ress in recent years. The observation of a time lapse of some 20 min between the beginning of the treatment and the patient reaching an optimum of hypalgesia have vaguely pointed to the participation of a humoral agent. Reports from Shanghai and Peking have given support to this notion. Thus using the classical cross-circulation experiment in rabbits acupuncture to only 1 animal resulted in a state of hypalgesia in both animals. Similarly, applying cerebrospinal fluid from acupunctured to nontreated rabbits had a hypalgesic effect in the recipient animals. No less than 3 different groups[4-6] have extracted from brain tissue a pentapeptide - enkephalin - which was shown to have morphine-like effects. The striking resemblance between the effects of enkephalin and morphine was revealed by Mayer et al. These authors administered naloxone (a substance known to be a specific morphine antagonist) to cats and human patients undergoing acupuncture like stimulation and demonstrated a prompt suppression of the acupuncture effect[7]. It appears, therefore, that the transmitter set free by acupuncture and acting at one or several levels of the CNS may well be identical or related to enkephalin.

The place of acupuncture, as seen by 2 anaesthetists, might be the following. Needling alone in the absence of other analgesic agents will hardly ever become the method of choice for pain relief for surgical interventions. This has been recognized even in China. Muscular relaxation would usually be insufficient and has to be reached by adequate pre-medication. Even if the relief of pain is considered sufficient, the patient may still suffer from modalities other than pain, namely pressure, traction and changes of temperature. If acupuncture in combination with other interventions is used to substitute for general anaesthesia, it is indispensable to gain the collaboration of the patient by explaining the course of the surgical procedure and pointing out possible sensations which she or he may perceive at certain stages. A gentle surgical technique and the avoidance of blunt dissection contribute to the avoidance of pain.

The possibility of using acupuncture should be seriously considered whenever it appears highly desirable to decrease the over-all risk of a surgical intervention by using a minimum of narcotics.

Apart from its application in surgery, electro-acupuncture as well as transcutaneous stimulation of nerves enjoy an increasing popularity in the treatment of chronic pain[8]. It is often possible to place the method of transcutaneous nerve stimulation into the hands of the patient. However, this requires a preceding exploratory phase by a medically qualified person with an adequate theoretical background and much practical experience regarding the indications for this particular method.

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Work in progress at the Shanghai Institute of Physiology, Division of Acupuncture

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Most Western visitors to China reporting on acupuncture are concerned with the effectiveness of the method for pain relief during surgery, rather than with possible mechanisms underlying the method. A review by Birger Kaada[8] is an exception. Kaada, a Norwegian neurophysiologist, being well informed on Western work relating to pain perception, was in a position to critically evaluate the results he was shown in China, in 1973. In June 1976, the author of the present text had the opportunity to visit the Shanghai Physiological Institute, where most of this work relating to the neurophysiological mechanisms of acupuncture is being done. It was easy to communicate with Professor Hsiang-Tung Chang, a neurophysiologist with experience in laboratories of the United States, now a responsible member of the Acupuncture Division. In contrast to Kaada's detailed and critical review the present text is essentially a factual report which had been written up in July 1976 for private circulation.

Meanwhile, a number of biochemically oriented groups have made it appear likely that acupuncture signals release a substance - enkephalin - which has the ability to suppress pain signals. Moreover, a specific morphine antagonist - naloxone - has been shown to reverse the pain-relieving action of acupunc-

ture (for references, see Chen[13]). Progress in what might be called 'pain biochemistry' has been a powerful incentive to re-view our knowledge of the interaction at various levels of the central nervous system between pain signals and acupuncture signals. Since many of the relevant papers are written in Chinese (with English summaries) I was asked by Professor H. Mislin to bring my notes up to date, ready for publication in Experientia.

According to H.-T. Chang's own statement, he paid little attention to the initial reports in 1958 when acupuncture was first successfully applied to surgery in Chinese hospitals. As a result of occasional visits to operating theatres he gradually became convinced 'that there was something in it' and felt it as a challenge to elucidate the underlying mechanisms. Since 1966 acupuncture analgesia has been listed as a subject for intense investigation. What the visitor sees in Shanghai is a well-equipped neurophysiological laboratory, comparable to similar ones in Europe or in the USA. The relevant literature from abroad is known and quoted. The contribution by Chang and his colleagues concerns the functioning of certain parts of the central nervous system and the alteration in functions brought about by acupuncture signals.

Whenever possible human volunteers are used. Localization experiments, when performed with cats, rabbits and rats, require a great number of animals. While negative results are mentioned in the original papers they will not be referred to in the present report.

Pain threshold

The temperature of heated wires attached to the skin of volunteers is varied, and the pain threshold is determined with and without acupuncture. It might be argued that a 'collaborative' person would tend to produce the right answer. This, however, is not so easy, since the intensity of the typical sensation produced at the site of acupuncture does not closely correlate with its analgesic effect.

Another way of determining pain threshold is as follows[4]. A cotton wick soaked in 4 M NaCl is placed in a plastic tube of diameter 2.5 mm. This is applied to the skin, and d.c. pulses of 0.45 sec duration are applied between this 'different' electrode and a large indifferent electrode. A prick sensation underneath the different electrode is taken as the threshold for pain and expressed in mA. It seems likely that free nerve endings are stimulated when hypertonic solution has diffused through the epithelial layer of the skin by electrophoresis. Acupuncture, when properly performed, increases the prick threshold by at least one order of magnitude.

With animals (cat, rabbit) painful stimuli are applied by focusing a light beam on the snout; the time taken by the animal to turn its head is used as an index of pain sensation.

Essential peripheral mechanisms

According to empirical observations the analgesic effect of needling is best seen when the acupuncturist feels a certain stiffness of the muscle while moving the needle. By the patient, the feeling is often described as a combined deep sensation of soreness, heaviness, tightness and swelling. Local muscle activity, as judged from the electromyogram, is highly correlated with the patient's sensation (heavy, moderate, zero); the intensity of the EMG also correlates with the acupuncturist's feeling of stiffness (heavy, moderate, zero). It is concluded that the sensation reported by the patient and the manual feeling of the needle operator are both closely related to local muscle activity[5].

Direct or reflex stimulation?

In patients under lumbar anaesthesia the acupuncturist experiences no local stiffness, and there is no electrical activity of the muscle. It is concluded that muscle stiffness is a reflex phenomenon[5].

The technique of mechanical stimulation by needles

The needle is first introduced at right angles to a depth 2–3 cm within the muscle 'until the characteristic acupuncture sensation is felt'. A mechanical acupuncture manipulator then moves the needles by about 3–5 mm at a frequency of 2/sec, with a twirling angle of 90–165° 'depending on the individual tolerance'[4].

Identity of afferent nerve fibres

Skin nerves may be blocked by a local anaesthetic (procaine) without loss of the analgesic effect of acupuncture applied to the same spot. Applying local anaesthesia to the muscle nerves makes needling ineffective (experiments on volunteers; Chiang[4]). The

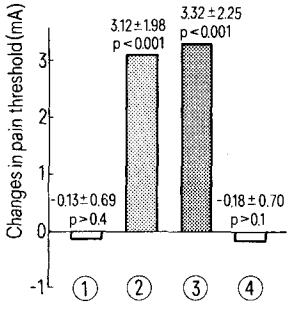

Fig. 1. Changes in pain threshold at different cutaneous points under conditions of rest ①, acupuncture ②, acupuncture following blockade of cutaneous nerves supplying the skin over the needling point ③, and acupuncture following blockade of the muscular nerves supplying the deep tissues underlying the needling point ④. (Chiang[4], fig. 3).

class of nerve fibres that is most important in carrying acupuncture signals is a problem still under investigation. Fibre groups I and II (Aα) as well as III (Aδ) were shown to respond to acupuncture-like manipulations of the m. tibialis anterior of the cat[3]. It is believed[3] that group II, signalling mainly from pressure receptors, is of particular importance. In a recent study[11] unit discharges could be obtained from C fibres (conduction velocity less than 2.5 m/sec) in the fossa poplitea of the cat. The majority of these fibres was activated by strongly pressing the tibialis muscle by means of a glass probe, yet not by stretching the muscle. A quarter of the C fibres responding to pressure also responded to needling of the muscle. When strong pressure or needling was discontinued, there was often the phenomenon of after-discharge lasting for many minutes (Wei et al.[11]). There is reason to assume that Ia and II fibres signalling mainly from muscle spindles play a certain role, while the importance of C fibres should by no means be overlooked. Much work is yet to be done before the question of fibre identity can be considered as fully answered. It would be desirable - but so far has proved to be impossible - to stimulate the population of C fibres electrically without simultaneously activating the thicker fibres within a peripheral nerve.

Ipsilateral or bilateral effect?

'Ho-ku' (a point on the left hand) was needled, and the pain threshold elevation was compared at 5 different sites on the left and right side of the body. The results indicate that unilateral acupuncture raises pain threshold on both sides. There seems to be a certain preference with respect to the segments, the effect on threshold elevation being larger in the segments needled[4].

Intestinal sensations and reflex muscular activity

The following method was used: Electrical stimulation of the left greater splanchnic nerve in cats; recording of action potentials from a dissected intercostal nerve, usually Th 11. A single stimulus applied to the splanchnic nerve resulted in efferent activity lasting for 8-30 msec. Activity of the muscle nerve was suppressed by electrical acupuncture, 100/sec applied through a needle in the animal's hindlimb. A short train of strong pulses applied to the splanchnic nerve resulted in pupillary dilation and in a rise of the blood pressure.

It seems important to mention that this reflex was tested in artificially respirated animals having no other 'anaesthesia' than a nerve-muscle blocker. Ether anaesthesia was only given during surgical interventions[8].

The pathway for the reflex from the splanchnic to the intercostal nerve

On decerebration the immediate effect of acupuncture was preserved, while the after-effect of electrical acupuncture was abolished. Transsection of the spinal cord at upper thoracic or lower cervical levels markedly increased the reflex response and made electroacupuncture of the hindlimb ineffective. It is assumed that interneurons at the spinal level are inhibited by descending impulses from a supraspinal site, and that acupuncture enhances descending inhibition[8].

Localization of pathways in the spinal cord

Bilateral lesion of white matter adjacent to the dorsal horn was most effective in removing inhibition of the spinal reflex mentioned above. It is suggested that this is the pathway for descending inhibition. Bilateral lesion of the ventrolateral funiculi at the height of Th_2 abolished the effect of electroacupuncture when applied to the hindlimb, while acupuncture applied to the forelimb was still effective in inhibiting the reflex from the splanchnic to the intercostal nerve. It is concluded that acupuncture signals have to reach a supraspinal site, from where they probably enhance descending inhibition[9].

Similarities between acupuncture and drug analgesia

Fentanyl in the 'intact' animal suppresses the above mentioned reflex when injected i.v. at 8 µg/kg. The

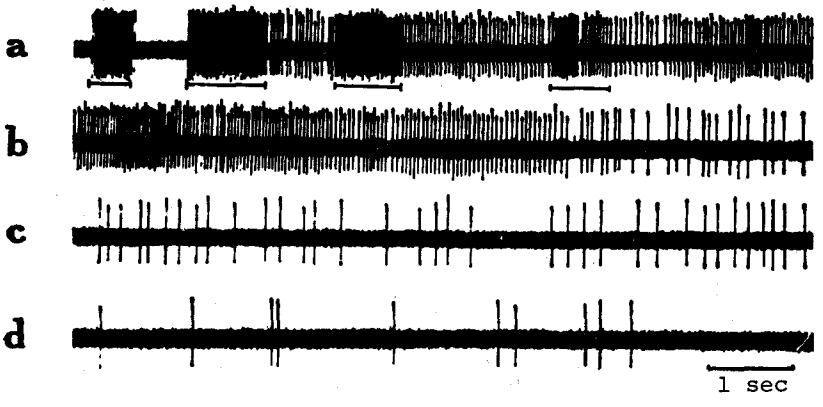

Fig. 2. Discharges from a single C fibre of the tibial nerve in the popliteal fossa of a cat. Pressure was applied 4 times to the m. tibialis anterior, trace a (4 marks). After-discharge of the same fibre is seen in the continuous traces b, c, d. (Wei et al.[11], fig. 3).

effect disappears when the white matter adjacent to the dorsal horn is destroyed. Both interventions thus seem to act by enhancing descending inhibition. It is stressed that different or additional pathways may well be involved for perception of pain and its suppression by acupuncture than for increased muscle tonus by signals from the splanchnic region and its inhibition by acupuncture[9].

Central structures mediating the suppression of the spinal viscero-somatic reflex by acupuncture

Lesion of the nucleus raphe magnus (median region of the medulla) almost completely abolished the inhibitory effect of electro-acupuncture, suggesting that this system is an essential relay between descending inhibition and ascending acupuncture signals[10].

Interaction at the spinal level

The spinal cord of cats was transsected at C_1, thus depriving it of the control by higher 'centers'. Recording microelectrodes were introduced into the dorsolateral fasciculus caudal to L_4. A single strong shock (stimulus for the whole population of fibres) applied to the ipsilateral sural nerve resulted in 2 bursts of activity in single units of the dorsolateral fasciculus. Repeating the same strong shocks, but simultaneously stimulating the same nerve by a train of weaker stimuli (presumably activating $A\alpha$-$A\delta$ fibres only) greatly reduced the rate of firing in a given fibre of the dorsolateral funiculus. There was but little after-effect, and not much latency for 'electroacupuncture' to become effective at the spinal level. Other nerves of the hindlimb could be used for electroacupuncture with somewhat less success than using 'the same nerve'; electroacupuncture applied to the radial nerve had practically no suppressive effect.

The authors assume that the units they have recorded from belong to the tractus spinocervicalis[7], having cell bodies in the dorsal horn, collecting signals from skin, muscle and viscera. They suggest that the site of inhibition is the dorsal horn. It is mentioned in the discussion that the authors have obtained excellent results in the relief of trigeminal pain by electrically stimulating the branch innervating the area of pain. In these experiments the C fibres were apparently not stimulated.

Interaction at the thalamic level

Glass microelectrodes of 0.5 µm tip diameter were pushed through the cortex into the thalamic region. The tips recorded extracellularly from single units, the amplitude of the spikes being of the order of 5 mV.

When recording from single units of the nucleus parafascicularis and stimulating the contralateral sciatic nerve by single shocks, discharge patterns could be found which are thought to be typical for the transmission of the sensation 'pain'. The criteria were as follows a) a long latency (order of 200 msec), b) a prolonged after-discharge, c) the lack of adaptation, d) a pronounced sensitivity to analgesic drugs. Neurons showing these characteristics could be activated by pinching the tail or by skin-pricking of the foot.

A strong stimulus to the sciatic nerve was required to obtain a pattern of unit activity with the criteria enumerated above, indicating that thin fibres must be excited to give the impulse pattern which is thought to be specific for pain.

It seems that 'pain signals' from different sites converge on the same unit at the thalamic level (Chang[1], fig. 5; hindlimb and tail). Under the influence of morphine the 'pain neurons' responded with a lower frequency and shorter duration of discharge; moreover, there was now the phenomenon of adaptation. It is speculated that morphine might decrease pain sensitivity by increasing the tendency to adapt.

Fig. 3. Response of a neuron in nucleus centralis lateralis to different kinds of stimulation. *a* absence of response to tactile stimuli, hair displacement (2 times) and light tappings (2 times) on the hind limb, *b* pin prick on hind limb, *c* forceful pinch of tail, *d* electrical stimulation of peroneal nerve (brief train of 4 pulses, not shown in the figure) (from ref. 1, fig. 5).

Morphine did not depress the activity of all neurons within the nucleus parafascicularis, an observation that strengthens the belief in the specificity of the pain neurons.

Much space is devoted to stating that diverse kinds of acupuncture-like stimulations have but a modest effect at the thalamic level[1]. Most convincing perhaps, is the inhibition of spontaneously discharging neurons of the nucleus parafascicularis by squeezing the tendon of Achilles.

In contrast to the rather insignificant action of acupuncture on the nucleus parafascicularis reported in 1973[1] I was shown evidence of marked inhibition of 'pain units' on the occasion of my visit in Shanghai. This inhibition was the result of directly stimulating the nucleus centralis medialis of the thalamus, which possibly is the receiving station for acupuncture signals. Inhibition can also be demonstrated in neurons of the nucleus parafascicularis by stimulating parts of the forebrain. The question then arises, whether or not the incoming acupuncture signals have to ascend to the forebrain in order to inhibit the structures of the parafascicular nucleus, thus making pain perception part of the reverberating circuit between cortex and thalamus, as already speculated by Chang[1] in 1973.

Fig. 4. Hsiang-Tung Chang in his laboratory in Shanghai, June 1976.

The centromedian nucleus of the thalamus also receives information about pain, but probably information that has been already processed. It may have the role of a thalamic association center[1]. Moreover, certain neurons in the nucleus centralis lateralis (about 50%) discharged in a fashion typical for 'pain': latency of 150–300 msec, after-discharges, sensitivity to analgesics. Trains of electric pulses applied to acupuncture points in the hindlimb inhibited the 'pain discharges' in 10 out of 18 cases. Electroacupuncture became most effective 2–5 min after its onset[2].

Humoral vs. reflex pathway of pain relief by acupuncture

Bilateral adrenalectomy was shown to have no effect on acupuncture analgesia[6].

The question as to a possible mediator set free in the periphery by needling has been answered by experiments on human volunteers. Occlusion of the left arm by a pressure cuff did not statistically diminish the effect of mechanical acupuncture at a point peripheral to the cuff. Pain threshold was compared a) before occlusion and b) 16 min after occlusion[4]. While the outcome of this experiment argues against a pain-relieving substance being released at the site of performing acupuncture, it does not rule out that specific transmitters are involved at the level of the central nervous system.

In fact, it has been reported by a group working at the Shanghai Institute of Materia Medica[12] that ^{3}H-5-hydroxytryptamine is released into perfused cerebral ventricles of rabbits undergoing acupuncture. A letter dated January 30, 1978, from Hsiang-Tung Chang adds the following information: 'These experiments[12] have largely been confirmed in our laboratory. In addition, selective distruction or stimulation of 5-HT containing raphe nuclei could reduce or enhance respectively the analgesic effect of acupuncture. The possible involvement of neurotransmitters in the process of acupuncture analgesia is further suggested by the following finding. In monkeys the reaction time for leverpressing to a nocuous stimulus was considerably prolonged during acupuncture. After the administration of a dose of naloxone the effect of acupuncture could be reversed, suggesting that an endogenous morphine-like substance might have been produced in the brain. Similar results were obtained in human subjects. The endogenous substances in those cases are believed to act as a neurotransmitter rather than an endocrine agent.'

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SPECIALIA

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Naphthoquinones in defensive secretion of an opilionid[1]

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26 January 1978

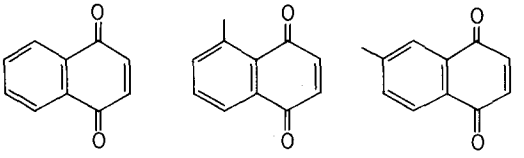
Summary. The defensive secretion of the opilionid *Phalangium opilio* contains 1,4-naphthoquinone and 6-methyl-1,4-naphthoquinone. Naphthoquinones are of rare occurrence in arthropods, having previously been reported only from certain tenebrionid beetles.

Previous studies of the defensive secretions of opilionids showed members of the suborder Laniatores to produce alkylated 1,4-benzoquinones and phenols[3–6] and members of the suborder Palpatores to produce short-chain acyclic compounds, including ketones, alcohols, and an aldehyde[3,7–10]. We here report on a species of Palpatores, *Phalangium opilio,* which is anomalous in that it produces naphthoquinones.

Phalangium opilio is a European species that has become established in parts of North America. Several hundred specimens of both sexes were collected in the vicinity of Ithaca, Tompkins County, New York, and 'milked' of secretion in the laboratory. For that purpose, small sections of capillary tubing were pressed against 1 of the 2 gland openings of each animal, thereby causing secretion from that gland to be squirted into the tube. Since the animals discharged from both glands at once, half their secretion was inevitably lost.

Gas chromatographic-mass spectrometric (GC-MS) analyses (2.4 m × 2 mm column, 2% OV-17 on Gas Chrom Q, 100–250 °C) showed the presence of 2 major components in the secretion of both sexes, in the ratio of 4–6:1. The principal component was identified as 1,4-naphthoquinone (**1**) on the basis of its characteristic mass spectrum (M^+ 158, 100%)[11]. The assignment was confirmed by co-injection with an authentic sample. It was calculated (gas chromatography; napthalene as internal standard) that a minimum of circa 0.5 µg of this quinone had been discharged per gland.

The mass spectrum of the 2nd component (M^+ 172, 100%) indicated that it was closely related to 1,4-naphthoquinone but that it contained an additional CH_2 unit. Comparison of its mass spectrum with that previously reported for 2-methyl-1,4-naphthoquinone[11] ruled out that particular isomer and left 5-methyl- and 6-methyl-1,4-naphthoquinone (**2** and **3**) as possibilities. These compounds were synthesized by Diels-Alder reaction of 1,4-benzoquinone with piperylene and isoprene respectively, and subsequent oxidation. The reaction of isoprene with benzoquinone was carried out most conveniently at 35 °C in aqueous medium, for under these conditions the addition product crystallized from the reaction mixture[12]. Following oxidation of the



1 **2** **3**