

- 1 The authors are indebted to Dr. M. Kárteszi, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, for the determination of plasma ACTH by radioimmunoassay in our patients, and to Prof. P. Vecsei, Department of Pharmacology, University of Heidelberg, providing us with highly specific antisera for corticosteroids RIA-s.
- 2 R.F. Bing and D. Schulster, *J. Endocr.* 74, 261 (1977).
- 3 J. Müller, in: Regulation of aldosterone biosynthesis. Monographs on endocrinology, vol. 5, p. 32. Springer-Verlag Berlin, Heidelberg, New York 1971.
- 4 J.F. Tait, S.A.S. Tait, J.D.M. Albano, B.L. Brown and F.L. Mendelsohn, in: Research on steroids, vol. 6, p. 19. Ed. H. Breuer, A. Hughes, A. Klopfer, C. Conti, P. Jungblut and L. Lerner. North-Holland Publ., Amsterdam/Oxford 1975.
- 5 R.L. Swallow and G. Sayers, *Proc. Soc. exp. Biol. Med.* 131, 1 (1969).
- 6 H.E. Falke, H.J. Degenhart, G.J.A. Abeln, H.K.A. Visser and R.J.M. Crougths, *Acta endocr.* 78, 110 (1975).
- 7 P. Vecsei, J. Onyechi, J. Hornung, R. Dietz, G. Mast, H. Hobler, *J. steroid Biochem.* 6, 383 (1975).
- 8 P. Vecsei, in: Methods of Hormone Radioimmunoassay, p. 393. Ed. M.B. Jaffee and H.R. Behrmann, Academia Press, New York 1974.
- 9 T.M. Connolly, P. Vecsei, D. Haak, K.H. Kohl, S. Abdelhamid, A. Ammenti, *Klin. Wschr.* 56, (Suppl. 1) 173 (1978).
- 10 D.T. Krieger, *Ann. N.Y. Acad. Sci.* 297, 527 (1977).
- 11 E. Gláz, R. Kiss, Gy. Lada, S. Vida, É. Katrics, E. Pécsi, in: Pathomechanism, clinical and therapeutic aspects of hyperaldosteronism, p. 43. Ed. E. Gláz. Boehringer, Mannheim Ltd. Publication, Budapest 1979.
- 12 Z. Kraiem, T. Rosenthal, R. Rotzak, B. Lunenfeld, *J. Steroid Biochemistry* 9, Abstr. 36 (1978).
- 13 J. Müller, in: Pathomechanism, clinical and therapeutic aspects of hyperaldosteronism, p. 21. Ed. E. Gláz. Boehringer, Mannheim Ltd. Publication, Budapest 1979.
- 14 E. Gláz and P. Vecsei, in: Aldosterone, p. 377. Pergamon Press, Oxford, and Akadémiai Kiadó, Budapest 1971.
- 15 H. Vetter, J.M. Bayer and W. Vetter, in: Frontiers of Internal Medicine 1974, p. 146. Ed. W.J. Alkan and R.D. Brunner. Karger, Basel 1975.

DISPUTANDUM

Acupuncture points and cutaneous nerves

J.P. Plummer

Department of Anatomy, University of Hong Kong, 4, Sassoon Road, Hong Kong (Hong Kong), 5 March 1979

Summary. In view of the number of workers who have confirmed the presence of cutaneous nerves beneath acupuncture points, a plea is made not to dismiss them too lightly or hastily at this point in the search for the mechanisms of acupuncture.

In a recent article in this journal Weidmann¹ surveyed the work in progress at the Shanghai Institute of Physiology, Division of Acupuncture. Quoting Chiang's work², Weidmann points out that skin nerves may be blocked by a local anaesthetic without loss of the analgesic effect of acupuncture applied to the same spot, whereas applying local anaesthetic to the muscle nerves makes needling ineffective.

As yet, persistent reports of a definite anatomical arrangement of structures at acupuncture points are lacking and indeed some of the findings are somewhat confusing if not contradictory. Recently Reichmanis and Becker³ have reviewed some of the anatomical findings reported in the literature.

A number of workers have noted the close association of points with different neural structures, and Gunn et al.⁴ proposed a classification of 70 acupuncture points according to their known neural structures, mentioning 3 types corresponding to: 1. the motor point of a muscle (35 points); 2. the focal meeting of superficial nerves in the sagittal plane (14 points); 3. points overlying superficial nerves or plexuses (21 points).

He is of course implying that at least some acupuncture points have some definite relationship with some superficial nerves.

Chiang² does not of course deny this and he injected local anaesthetic into a branch of the radial nerve which passes directly in the skin over Hoku (Co 4, L.I.4). Does this mean that this nerve has nothing to do with this acupuncture point but just happens to be passing here by chance?

Matsumoto and Lyu⁵ have reported the correspondence between 33 acupuncture points and the usual points injected with local anaesthetic to produce regional/local nerve block.

Bossy et al.⁶ performed dissection on 201 acupuncture points and reported macroscopic findings as follows: 58 points (29%) revealed a superficial cerebrospinal nerve, 74 points (37%) revealed a vasculo nervous pedicle, 69 points (34%) revealed a vascular element, mostly venous. They only dissected to a depth of 5 mm, and were thus only reporting 'supra-aponeurotic macroscopic elements'. However they claim that 132 points out of 201, i.e. 66%, revealed macroscopic superficial nerves. Is it just coincidence that these nerves are passing by acupuncture points? A group of workers in Shanghai⁷ have performed dissection on 324 points, not only superficially but also into deeper tissues as well. 8 cadavers, 49 upper limbs and 24 lower limbs were used in their studies although they do not state on how many of the parts each individual point was dissected. They reported finding macroscopic neural structures (nerves) beneath every point except one. Details of their findings are as follows:

superficial (cutaneous) nerves: 304 points

deep nerves: 170 points

both superficial and deep nerves: 149 points

The one remaining point together with 21 other important points were examined microscopically after staining and it was found that at each of these 22 points, from the skin down to deeper tissues (including muscle), in every layer there was an abundance of concentrated nerve bundles and fibres of varying diameters, and numerous dense nerve endings. Note especially that they found macroscopic evidence of cutaneous nerves at 304 of the 324 points examined i.e. 94%. Unfortunately fuller details have not yet been published to this author's knowledge.

My own studies including macroscopic dissection, microscopic sections and clinical examination have also shown that many acupuncture points have cutaneous nerves pass-

ing beneath them, and in fact a branch of the nerve seems to actually pierce the dermis right at the point⁸. In any case I should like to urge that the presence of cutaneous nerves beneath so many acupuncture points not be dismissed too hastily at this point in our present knowledge (or lack of it) of the mechanisms of acupuncture.

- 1 S. Weidmann, *Experientia* 34, 964 (1978).
- 2 C.Y. Chiang, *Sci. sin.* 16, 210 (1973).
- 3 M. Reichmanis and R.O. Becker, *Comp. Med. East West*, 6, 67 (1978).
- 4 C.C. Gunn, F.G. Ditchburn, M.H. King and G.J. Renwick, *Am. J. Chin. Med.* 4, 183 (1976).
- 5 T. Matsumoto, *Am. Surg.* 41, 11 (1975).
- 6 J. Bossy, J.C. Maurel and G. Godlewski, *Bull. Ass. Anat.* 59, 357 (1975).

- 7 Shanghai No. 1 medical college acupuncture anaesthesia group, affiliated with Chung Shan Memorial Hospital acupuncture anaesthesia group. *Liberation Daily News*, January 5, 1972, in Chinese.
- 8 J.P. Plummer, *Observations at Acupuncture Points*. In preparation.
- 9 H.T. Chang, *Acupuncture analgesia today*. *Chin. med. J.* 92, 1 (1979).

Note added by S. Weidmann, Berne: The report by Chiang et al.² seems to be undisputed as an experimental fact. However, from the way I quoted this work¹ readers might indeed have got the impression that acupuncture points in relationship with cutaneous nerves do not exist. The present contribution by an anatomist is taken as a valuable comment to show that the afferent pathways taken by acupuncture signals are incompletely understood, as recently re-stated by Chang⁹.

PRO EXPERIMENTIS

Tangential flow filtration of *Bordetella pertussis* submerse cultures

A. Valeri, G. Gazzei and G. Genna

Research Centre, ISVT Sclavo, I-53100 Siena (Italy), 23 January 1979

Summary. A procedure is reported for the large scale separation of *Bordetella pertussis* microorganisms from liquid culture media by tangential flow filtration (cross flow filtration) using anisotropic membranes with a cut-off limit of 1×10^6 daltons, and microporous membranes with a pore size of $0.22 \mu\text{m}$.

The large scale separation of microorganisms from culture broth in vaccine preparation is generally achieved using methods that require complex and very expensive apparatus (continuous flow centrifugation, special filtration units with filter aids) or which are very time-consuming with consequently increased risks of contamination (repeated batch centrifugation).

Filtration techniques with a flow parallel to the filtering surface offer a new approach to the separation of solids from the liquid phase, and may also be applied to bacterial suspensions¹. A tangential flow filtration system, which presents some particular advantages, has been used successfully in this laboratory for the concentration and purification of influenza viruses, using anisotropic membranes with a high molecular weight cut-off².

In this paper we report the results obtained using this system with 2 different types of membranes, for the collection and large-scale concentration of *Bordetella pertussis* cultures: the centrifugation technique was adopted for comparative purposes.

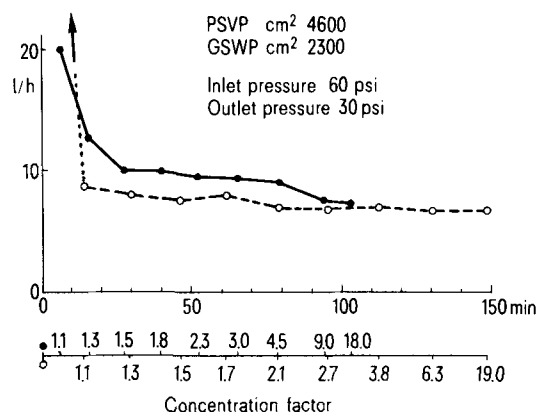
Results and discussion. These studies were performed on the Pellicon Cassette® System equipped with a piston pump, using anisotropic PSVP molecular filtration membranes with a cut-off limit of 1×10^6 daltons and a surface area of 4600 cm^2 , or $0.22 \mu\text{m}$ GSWP microporous membranes with a surface area of 2300 cm^2 (Millipore, Bedford, Mass., USA). Molecular filtration membranes consist of a relatively dense skin $0.02\text{--}0.03 \mu\text{m}$ thick (the actual filtrating part), supported by a porous substructure about $150 \mu\text{m}$ thick, and this asymmetry, in contrast with the homogeneous structure of microporous filters, confers an anisotropic configuration to the membrane; because of these differences in design, the performance of the 2 types of membranes is quite different.

Batches of *B. pertussis* strain 509 submerse cultures, each composed of about 20 l, were concentrated to 1 l by forced tangential flow through the membranes, or were processed

using the Sorvall mod. RC-3 centrifuge. The yield of the process was determined by the Opacity Units method (OU)³, and the quality of the retentate and filtrate was evaluated by the Mouse Toxicity Test (MTT)⁴. The readings were taken, as shown in the table, using previously established levels of toxicity.

The figure shows a typical performance of the membranes during the concentration phase at constant pressure.

The flow levels of the filtrate per unit of time and surface area, obtained in these tests, appear to be higher when employing the microporous membranes; mean values of $0.527 \text{ ml min}^{-1} \text{ cm}^{-2}$ were obtained with the GSWP type, as opposed to $0.0359 \text{ ml min}^{-1} \text{ cm}^{-2}$ with the PSVP type.



Filtrate flow levels during the concentration of *B. pertussis* by tangential filtration using PSVP anisotropic (●) and GSWP microporous (○) membranes. Starting volumes: 18 l for PSVP membranes and 19 l for GSWP membranes. The high initial flow (not estimated) and the rapid passage to a steady flow rate in the case of the microporous membranes, are indicated by the arrow and the dotted line.