la possibilité de donner à ce mode de test une étendue plus large (dosage de la cholinestérase).

EINE NEUE METHODE FÜR DIE BIOLOGISCHE ERPROBUNG VON CURARISIERENDEN MITTELN

ZUSAMMENFASSUNG

BOOK REVIEW


Much important information, relevant to the anaesthetist, has been distorted by the repetition of half-truths from one textbook to another. Tracing back to the (absence of) source is interesting but discouraging. Against this background, Cardiac Output and Regional Blood Flow is refreshing in its sheer authority. The authors have worked in the field for over a decade and much of the material is their own, although the work of others is quoted in abundance (about 700 references). Nevertheless this constitutes no lifeless catalogue of names but the significance of each study is revealed by the succinct comments of the authors.

The first chapter deals with methods of measurement of cardiac output, and is concerned with general principles rather than technical details. It is followed by valuable reviews of measurements in resting and exercising man. The fourth chapter deals with methods of study of the regional blood flow. This again is largely concerned with general principles and particularly the significance of the A-V oxygen difference. Splanchnic, renal, cerebral, coronary and limb blood flow are considered but it is disappointing to find no account of electromagnetic or sonic flowmeters, neither is there any mention of the difficulties in deriving volume flow rate from linear flow rate at a particular distance from the centre of the vessel. The chapters on the distribution of the cardiac output at rest and at exercise are immensely valuable, and one wishes that it had been possible to write a third chapter on distribution during anaesthesia.

The rest of the book is a systematic account of cardiac output and its distribution in patients with disorders of the circulatory and respiratory systems. This concerns all anaesthetists, but particularly those dealing with cardiac surgery. The book provides a sound basis for the practical management of circulatory problems under the difficult conditions encountered during surgery on those with severe cardiac disease.

This book is a model of what a monograph should be. It is strongly recommended for anaesthetic departmental libraries, and anaesthetists concerned with the care of patients suffering from cardiorespiratory disorders will probably wish to purchase their own copies.

J. F. Nunn