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# THE EFFECT OF INJECTED SOLUTIONS ON THE CELL CONTENT OF THE CEREBROSPINAL FLUID

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An account has been given in an earlier paper (Bedford, 1946) of the effect of the introduction of isotonic sodium chloride solution into the cisterna magna on the cell content of the cerebrospinal fluid of dogs anaesthetized with "nembutal" (Abbott). The duration of these experiments was approximately five hours. Polymorphonuclear leucocytes, generally unaccompanied by other reactive cells, were present at the end of nine out of thirteen experiments. The introduction under the same conditions of Ringer's solution (Dale's formula) or of distilled water did not cause reactive cells to appear in the cerebrospinal fluid. In the experiments now reported, the changes in cell count have been investigated 24 hours after the introduction of an isotonic solution of sodium chloride into the cisterna magna, and a comparison has been made with the effects produced after a similar period of time by solutions of procaine, amylocaine, and amethocaine hydrochlorides. A study has also been made of the effect of simple puncture of the cisterna magna, the introduction of distilled water and of Ringer's solution.

### *Experimental procedure*

Except for slight modifications, the experimental procedure was similar to that used in earlier experiments (Bedford, 1946). In this series of experiments the animals were anaesthetized with ether. They were allowed to recover from the anaesthetic after the solution under investigation had been introduced and the needle withdrawn. It was necessary, however, after the introduction of local anaesthetics to maintain respiration with the pump until the onset of spontaneous respiration. The animals were again anaesthetized with ether twenty-four hours later and a sample of cerebrospinal fluid removed for cytological examination. The films were fixed by exposure to iodine vapour after the technique of Kubie and Smith (1925) and lightly stained with methylene blue. The use of this technique has greatly facilitated cellular differentiation. In the present series of experiments a finer needle has been used for cisternal puncture; as a result, detached mesothelial cells have been observed only on rare occasions in specimens of cerebrospinal fluid, and the possibility of post-operative leak from the subarachnoid space has been reduced. The puncture was made without previous incision directly through the shaved and sterilized skin. An experiment was discontinued if the initial sample of cerebrospinal fluid contained cells of any kind. The solutions were made up with freshly distilled water from an all-glass still. Although the individual solutions were not tested for the presence of pyrogens, repeated examination of distilled water from this still has never revealed their presence. The solutions were sterilized, without delay, in an autoclave at a pressure of 12 lb. per square inch for half an hour. The Ringer's solution was boiled for 20 min. at atmospheric pressure. Care was taken to sterilize the solutions in alkali-free glass containers. A constant volume (1.5 c.c.) of solution, at room temperature, was introduced into the subarachnoid space throughout the experiments.

## RESULTS

### *The effect of ether anaesthesia*

Four dogs were anaesthetized with ether; one for a period of 15 minutes, two for 30 minutes, and one for one hour. The ether was administered by means of a pump through a catheter, introduced into the trachea. In all four dogs, samples of cerebrospinal fluid removed 24 hours later under ether anaesthesia were free from cells.

*The effect of simple puncture and of distilled water*

The effect of simple puncture of the cisterna magna was studied in ten dogs. After introduction, the needle was allowed to remain *in situ* with the stilette inserted for ten minutes. At the end of this period, the needle was withdrawn and the animal allowed to recover. The results obtained 24 hours later are summarized in Table I,

TABLE I

THE EFFECT OF SIMPLE PUNCTURE OF THE CISTERNA MAGNA AND OF DISTILLED WATER ON THE CELL CONTENT OF THE CEREBROSPINAL FLUID

| Simple puncture | | Distilled water (1.5 c.c.) | |
|---|---|---|---|
| Wt. of dog in kg. | White cells per cu.mm. C.S.F. after 24 hours | Wt. of dog in kg. | White cells per cu.mm. C.S.F. after 24 hours |
| 11.0 | 40 | 10.0 | 480 |
| 8.0 | 70 | 9.0 | 450 |
| 7.5 | 60 | 8.5 | 450 |
| 11.0 | 70 | 10.0 | 345 |
| 8.0 | 20 | 7.5 | 420 |
| 8.6 | 40 | 6.0 | 400 |
| 12.5 | 12 | — | — |
| 7.5 | 40 | — | — |
| 8.0 | 0 | — | — |
| 6.5 | 12 | — | — |
| Mean ± S.D. | 36 ± 8 | | 424 ± 19 |

where it will be noticed that white cells were present at the end of all but one experiment. The average number of cells per cu.mm. of cerebrospinal fluid was 36. The effect of the introduction of distilled water was studied in six animals. The results are summarized in Table I, where it will be seen that the average number of white cells at the end of the experiment was 424, and the standard deviation of the average 19.

*The effect of isotonic sodium chloride and of Ringer's solution*

The effect of isotonic sodium chloride solution (0.9 g. NaCl/100 c.c.) was studied in nine experiments and that of Ringer's solution (Dale's formula) in six experiments. The *p*H values of the solutions after sterilization were 6.8 and 7.4 respectively, as determined by indicators. The results of the experiments are summarized in Table II.

*The effect of solutions of procaine, amylocaine, and amethocaine hydrochlorides*

The effect of procaine was studied in ten experiments; five experiments were performed with

TABLE II

THE EFFECT OF ISOTONIC SODIUM CHLORIDE SOLUTION AND OF RINGER'S SOLUTION (DALE'S FORMULA) ON THE CELL CONTENT OF THE CEREBROSPINAL FLUID AFTER 24 HOURS

| Normal Sodium Chloride Solution (1.5 c.c.) | | Ringer's Solution Dale's Formula (1.5 c.c.) | |
|---|---|---|---|
| Wt. of dog in kg. | White cells per cu.mm. C.S.F. after 24 hours | Wt. of dog in kg. | White cells per cu.mm. C.S.F. after 24 hours |
| 7.5 | 1,000 | 9.0 | 740 |
| 5.5 | 750 | 6.0 | 490 |
| 8.0 | 1,500 | 8.0 | 720 |
| 7.0 | 750 | 7.0 | 690 |
| 6.0 | 700 | 7.5 | 470 |
| 8.5 | 1,500 | 6.5 | 680 |
| 7.0 | 800 | — | — |
| 8.5 | 1,600 | — | — |
| 7.5 | 1,000 | — | — |
| Mean ± S.D. | 1,067 ± 109 | | 632 ± 49 |

amylocaine and a similar number with amethocaine. Procaine and amylocaine were administered in a 1 per cent (w/v) concentration in distilled water. The depressant action of amethocaine on respiration was so powerful and prolonged that it was found convenient to use a solution not stronger than 0.25 per cent; even after the introduction of a solution of this concentration, respiration was frequently paralysed for half an hour or longer. The *p*H values of the procaine, amylocaine, and amethocaine solutions after sterilization were 5.9, 5.9, and 6.0 respectively. The results of these experiments are summarized in Table III.

It will be seen that the average number of cells per cu.mm. of cerebrospinal fluid at the end of the experiments was approximately the same with all three drugs.

## Discussion

It would seem from the above experiments that the introduction into the subarachnoid space of isotonic sodium chloride solution, procaine, and amylocaine hydrochlorides in 1 per cent (w/v) and amethocaine hydrochloride in 0.25 per cent (w/v) concentration in distilled water excites reactions of approximately equal intensity as determined by the number of white blood corpuscles in the cerebrospinal fluid after 24 hours. Ringer's solution caused a more intense reaction than distilled water, but not so powerful as that of isotonic sodium chloride solution; it occupied a position intermediate between the two. The standard deviations of the averages obtained in these experiments indicate that the above differences in

TABLE III

THE EFFECT OF PROCAINE, AMYLOCAINE AND AMETHOCAINE HYDROCHLORIDES ON THE CELL CONTENT OF THE CEREBROSPINAL FLUID

| 1% (w/v) Procaine (1.5 c.c.) | | 1% (w/v) Amylocaine (1.5 c.c.) | | 0·25% (w/v) Amethocaine (1.5 c.c.) | |
|---|---|---|---|---|---|
| Weight in kg. | White cells per cu.mm. C.S.F. after 24 hours | Weight in kg. | White cells per cu.mm. C.S.F. after 24 hours | Weight in kg. | White cells per cu.mm. C.S.F. after 24 hours |
| 6.5 | 1,340 | 6.2 | 1,600 | 8.5 | 670 |
| 10.5 | 840 | 12.0 | 800 | 7.5 | 1,490 |
| 12.0 | 1,500 | 12.5 | 810 | 8.0 | 730 |
| 8.0 | 810 | 7.5 | 1,090 | 9.5 | 1,360 |
| 10.0 | 900 | 12.0 | 700 | 6.5 | 1,500 |
| 7.0 | 1,200 | — | — | — | — |
| 8.5 | 750 | — | — | — | — |
| 7.5 | 825 | — | — | — | — |
| 9.0 | 1,180 | — | — | — | — |
| 8.5 | 1,010 | — | — | — | — |
| Mean ± S.D. | 1,035 ± 81 | | 1,000 ± 163 | | 1,150 ± 185 |

intensity of reaction are statistically significant. The lack of correlation between the weight of the animal and the reaction provoked by a constant volume of solution was unexpected. It can, however, be accounted for in several ways. The dogs used in these experiments were mongrels; little selection has been possible and consequently marked variation was noticed in the size and shape of the cisterna magna of dogs of the same weight. Variations will accordingly occur in the degree of dilution of solution immediately after introduction and in the rate at which it leaves the cisterna magna. An important cause of lack of correlation between body weight and reaction to a given volume of solution is leakage through the puncture hole after withdrawal of the needle. It has been possible to demonstrate that the puncture hole through the dura and the arachnoid does not always close after withdrawal of the needle; the longer the needle remains inserted, the more likely is leakage to occur. A leaking puncture hole can produce two important results: if the pressure in the cisterna magna is high, as normally occurs when the animal is inclined towards the "head-down-tail-up" posture, fluid escapes, often at a considerable rate, from the cisterna magna into the extradural region. On the other hand, if the animal is inclined towards the "head-up-tail-down" posture, the pressure in the cisterna magna falls and aspiration may take place from the extradural region. The fluid aspirated generally consists of blood and tissue fluid, which are known (Hammes, 1944) to have a marked irritant action on the meninges. These phenomena have frequently been demonstrated on the living animal during the course of the experiments. Their occurrence can be avoided to a certain extent by the use of a fine needle and by careful avoidance of any disturbance of the needle after introduction.

The cells present in the cerebrospinal fluid at the end of the experiments consisted almost entirely of polymorphonuclear leucocytes; in many instances they were the only cells detected. Lymphocytes rarely formed more than 3 per cent of the total white cells.

It is improbable that the cellular reaction provoked by local anaesthetics is a *p*H response. Experiments *in vitro* have shown that the solutions rapidly assumed the *p*H of the cerebrospinal fluid on the addition of a relatively small volume of the latter fluid. Injected solution, withdrawn a few seconds after introduction into the cisterna magna, was generally found to have acquired the *p*H of cerebrospinal fluid.

In spite of technical difficulties, the experiments would appear to demonstrate that distilled water is a less powerful irritant to the meninges than isotonic sodium chloride solution and thereby to confirm the results of earlier experiments (Bedford, 1946), that isotonic sodium chloride solution, procaine, and amylocaine hydrochlorides in a 1 per cent (w/v) and amethocaine hydrochloride in a 0.25 per cent concentration in distilled water excite reactions of approximately equal intensity.

The results of the experiments raise doubt as to the advisability of using simple sodium chloride in order to render isotonic solutions required for introduction into the subarachnoid space.

SUMMARY

1. A study has been made of the cell content of the cerebrospinal fluid 24 hours after the introduction of procaine, amylocaine, and amethocaine into the cisterna magna of dogs and the effects of these drugs have been compared with those produced by distilled water, isotonic sodium chloride and Ringer's solution over a similar period of time.

2. Isotonic sodium chloride solution, 1 per cent procaine and amylocaine hydrochlorides and 0.25 per cent amethocaine hydrochloride in distilled water excited reactions of approximately equal intensity. Ringer's solution caused a more intense reaction than distilled water, but less than that of isotonic sodium chloride solution.

3. The results of these experiments raise doubt as to the advisability of using simple sodium chloride in order to render isotonic solutions required for introduction into the subarachnoid space.

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