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Abstract #3

Nuclear Medicine and Acupuncture: A Study on the Migration of Radioactive Tracers after Injection at Acupoints

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Objective: This paper reports on the authors' investigation of the pathways of acupuncture meridians in the human body through the injection of radioactive tracers (isotopes) at acupuncture points.

Design: The radioactive tracer used was the most common radioactive tracer, techetium-99m (^{99m}Tc), as sodium pertechnetate. The experiment was conducted with a gamma camera, a Siemens SAM (small-area mobile) digital scintillation camera. Image analysis was conducted by a computer system built into the camera. Morphological studies and quantitative dynamic studies were conducted.

The morphological studies consisted of analytical and differential studies. For the analytical studies, the radioactive tracer is injected at a control point located outside any acupoint. Then, another injection is given at an acupoint.

The differential analysis was conducted in order to establish the specific and unique characteristics of the pathways observed in the analytical studies and thus eliminate a vascular or lymphatic explanation. To investigate the vascular pathways, two radiotracers of different energies and therefore discernible by spectrometry were utilized: Technetium-99m was injected as an acupoint and Thallium (^{201}Tl) was injected in a small vein situated next to that acupoint. To study the possible relationship between the lymphatic pathways and those demonstrated by the radiotracer, the same dose (20 MBq) and volume (0.05ml) of pertechnetate was simultaneously injected at an acupoint and the first interdigital space of the foot. A quantitative study of the previous data was conducted after selecting two mirror regions of identical shape and size on the leg along the Liver meridian (an acupuncture meridian) and similar "background noise regions" outside the pathways.

Sequential study and stimulation studies were conducted as part of quantitative dynamic studies. The goal of the sequential study was to evaluate the speed of radiotracer migration along preferential pathways. In healthy control subjects and patients with unilateral renal pathology, two sodium pertechnetate injections of identical volume and activity were given simultaneously

at the left and right acupoints K-7. In the stimulation study, mechanical, electrical, and thermal stimulation were performed on certain acupoints after the injection of radiotracers to study the migration of the radiotracers.

Laboratory experiments conducted in collaboration with the Cytology Laboratory of the Military Hospital of Percy in Paris tested modifications of granulocyte membrane potentials during stimulation of an acupoint using either a needle or a laser beam. The cell membrane potential was measured with a fluorometric method on blood sampled one minute after the end of injections or stimulations, and compared with control blood from the same subject.

Setting: The work was conducted on patients from the Department of Urology and from the Acupuncture Department of Biophysics and Nuclear Medicine from the Necker Hospital in Paris. Each experiment was repeated several times.

Patients and Other Participants: The work was conducted on over 250 healthy control subjects and on 80 patients with renal pathology.

Interventions: Not applicable to this study.

Main Outcome Measures: The authors expected to find that the preferential pathways taken by the radiotracers coincide with the acupuncture meridians as described in Chinese traditional medicine and that these pathways are distinguishable from either lymphatic or vascular routes.

Results: Morphological studies found those tracer migrations from acupoints in both healthy and sick patients followed the same identical pathways with those described as "meridians" in Chinese traditional medicine. The results suggest that these pathways are different from vascular and lymphatic pathways.

The quantitative dynamic studies found that in injections at bilateral K-7, there was a faster diffusion on the healthy side, and slower diffusion on the diseased side. In inflammatory organ disease, there was increased migration speed of the radiotracer in the meridian of the related organ. A reduced tracer migration speed is indicative of a degenerative disease, such as cancer. Such findings could be used as the basis of a therapeutic evaluation or diagnosis. The laboratory experiments with cell membranes suggests that acupoint stimulation could be used to provoke constant and reproducible changes in cellular physiology.

Conclusion: The migration speed and patterns of a radioactive tracer along pathways which coincide with the Chinese acupuncture meridians show that these routes have neither a vascular nor a lympathic origin. These pathways are very likely related to the connective tissue diffusion following the neurovascular bundles along the extremities. Findings suggest the hypothesis of the intervention of a neurochemical mechanism in information transmission.

Abstract #3

Illustration of Radioactive Tracers

Figure 1.

Top: Injection at a control (non-acupuncture) point: No preferential tracer migration was observed during the 307 seconds following injection. *Bottom:* Injection at an acupoint: A preferential migration proceeds at a speed of 6 cm per minute toward the trunk.

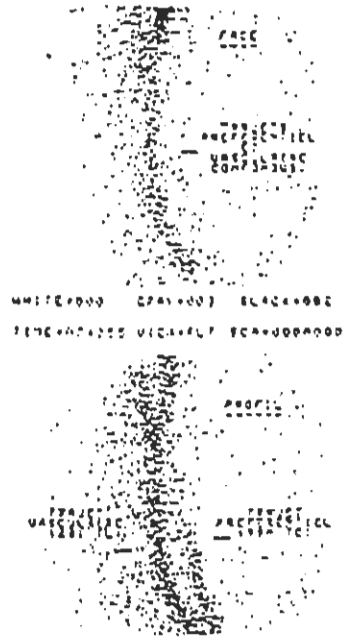


Figure 2.

Top: Anterior view: After injection of ^{99m}Tc at acupoint LV-2 and injection of ^{201}Tl (thallium) in a small vein next to the acupoint. No significant difference was noted between the two pathways. *Bottom:* A profile view clearly shows the separate and distinct routes.

Figure 3.

After injection of the radiotracer at GB-36 both retrograde and anterograde migration is observed which is incompatible with the vascular migration hypothesis. Migration following the Urinary Bladder and Gallbladder meridians in one direction is also noted.

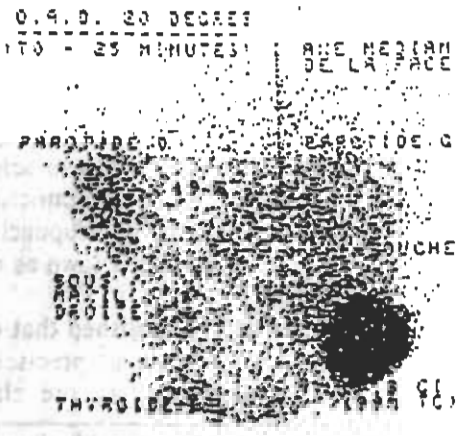


Figure 4.

After injection at LI-18 the tracer migrates to the upper lip crossing the median facial line in the opposite direction of venous circulation. 25 minutes after injection, the preferential pathway is still visible and uptake appears in organs for which ^{99m}Tc has a high affinity, e.g., salivary glands, thyroid.

er than the criteria for nuclear stability. Because of this characteristic they are able to transform spontaneously, emitting an alpha ray if the nucleus is heavy or a beta ray if the nucleus is light, which is the case with the indicators used in nuclear medicine. These emitted rays possess an energy which corresponds either to the totality of the excess in the unstable nucleus, or to a fraction of that energy. This leaves the nucleus in an excited state in which it emits a gamma ray in its transition to a basic state.

This gamma ray, used for the detection of the radioactive tracer, has a greater ability to penetrate biological tissues than that of beta rays. Therefore, the detection of these gamma rays allows us to follow the migration of radioactive tracers. The most common radioactive tracer used in nuclear medicine is technetium-99m (^{99m}Tc), as sodium pertechnetate.

The detection of gamma rays is done with a gamma camera. In this study we used a Siemens SAM (small area mobil) digital scintillation camera with a memory capacity of 256 x 256 pixels, 8 bits each, associated with two image planes of the same dimension. Image analysis was conducted by a computer system built into the camera.

Gamma photons are absorbed into a thallium-activated sodium iodide crystal in which they produce a scintillation. Locating the sites of scintillation within the crystal is accomplished by the intermediary of a matrix of photo-amplifiers which are connected to an electronic device which produces three signals. Signals x and y represent the site of interaction in the crystal and z corresponds to the energy of the incident gamma photon on which the spectrometric determination is made. Signals x and y constitute the co-ordinates whose spacial location is homothetic to that of the interaction site in the crystal, itself a reflection of the site of emission in the selected organ [or site].

After an acupuncture point (acupoint), is located with anatomical landmarks, palpation and measurement of local impedance, the radioactive tracer is injected into that point with hypodermic needles (0.5 mm), at a depth of 3-5 mm as determined by a special sensation [deqi] felt by the subject. The injected volume must be as small as possible, approximately 0.05 ml, with a radioactivity of 10-20 MBq.

These efforts have led in two directions:

1. Morphologic studies, and
2. Quantitative dynamic studies.

Our work was conducted on over 250 healthy control subjects and on 80 patients with renal pathology from the Department of Urology and from the Acupuncture Department of Biophysics and Nuclear Medicine from the Necker Hospital in Paris. Each experiment was repeated several times.

I. Morphological Studies

A. Analytical Studies

Initially, the radioactive tracer is injected at a control point located outside any acupoint. No preferential migration was observed during the 307 seconds of the experiment and only a very slight centrifugal diffusion of the tracer was noted around the injection point (see Figure 1, top).

Conversely, when the injection is performed at an acupoint, a linear migration was noted which, in the case of an extremity, progresses preferentially toward the trunk. After injection at acupoint K-7 (Fuliu, located on the medial aspect of the leg, above and behind the medial malleolus), the migration distance is 30 cm proximally (toward the trunk) from the site of injection after 345 seconds (Fig. 1, bottom).

The tracer migrations from acupoints located on both the upper and lower extremities have always been found to follow identical pathways in healthy control subjects as well as in sick subjects. The pathways demonstrated in these migrations coincide with those described as "meridians" in Chinese traditional medicine.

Hyperconcentrations of the radioactive tracer could be seen along the pathways. Their positions, in relationship to anatomical landmarks, correspond to acupoints distal to the site of injection on the same meridian.

Portions of the twelve meridians located on the upper and lower extremities, and described in Chinese traditional medicine, also demonstrated the same results. Because of the weak energy emitted by the gamma photons of technetium-99m (140 kilo volts), it is difficult to visualize the portions of the meridians which run deep in the trunk. The thickness of the tissues absorbs too many photons and prevents the formation of interpretable images.

Quantitative analyses performed on images and blood sampled up to 60 minutes after injection, in both healthy and ill subjects, show that less than 5% of the radiotracer injected at the acupoint migrates along the meridian. The remainder of the tracer follows the normal rules of diffusion and is slowly resorbed by the vascular bed surrounding the site of injection, but at a rate slower than that observed along the preferential pathways. The diffusion of the radiotracer has such a low concentration that it does not produce a signal-to-noise ratio sufficient to constitute an interpretable image. At 15-20 minutes after injection, however, uptake at the vascular level appears in organs for which the tracer, technetium-99m, has a high affinity such as the thyroid and salivary glands.

In addition to experiments with ^{99m}Tc, similar results have been obtained using other radioactive tracers, such as xenon-133, thallium-201, or bichloride of mercury-197 (which has an affinity for the kidneys). In man, no different molecular migratory behavior between neutral molecules, anions or cations has been shown.

B. Differential Analysis

A differential analysis was conducted in order to establish the specific and unique characteristics of the observed pathways and thus eliminate a vascular or lymphatic explanation.[9]

1. Vascular Pathways

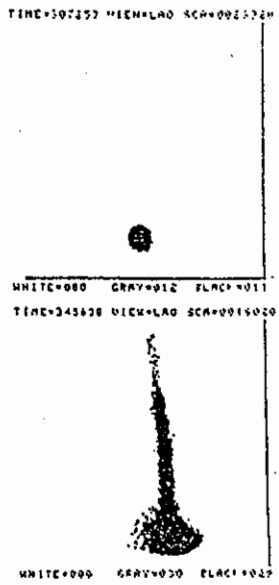
Experimental data suggests that these pathways do not correspond with vascular routes. The speed of migration along the preferential pathways is slow, approximately 3-5 cm per minute and incompatible with transport along a vascular route. In addition, this migration speed is identical whether ^{99m}technetium or a radioactive tracer such as ^{197}Hg-bichloride is used. Renal counts show no significant differences in either appearance-time or in the radioactive concentration of the two tracers. If the route were vascular, these values would reveal a rapid and significant concentration of the radiotracer.

If a tracer is injected randomly, partially intravenously, the observed pathway immediately follows the venous route and disappears in less than a minute, while the migration observed along a meridian requires several minutes to form and disappears very slowly over several dozen minutes.

The hypothesis of rapid diffusion towards the vascular bed has also been rejected. The injection of [^{99m}Tc] pertechnetate at the acupoint Liver-3 (Taichong, located next to the dorsal vein of the foot in the angle between the first and second metatarsal bones), creates a mantle around it. A slow axial pathway of migration can be observed, conforming to the classically described route of the meridian, rather than a more rapid and transversely oriented flow as would be expected via the vascular anatomy.

In order to eliminate the hypothesis of a vascular migration, two radiotracers of different energies and therefore discernible by spectrometry have been utilized: ^{99m}Tc and ^{201}Tl (Thallium). Technetium-99m was injected at an acupoint and ^{201}Tl was injected in a small vein situated next to that acupoint.

An anterior view does not show any significant difference between the pathway



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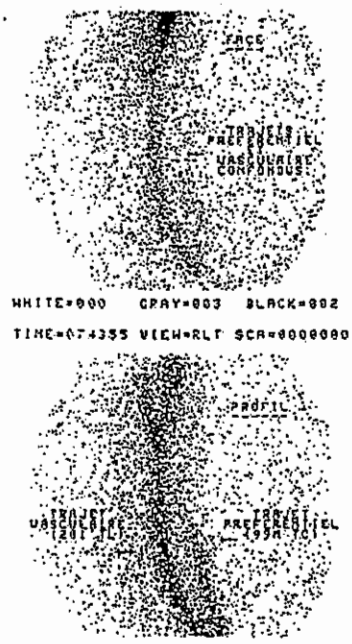

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Top: Anterior view: After injection of ^{99m}Tc at acupoint LV-2 and injection of ^{201}Tl (thallium) in a small vein next to the acupoint. No significant difference was noted between the two pathways. *Bottom:* A profile view clearly shows the separate and distinct routes.

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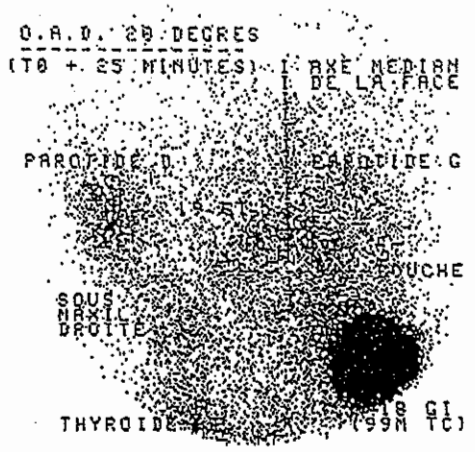

Figure 4.
After injection at LI-18 the tracer migrates to the upper lip crossing the median facial line in the opposite direction of venous circulation. 25 minutes after injection, the preferential pathway is still visible and uptake appears in organs for which ^{99m}Tc has a high affinity, e.g., salivary glands, thyroid.

of the meridian and the vascular route. However, a profile view obtained at a 90 degree angle from the previous image reveals that the "meridian" pathway is distinct from the vascular pathway. Here again, we can observe that the pathway of the meridian is slow to appear and disappear, whereas the vascular pathway images are immediately formed and fade very rapidly (Figure 2). This experiment, which distinguishes the meridian pathway from the vascular route, demonstrates the specificity and uniqueness of these pathways.

An injection of a tracer at acupoint Gallbladder-36 (Waiqiu, located on the lateral aspect of the leg at mid-calf), frequently shows both retrograde and anterograde migration on the meridian pathway, incompatible with the vascular migration hypothesis (see Figure 3). This bidirectional migration is also found when the injection is performed at other acupoints, for example at Stomach-41 (Jiexi) on the dorsal surface of the foot at the tibio-tarsal articulation.

When the tracer is injected at left Large Intestine-18 (Futu, located between the two heads of the sternocleidomastoid muscle level with the laryngeal prominence), it migrates toward the upper lip, which is in the opposite direction of the venous circulation. This migration traverses the median facial line at the nasal base to reach the region of acupoints LI-19 and LI-20 (Kouheliao and Yingxiang) on the right (see Figure 4).

2. Lymphatic Pathways

Qualitative Study: To study the possible relationship between the lymphatic pathways and those demonstrated by the radiotracer, the same dose (20 MBq) and volume (0.05 ml) of [^{99m}Tc] pertechnetate was simultaneously injected at an acupoint and at the first interdigital space of the foot, an area for the study of isotope lymphography of the lower limb. The tracer is used in its colloidal form (rhenium sulfur labeled with ^{99m}Tc).

This anatomical region is the location of the Liver meridian which starts behind the lateral proximal ungual angle of the big toe, then continues along the lateral aspect of this toe to the space between the first and second metatarsal bones on the dorsal side of the foot. This is the site of two important acupoints: LV-1 (Dadun), located at the lateral aspect of the big toe, level with the transverse depression indicating the junction of the nail and its matrix; and LV-2 (Xingjian), located at the lateral aspect of the big toe, distal to the metatarsophalangeal articulation of the big toe, at the base of the first phalanx, thus at the level of the first interdigital space.

In this experiment, 20 MBq ^{99m}Tc (colloidal) was injected at acupoint LV-2 on the right foot. Simultaneously, an identical dose (non-colloidal) was injected in the interdigital space of the left foot, at a similar depth (approximately 3 mm), and with the needle being slightly angled out and down to avoid the Liver meridian as much as possible.

Keeping in mind the weak fraction of the tracer which migrates from the acupoints, these points are kept out of the detector's field to avoid any risk of saturation of the image. Measurement started five minutes after the injections and continued for 2 minutes.

This produced a clear pattern of tracer migration in the right leg in accordance with the Liver meridian originating from acupoint LV-2. However, there is practically no defined migration in the left leg where the injection was performed according to isotope lymphography technique but using a noncolloidal tracer (see Figure 5).

Quantitative Study: Additionally, a quantitative study of the previous data was conducted after selecting two mirror regions of identical shape and size (see regions 1 and 2, Fig. 5) on the leg along the Liver meridian, and two similar "background noise" regions outside the pathways (regions 3 and 4, Fig. 5). The "counts"

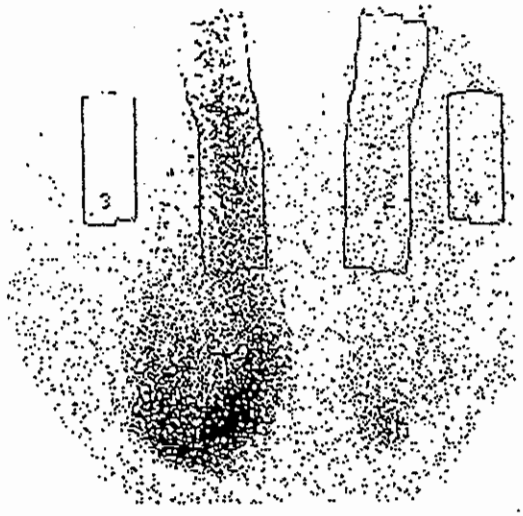

Figure 5.
After injection of ^{99m}Tc at LV-2 there was a clear pattern of tracer migration along the right leg (left side of picture) while no migration was observed along the left leg after injection of a radiotracer at a non-acupoint (the first interdigital space).

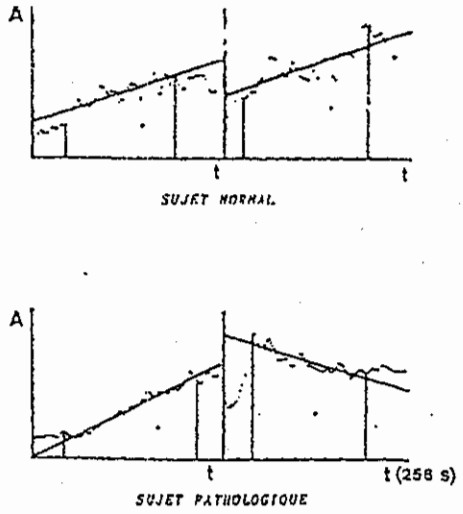

Figure 6.
Sequential study of radiotracer migration speed after injection at left and right acupoint K-7. *Top:* Migration speeds are almost identical on both sides in a healthy subject. *Bottom:* Faster migration was observed on the side of left renal pathology than on the healthy side.

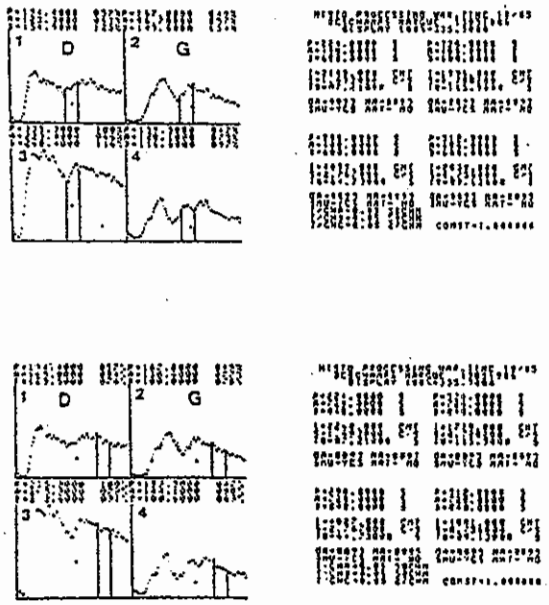

Figure 7.
Two-minute laser stimulation at left acupoint K-2: A marked variation in the rate of tracer migration was noted simultaneously with stimulation both homolaterally (top tracings 2 and 4) and contralaterally (top tracings 1 and 3). A second stimulation four minutes after the first does not show any response (lower tracings).

obtained on the background areas are identical. However, there was a great difference in counts in the region of the Liver meridian depending on the site of injection: LV-2 or interdigital space. The ratio, after normalization and background subtraction, is 7.5 in favor of the injection side performed at the acupoint.

Images taken one and two hours later, revealed no uptake in the lymphatic nodes and the pelvis on the right side where the injection was performed at acupoint LV-2, whereas the lymphatic chain appeared on the left side where the injection was performed according to the standard lymphography technique.

These results suggest that the pathways of the meridians and the lymphatic routes do not coincide. In the above experiment, the minimal activity seen in the left-leg interdigital space injection (13.5% of the right leg), realistically corresponds to a fraction of tracer which has diffused towards the Liver meridian.

This morphological study allows us to affirm, contrary to certain authors,[10] that the preferential pathways evidenced by radioactive isotopes:

- have unique characteristics,
- are coincident with the meridians described in Chinese traditional medicine,
- are distinguishable from vascular and lymphatic routes.

To what do these pathways correspond? The current state of research in this area and most notably the work centered on the contralateral responses to certain stimulation, leads us toward the hypothesis of a neurochemical process.

II. Quantitative Dynamic Studies

A. Sequential Study

The goal of this work was to evaluate the speed of radiotracer migration along preferential pathways. In healthy control subjects and patients with unilateral renal pathology, two sodium pertechnetate injections of identical volume and activity were given simultaneously at the left and right acupoints K-7 (described previously). The image acquisition, starting at injection time, consisting of 128 sequential images of 64 x 64 pixels (2, 4 or 8-second images at 256, 512 or 1024 seconds), constitutes a dynamic study which allows:

1. The summation of the 128 images to create a new image which defines areas of interest corresponding either to a section of the left and right meridians or to the injected points.

2. The graphing of data resulting from emitted impulses in the above areas. This data quantifies the evolution of radioactivity in relation to time (time-activity) and reflects the tracer's output in the regions of interest on meridian pathways.

Results were compared between healthy control subjects and patients with left renal pathology. For normal subjects the median slope of the resulting graph are the same on both sides (see Figure 6, top). Conversely, for a subject presenting with unilateral renal pathology, the slopes are very different and steeper on the pathological side (see Figure 6, bottom) as revealed by sequential images. Such an asymmetric response in a patient with unilateral renal pathology is also found in reference zones relative to the injection points.

The time-activity graphs generated from injection at bilateral K-7 show a faster diffusion on the right than on the left, in accordance with more rapid tracer migration on the healthy right side than on the diseased left.[11] These differences in output of the radiotracer in the preferential pathways correspond to acupuncture meridians and are consistent in every pathological case examined. A constant factor in inflammatory organ disease is found in the increased migration speed of the radiotracer in the meridian of the related organ. Conversely, a reduced tracer migration speed is indicative of a degenerative disease, e.g., cancer. Additionally, migrations lacking continuity, showing accumulation, are indicative of pathology. Such findings can be used as the basis of a therapeutic evaluation or diagnosis and will have a

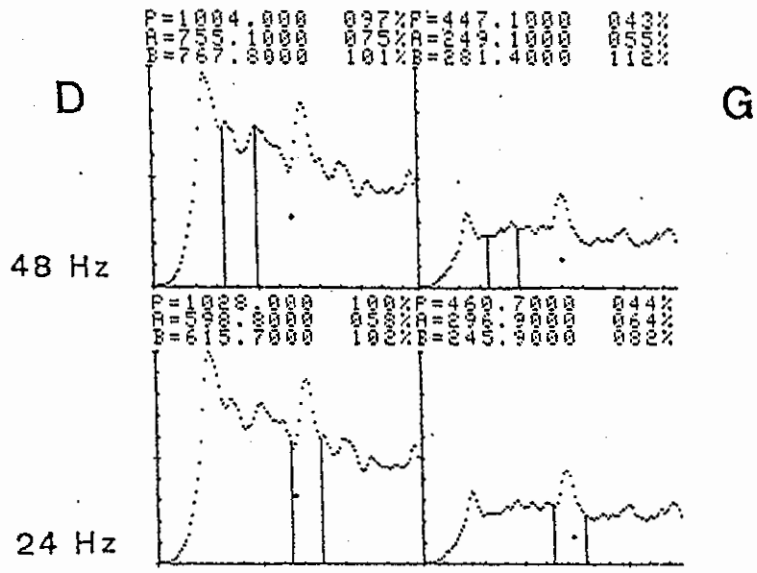

Figure 8.
Five minutes after injection of the radiotracer, a two-minute laser stimulation at left acupoint K-2 with a beam cutting frequency of 48 Hz (top tracings) fails to give a response. At the ninth minute, an identical laser stimulation at right K-2 with a cutting frequency of 24 Hz shows a very positive response both homo- and contralaterally.

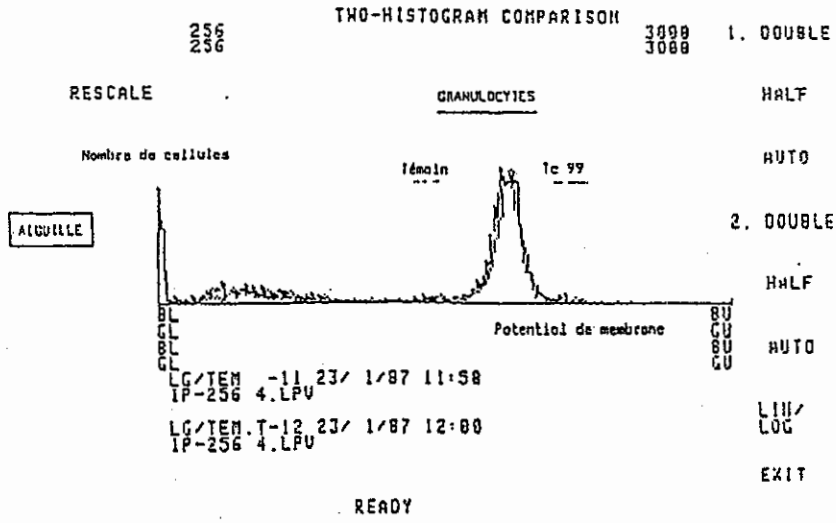

Figure 9.
Simple injection of the radiotracer at an acupoint does not cause any change in the granulocyte membrane potential compared to control blood.

place in the context of early detection and preventive medicine.

B. Stimulations

It was of interest to us to evaluate the effect of acupoint stimulation on the migration of these tracers since such stimulation is typically performed in the clinical practice of acupuncture. Stimulation can be:

- *mechanical*, using acupuncture needles;
- *electrical*, with an alternating or continuous current;
- *thermal*, by applying a source of heat to the acupoint, a process known as "moxibustion" in Chinese medicine.

All such stimulations can generate a subjective response from the patient. In order to avoid this risk, we used a laser beam (produced by a 28 mW helium-neon tube) as a source of stimulation because it does not provoke a sensation.

Two injections of sodium pertechnetate (^{99m}Tc) of identical activity (20 MBq) and volume (0.05 ml) were simultaneously performed at acupoint K-7, right and left. Acquisition consisted of 128 sequential images of 8 seconds each for a duration of 1016 seconds. In areas of interest defined on the preferential pathways, time-activity graphs were constructed.

Eight minutes after injection, a two-minute stimulation by laser was performed unilaterally at acupoint K-2 (Rangu) on the left, located distal to K-7. This point is located on the medial aspect of the foot, at the lower internal extremity of the scaphoid and first cuneiform articulation. A marked variation of the tracer's rate of migration was noted during stimulation, not only on the left side, but also on the right side (see Figure 7).

There was no response to a second stimulation, 12 minutes after the first one: the hypothesis of a refractory period described in Chinese traditional medicine should be considered. Homo- and contralateral responses are very important when we consider the reputed chronobiological "activity" of the acupoint.

Also, it is known that the efficacy of irradiation by low-power laser beam, particularly in rheumatology, is best when the beam cutting frequency is low, around 20 Hz. The same seems to apply to the efficacy of laser stimulation of an acupoint.

After tracer injection at K-7 (left and right), two 2-minute unilateral laser stimulations were performed, one at the fifth minute at K-2 left, and the other at the ninth minute at K-2 right. The cutting frequencies were 48 and 24 Hz, respectively. The resulting graphs show that the 48 Hz stimulation failed to produce a response, whereas a lower frequency significantly modified the tracer's rate, both homo- and contralaterally, with a synchronized change of relatively similar amplitude (see Figure 8). Thus, a low cutting frequency, around 20 Hz, seems to be optimal to provoke a response to stimulation.

Stimulation trials were conducted after tracer injection at acupoint LI-4 (Hegu) (left and right) located between the first and second metacarpal bones on the dorsal side of the hand. At the 320th second, an inflatable armband was secured above the biceps of the right arm, inflated to a pressure at least fifty percent of the systolic arterial blood pressure, and maintained until the 640th second. While the armband was in place, a 2-minute needle stimulation was performed from the 400th to the 520th second at LI-11 (Quchi), located between the injection site and the armband, level with the lateral side of the elbow.

The time-activity graph showed a right-sided interruption of the tracer's flow, coinciding with the constriction due to the armband, and no response to stimulation. On the left side, without armband compression, no response to armband constriction (of the right biceps) and a positive response to stimulation was revealed by a modification of the tracer's migration rate.

Thus, a constriction constitutes an efficient obstacle to the rate of the tracer in

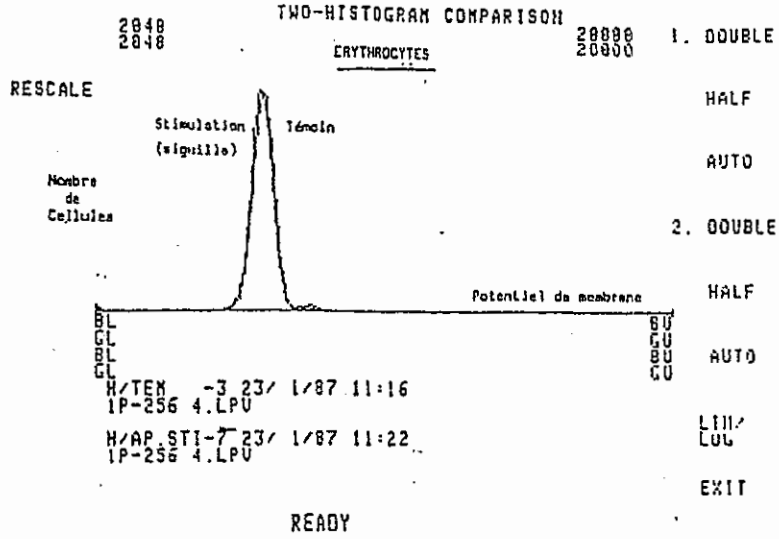

Figure 10.
Stimulation of an acupoint shows no change in erythrocyte membrane potentials.

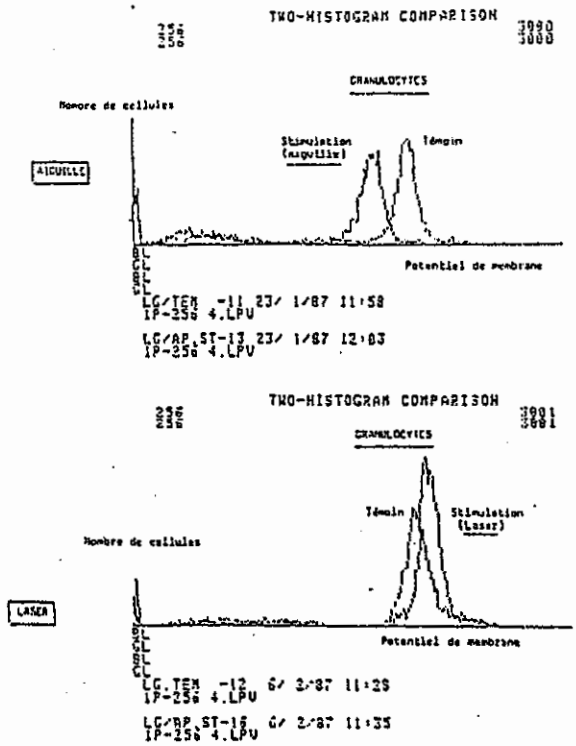

Figure 11.
Stimulation of an acupoint causes a statistically significant change in granulocyte membrane potentials which varies inversely with mechanical (needle) or laser stimulations.

preferential pathways in connective tissues, but does not hinder the simultaneous contralateral transmission of information. This contralateral response further supports the hypothesis of the intervention of a neurochemical process.[12]

C. Biological Modifications:

Laboratory experiments conducted in collaboration with the Cytology Laboratory of the Military Hospital of Percy in Paris[13] showed modifications of granulocyte membrane potentials during stimulation of an acupoint using either a needle or a laser beam. The cell membrane potential was measured with a fluorometric method on blood sampled one minute after the end of injections or stimulations, and compared with control blood from the same subject.

The simple injection of a radiotracer at an acupoint did not cause any modification in the granulocyte membrane potential, compared to control samples (see Figure 9). However, "stimulation" of an acupoint did cause a statistically significant change in the granulocyte membrane potential with no change in the erythrocyte membrane potential (see Figure 10). It was observed that the change in granulocyte membrane potentials induced by needle and laser stimulation varied inversely (see Figure 11).

Finally, no modification of erythrocyte or granulocyte membrane potentials was noted when stimulating a placebo point. These findings suggest the ability of acupoint stimulation to provoke constant and reproducible changes in cellular physiology.

Conclusion

The migration speed and patterns of a radioactive tracer along pathways which coincide with the Chinese acupuncture meridians show that these routes have neither a vascular nor a lymphatic origin. These pathways are very likely related to connective tissue diffusion following the neurovascular bundles along the extremities. In this manner, according to Björn Nordenström (Karolinska Institute in Stockholm, Sweden),[14] interstitial spaces would constitute a preferential ionic pathway corresponding to the meridians described in acupuncture.

During the stimulation of acupoints, stimulation without which acupuncture does not have an effect, a rapid and simultaneous response is noted both on the stimulated and the contralateral meridians. These responses are identical in time of manifestation and also in amplitude and duration. This simultaneous contralateral response suggests the hypothesis of the intervention of a neurochemical mechanism in information transmission.

Acknowledgment

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