instrument, suggest that the development of the technique for the non-destructive examination of skin would be fruitful for studies of pigmentation, the nature of infected areas, the presence or absence of secretions, and similar effects[6].

[6] This work was supported by Public Health Service Grant No. AP00211-01A2. The cooperation of the personnel of Block Engineering Inc. is gratefully acknowledged.

Zusammenfassung. Es wird ein Instrument auf Basis der Interferenzmethode, das häufig wiederholte IR-Spektren summieren kann, entwickelt und zur Spektralbeobachtung der IR-Emission der menschlichen Haut benützt.

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A Technique of Integumental Grafting for Aging Studies

Skin transplantation as a tool in aging studies has been employed in the past[1,2]. The inherent disadvantages in the conventional skin grafting methods are: a temporary state of ischemia prior to vascularization of the transplant, an upper limit in the size of the graft in order to avoid necrosis, and the requirement for a careful preparation of the graft bed[3]. The result is that grafts large enough for multiple biochemical analyses are difficult to obtain and the effect of temporary ischemia on their chemical composition enters as a variable.

We are reporting on a technique, which allows an exchange of integumental grafts between old and young rats and in which the above-mentioned problems with the more conventional grafting techniques can be avoided. It consists of a 2-step procedure, the first being the establishment of a parabiotic union which is later followed by separation.

Female rats of the Fischer strain were used. The grafting process was usually carried out between a young (6 months old) and an old (18 months old) animal. Surgery was performed under chloral hydrate anesthesia (270 mg/kg i.p.). Following the removal of the hair a lateral incision was made in both participants and corresponding edges were united with surgical clips. The more intimate union of the conventional parabiotic techniques proved definitely detrimental and should be used only if rotation of the animals and the resulting tension on the skin is to be avoided. The success of this method depends on the administration of antibiotics. Each animal received 60,000 U of long-acting penicillin (Longicil – Fort Dodge Laboratories) by i.m. injection. Clips were usually removed after 12–14 days and the animals were thereafter left to themselves up to 2 months. While separation can be accomplished earlier, delaying this step seems to increase the chances for the survival of larger grafts. Figure 1 shows a parabiotic pair to be separated.

In the second step a pair of incisions are made under chloral hydrate anesthesia in the dorsal and ventral layers of the skin connecting the animals. The incisions curve away from each other and from the original suture line in the direction of parabionts. Each animal is thereby left with an open surface to be covered with the now completely healed flap derived from its parabiotic pair. As a result one of the animals is covered ventrally and the other dorsally, with respect to the original suture line. In this step the cut surfaces are brought into apposition and the wound is closed with individual stitches. This gives a larger intact graft area than closure with clips would allow.

Figure 2 shows the appearance of the previous pair after separation. At this stage the same dose of antibiotic as before is administered again. Stitches are either removed later or are lost spontaneously during the healing process. The final appearance of a separated and completely healed pair is shown in Figure 3.

An outline of this method omitting experimental details appeared in 1964 as a news release[4]. Some time later a Russian team[5] reported on a technique very similar in

Fig. 1. Parabiotic pair made ready for separation.

[1] G. BRAND, P. J. BAKER, W. L. BEAM, and P. HAGEMAN, Fedn. Proc. Am. Socs. exp. Biol. *2*, 200 (1964).
[2] P. L. KROHN, Proc. R. Soc., London *157*, 128 (1962).
[3] R. E. BILLINGHAM, in *Transplantation of Tissues and Cells* (Eds., R. E. BILLINGHAM and W. K. SILVERS; The Wistar Institute Press, Philadelphia 1961), p. 9.
[4] G. C. RING, Med. Trib. *5*, 11 (1964).
[5] A. G. LAPCHINSKY, G. V. MEDVEDEVA, I. D. GADALINA, V. I. SUSLIKOV, and A. G. EINGHORN, Ann. N.Y. Acad. Sci. *120*, 435 (1964).

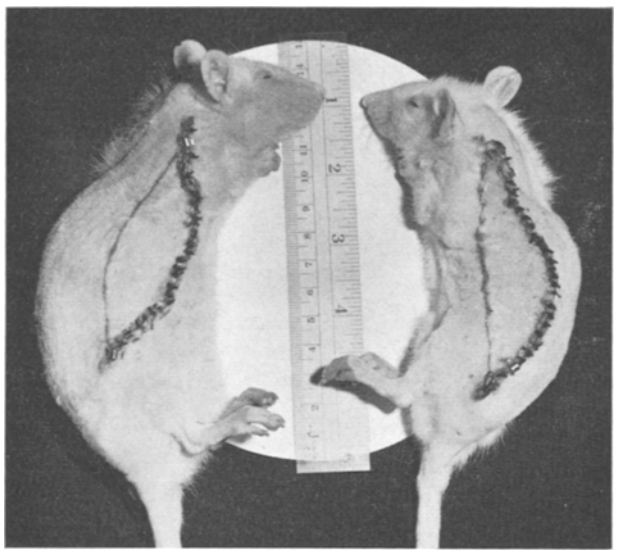

Fig. 2. Parabiotic pair after separation. Suture lines outlined in ink for contrast.

Fig. 3. Healed pair. Graft outlined in ink.

principle but differing in detail. They, however, reported high mortality rates despite extensive postoperative care. In our view this is due to the fact that parabiosis and skin grafting were carried out simultaneously, thereby increasing surgical trauma and necessitating muscle to muscle parabiosis. In the method described above, surgical trauma in the parabiotic step is minimized and graft healing can take place unencumbered by a parabiont. We also found the use of massive doses of antibiotics essential for success. With the method described above, postoperative mortalities are negligible[6,7].

Zusammenfassung. Eine Methode zur Ausführung ausgedehnter Hauttransplantationen bei Ratten, die einer vorübergehenden Parabiose unterworfen wurden, wird beschrieben. Die Methode soll zur Untersuchung von Altersveränderungen der Haut benutzt werden.

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Schnelles Einfrieren von Geweben und Flüssigkeiten

Beim langsamen Einfrieren von Geweben und Flüssigkeiten (wie zum Beispiel Blut) können durch die Bildung grösserer Eiskristalle morphologische Veränderungen und Substanzverschiebungen eintreten. Daher ist für die Zellen- und Gewebetrennung sowie gewisse Fragen der Histochemie ein schnelles Einfrieren von grosser Bedeutung.

Dem schnellen Einfrieren stehen zwei physikalische Eigenschaften der Materie entgegen: Bereits gefrorene Organteile sind schlechte Wärmeleiter, so dass trotz Anwendung tiefster Temperaturen ein schnelles Durchfrieren nicht ohne besondere Massnahmen zu erreichen ist. Weiterhin wird ein schnelles Einfrieren durch das Leidenfrostsche Phänomen behindert. Bei der Herstellung histologischer Präparate umgeht man diese Schwierigkeiten, indem man ausserordentlich kleine Objekte in zuvor tiefgekühlten Flüssigkeiten, die keine Gasentwicklung zeigen, einfriert.

1952 wurde von uns[1] eine Methode angegeben, die es gestattet, grössere Gewebemengen relativ schnell einzufrieren. Dabei werden kleinere Organstücke gemeinsam mit fester Kohlensäure in einem Starmix gemahlen. 1964 veröffentlichten MOLINE und GLENNER[2] eine Methode, bei welcher die Einfriergeschwindigkeit für kleinere Organstücke 10–20 sec beträgt. Mit Hilfe eines von uns entwickelten Einfriergerätes, das nachfolgend beschrieben wird, können Gewebe im Bruchteil einer Sekunde eingefroren werden.

Es ist bekannt, dass man Gewebe relativ schnell einfrieren kann, wenn man sie zwischen zwei tiefgekühlte

[1] M. BEHRENS, Hoppe-Seyler's Z. *291*, 245-246 (1952).

[2] S. W. MOLINE und G. G. GLENNER, J. Histochem. Cytochem. *12*, 777 (1964).