THE EFFECT OF SERUM FROM JAUNDICED PATIENTS ON THE ACTION OF RELAXANTS

BY

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SUMMARY

The action of tubocurarine, gallamine, laudexium, suxamethonium and decamethonium was reduced in the presence of serum from jaundiced patients when used to antagonize the contracture produced by acetylcholine on the rectus abdominis muscle of the frog; that of the tropeine derivatives DF596 and DF648 was enhanced. Increased protein binding provides the most likely explanation for the reduced effectiveness of most relaxants but the enhanced activity of the tropeine derivatives cannot be explained on this basis. The latter drugs, however, are destroyed by serum esterases and since a low serum cholinesterase is common in jaundice this could account for their increased effectiveness.

It is general experience among anaesthetists that patients with liver disease are sometimes remarkably resistant to curare. This resistance was first described by Dundee and Gray (1953) and later by Haselhuhn (1957) who studied a group of forty-one patients with advanced liver disease.

One obvious possibility was that blood from patients with liver disease contained a factor, absent in normal blood, which antagonized the action of tubocurarine. Such a possibility could be investigated by a suitable means of biological assay. Relaxant drugs are known to antagonize the contracture of the frog rectus abdominis muscle in response to acetylcholine and it was planned to study the effect of normal serum and serum from patients with jaundice or liver disease on such antagonism. The relaxant drugs tested were tubocurarine, gallamine, laudexium, suxamethonium and decamethonium, as well as two synthetic compounds DF596 and DF648 not yet released for clinical use (Haining, Johnston, and Smith, 1959).

METHOD

The relaxant drugs were assayed by the method of García de Jalon (1947) in which the drugs were used to antagonize the contracture produced by acetylcholine in the rectus abdominis muscle of the frog. The muscle was carefully dissected, removed from the frog with the minimum amount of interference and immersed in a water-bath containing 4 ml of frog Ringer solution (NaCl, 6 g/l; KCl, 0.15 g/l; CaCl₂, 6 H₂O, 0.3 g/l; NaHCO₃, 0.59 g/l; glucose 1 g/l) aerated with a gas mixture containing 95 per cent oxygen and 5 per cent carbon dioxide at room temperature. With one end fixed to the bottom of the bath the free end of the muscle was attached to a suitable lever recording on a smoked drum.

In a preliminary series of experiments acetylcholine was added to the water-bath containing the test solution and left in contact with the muscle for a fixed period at the end of which the bath was drained and the muscle washed, usually three times. All these operations were carried out by hand and under the conditions described it proved impossible to maintain a constant acetylcholine response, presumably because of the large number of variables involved. Accordingly an automatic bio-assay apparatus of the type described by Boura, Mongar and Schild (1954) was introduced to control the timing of operations in the assay cycle. This cycle, which lasted approximately 7 minutes, followed a fixed pattern: (1) a rest period of 2.5 min; (2) Ringer solution was drained from the bath automatically at the end of the rest period and replaced by Ringer...
containing the solution under test; (3) second rest period to acclimatize the rectus muscle to the new solution; (4) drum switched on and baseline recorded for 1 min; (5) 1 μg acetylcholine added by hand; (6) contact period during which the response was recorded for 1 min; (7) preparation washed three times. The sequence of events was then repeated.

The most suitable dose of acetylcholine was found by trial and error to be 0.5–1.5 μg. The most satisfactory time cycle lasted 6–8 min.

Usually several varying responses to acetylcholine in Ringer solution were obtained before the response achieved uniformity, designated the standard response. At least two equal consecutive contractures (standard responses) were obtained before the addition of the serum under test. Contractures were recorded in response to acetylcholine in the presence of normal serum in the Ringer bath (0.2 ml in 4 ml of Ringer solution), in the presence of abnormal serum, in the presence of relaxant drugs, and in the presence of normal or abnormal serum plus a relaxant drug.

The relaxant drugs tested were tubocurarine 0.5–1 μg/4 ml, gallamine 3 μg/4 ml, laudexium 0.1 μg/4 ml, decamethonium 3 μg/4 ml, suxamethonium 1–3 μg/4 ml, and two synthetic compounds, DF596 1–2 μg/4 ml and DF648 1 μg/4 ml.

The sera tested were derived from normal healthy volunteers, from patients with obstructive, hepatic and haemolytic jaundice and from patients with liver cell disease without jaundice.

Froth formation in the presence of serum was prevented or reduced by adding a small drop of silicone antifoam to the Ringer-bath.

**RESULTS**

The presence of serum in the Ringer solution did not alter the height of the contracture of the frog rectus produced in response to 1 μg acetylcholine except on rare occasions when a slight decrease was noted.

All relaxants antagonized the action of acetylcholine on the frog rectus less effectively in the presence of serum. In the case of suxamethonium both the suxamethonium contracture and the anti-acetylcholine action were reduced when serum was present in the Ringer solution.

Occasionally the standard response to acetylcholine was enhanced immediately after a relaxant had been removed from contact with the muscle. Only one response was enhanced, usually the first contracture after the relaxant had been washed out, but once or twice it did not appear until the second contracture (fig. 1).

The competitive blocking agents tubocurarine (fig. 2), gallamine and laudexium invariably antagonized the acetylcholine contracture in the presence of normal serum. With gallamine and tubocurarine, in most instances, this antagonism was less marked when the serum was derived from jaundiced patients, but sometimes it was unchanged and in one instance the presence of jaundiced serum enhanced the action of tubocurarine. The antagonistic effect of jaundiced serum on laudexium was both less obvious and less frequent. Details are set out in table I.

**Table I**

*The effect of muscle relaxant drugs on the height of the contracture of frog rectus muscle in response to acetylcholine in the presence of serum from jaundiced patients.*

*Acetylcholine dose level: 0.5–1.5 μg/4 ml Ringer.*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose μg/4 ml Ringer solution</th>
<th>No. of jaundice sera tested</th>
<th>Effect antagonized</th>
<th>No change</th>
<th>Effect enhanced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubocurarine</td>
<td>0.5–1</td>
<td>32</td>
<td>23</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Gallamine</td>
<td>3</td>
<td>18</td>
<td>13</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Decamethonium</td>
<td>3</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>Suxamethonium</td>
<td>1–3</td>
<td>14</td>
<td>9</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Laudexium</td>
<td>0.1</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>DF596</td>
<td>1–2</td>
<td>8</td>
<td>1</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>DF648</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>
Fig. 1
Showing the enhanced response to acetylcholine after previous contact with a relaxant drug. The reduced effect of gallamine in antagonizing the acetylcholine induced contracture in the presence of jaundiced serum is also illustrated.
A: normal serum and 1 μg gallamine.
B: hepatic jaundiced serum and 1 μg gallamine.

Fig. 2
Showing the inhibiting effect of serum from a patient with hepatic jaundice on the action of tubocurarine.
A: normal serum and 1 μg tubocurarine.
B: hepatic jaundice serum and 1 μg tubocurarine.

Fig. 3
Showing the slight contracture produced by decamethonium as well as the reduced blocking action of the drug in the presence of jaundiced serum.
A: normal serum and 1 μg decamethonium.
B: obstructive jaundiced serum and 1 μg decamethonium.
The depolarizing agents decamethonium (fig. 3) and suxamethonium (fig. 4) are both capable of producing contractures of the frog rectus muscle similar to those produced by acetylcholine. In practice this rarely occurred with decamethonium since the minimum dose required to antagonize the acetylcholine contracture has little or no stimulant effect of its own. With suxamethonium, however, the antagonist dose and the stimulant dose were approximately equal and this led to difficulties in interpretation. Nevertheless, it was possible on a number of occasions to eliminate the stimulating effect of the drug while retaining its antagonist action. As with the competitive blocking drugs the depolarizing agents were usually less active in the presence of jaundiced serum than in the presence of normal serum. In one instance the effect of suxamethonium was enhanced when jaundiced serum was present.

The tropeine derivatives DF596 (fig. 5) and DF648 (fig. 6), though classified as competitive blocking agents behaved quite differently from tubocurarine and gallamine in the presence of jaundiced serum. Almost invariably the extent of the block produced by both drugs was greatly enhanced when jaundiced serum was used (table I) and a similar effect was produced in the presence of serum from patients with liver disease.

**FIG. 4**

Showing the slight contracture produced by suxamethonium (at A) on which is superimposed the reduced acetylcholine response. At B, the suxamethonium induced contracture is virtually absent but the acetylcholine response is only slightly inhibited.

A: normal serum and 2 \( \mu g \) suxamethonium.
B: obstructive jaundiced serum and 2 \( \mu g \) suxamethonium.

**FIG. 5**

Showing that the presence of jaundiced serum enhances the blocking action of DF596.

A: hepatic jaundice serum and 1 \( \mu g \) DF596.
B: normal serum and 1 \( \mu g \) DF596.
THE EFFECT OF SERUM FROM JAUNDICED PATIENTS

FROG RECTUS RESPONSE

1 µg Acetylcholine

A: normal serum and 1 µg DF648.
B: hepatic jaundiced serum and 1 µg DF648.

Fig. 6

Showing that the neuromuscular blocking action of DF648 is enhanced by serum from a patient with hepatic jaundice.

Serum from all types of jaundice modified the antagonism of relaxants in the same way, but only occasionally was it found that one serum would modify all relaxants; commonly at least one of the group would be unaffected.

Of five sera derived from patients with liver disease but not jaundiced, none antagonized the action of tubocurarine but all five enhanced the action of DF596. Of four tested on gallamine, three produced antagonism. Two sera out of four antagonized decamethonium. The effect on suxamethonium was less clear; only one had an antagonistic effect and two appeared to enhance the action of the drug.

DISCUSSION

Antagonism to tubocurarine in patients with liver disease was first reported by Dundee and Gray (1953) who suggested that such patients might have motor end-plates more sensitive to acetylcholine and therefore more resistant to tubocurarine. This suggestion was based on the fact that patients with liver disease have a lowered serum pseudocholinesterase (Wilson, Calvert and Georghegan, 1952) and on the observation by Heymans, Verbeke and Votava (1948) that when blood pseudocholinesterase is lowered there is an increased responsiveness to acetylcholine.

The resistance to tubocurarine seen in our experiments could be connected with lowered pseudocholinesterase levels in the serum but cannot be due to the influence of a diseased liver on end-plate cholinesterase since the frog-rectus method does not test the sensitivity of the patient's motor end-plates and since many of the sera tested were from patients without liver disease. Furthermore, such an explanation cannot account for the weakened effect of the depolarizing drugs, decamethonium and suxamethonium; still less can it account for the enhanced effect of the competitive blocking substances DF596 and DF648.

Our evidence suggests that there is a factor present in serum capable of interfering with the mechanism of neuromuscular block. The fact that serum, jaundiced or otherwise, does not affect the response to acetylcholine whereas both normal and jaundiced sera modify the antagonistic action of the relaxants implies that this unknown factor reacts with the blocking drug and not with the mechanism of neuromuscular transmission.

Specific evidence in favour of such a reaction comes from the work of Aladjemoff, Dikstein and Shafrir (1958) who showed that in two healthy patients resistant to tubocurarine the plasma level of the drug was significantly higher than the levels in patients not resistant and given the same dose. They went on to show that a dose of tubocurarine previously incubated with bovine globulin or human albumin was much less effective than the same dose untreated when injected into dogs. Aladjemoff and her colleagues inferred from this that in patients resistant to tubocurarine the drug becomes bound in excessive amounts to plasma protein so that it is unable to diffuse out of the vascular system in sufficient concentration to establish effective neuromuscular block.

Increased protein binding probably provides the most likely explanation for the reduced effectiveness shown by the majority of the relaxant drugs tested in the presence of jaundiced serum. The fact that resistance to the action of relaxants is not confined to patients with liver disease makes it easier to accept this interpretation since a com-
mon factor in jaundice, whether of obstructive, hepatic or haemolytic origin, is a disturbance in the plasma protein content. The more equivocal results obtained with sera from patients with liver disease but no jaundice could be explained by the absence of any significant disturbance in plasma protein content.

The only complicating factor is the enhanced action of the tropeine derivative DF596 and DF648 which cannot be explained by increased protein binding. These drugs, however, were synthesized on the basis of a susceptibility to the action of serum esterases (Haining, Johnston and Smith, 1960). Thus although the low serum cholinesterase previously discussed cannot account for the antagonism described it could explain the increased effectiveness of the tropeine derivatives.

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REFERENCES


