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Dialytrode for Long Term Intracerebral Perfusion in Awake Monkeys

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Abstract—A method is presented for the establishment of long term electrical and chemical communication to and from the brain. The *external dialytrode* is formed by a push-pull cannula ending in a small permeable bag, plus seven contacts spaced alongside. The *transdermal dialytrode* consists of a push-pull cannula ending at one side on a permeable bag and at the other side on two puncturable reservoirs placed subcutaneously. *In Vitro* experiments showed that the rate of passage of ^{3}H-tyrosine through the dialytrode membrane was about 0.5 % per hour. By applying positive or negative pressures, exchange rates of 2-4 µl/minute could be obtained. *In Vivo* functional evidence of passage of chemicals was obtained in rhesus monkeys implanted with dialytrodes: (a) Perfusion of L-glutamate (1 M) through the amygdala, induced typical glutamate afterdischarges; (b) Aminoacids, such as glutamine, asparagine, serine, glutamate, glycine, and α-alanine, were collected in amygdala perfusate; (c) Glycoproteins, such as fucose, mannose, glucose, galactosamine and galactose were collected in caudate nucleus perfusate; (d) Two way exchange, to and from the brain, was demonstrated by curves of washout and by local biosynthesis of amino acids from labelled glucose. *Histological studies* show good biological tolerance of dialytrodes and perfusion procedures. Transdermal dialytrodes remain functional for extended periods, are unobtrusive when not in use, and readily available when desired. This system may provide a new diagnostic and therapeutic procedure in man, to obtain neurochemical information from, and to deliver drugs to specific structures of the brain.

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Introduction

In order to investigate intracerebral regional pharmacology and local neurochemistry, several authors have described "push-pull" cannulas for the perfusion of liquids through the brain (16, 23, 28) and single cannula for intracerebral injection of drugs [14, 17, 18, 19, see bibliography in Delgado (6, 8)]. Direct administration of drugs into the ventricles or into the brain has been used for therapeutical purposes in man (24, 25, 26, 30). Diffusion of anesthetics and drugs through a silicone rubber membrane placed at the tip of a push-pull cannula (chemitrode) has been proposed for the administration of chemicals to the brain (15, 20).

Several years ago we described the "Chemitrode" System (13), consisting of two small (#27) cannulas in juxtaposition plus an array of contacts placed alongside permitting: (*a*) electrical recording, (*b*) electrical stimulation, (*c*) injection, (*d*) collection, (*e*) perfusion of fluids into discrete areas of the brain in unanesthetized animals. The method has already proved its practicality (1, 2, 4, 6, 9, 10, 11, 12, 13, 29).

The above described methods for intracerebral injection and perfusion have several common disadvantages: risk of infection, blocking of the cannulas by clotting of tissue fluids and a possible poor recovery of perfusate.

In order to overcome these problems we have developed the system named "Dialytrode" (7, 8), which is described in the present paper. The External Dialytrode is similar to the Chemitrode, with its tip closed by a small porous bag which forms a barrier for microorganisms and tissue cells, while permitting the passage of fluids and chemicals. The Transdermal Dialytrode is a totally subcutaneous device, permitting injection and collection of fluids, plus electrical stimulation and recordings. This last instrument may be suitable for therapeutical applications to man.

Methods

Description of Dialytrodes.

A. — *The External Dialytrode* is formed by two stainless steel tubing #27, soldered together, with one tip ending 1 mm shorter than the other, inside a polysulfone bag 5 mm in length by 1 mm in diameter. In addition there is an array of seven contacts of teflon insulated stainless steel wire #36 (5 mils in diameter), with 1 mm exposed tips spaced 3 mm alongside the cannulas (Fig. 1). The leads end in a seven pin Winchester socket for the establishment of connections with instrumentation. The length of the dialytrode tubings is predetermined to reach the desired depth inside the brain.

B. — *The Transdermal Dialytrode* is formed by two pieces of teflon tubing cemented together, with one tip ending 1 mm shorter than the other in a 5 by

1 mm polysulfone bag. The other ends of the tubing terminate in two separate rubber reservoirs covered by sylastic and measuring 10 mm in length by 3 mm in diameter (see Fig. 2).

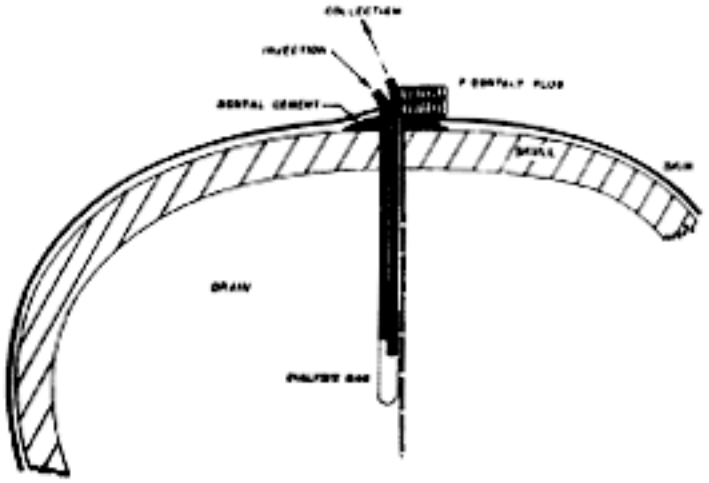

FIG. 1

External dialytrode consists of a push-pull cannula ending in a permeable polysulfone bag, plus seven contacts. This instrument permits electrical stimulation and recording, plus chemical injection and collection.

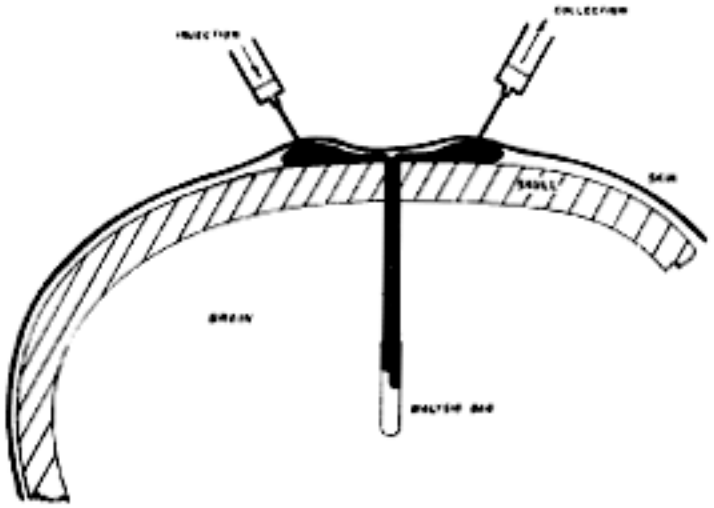

FIG. 2

Transdermal dialytrode consists of a push-pull cannula ending at one side on a permeable bag, and at the other side on two reservoirs. The instrument permits long term transdermal delivery and collection of both electrical and chemical information.

Surgery for implantation.

Under diabutal anesthesia (36 mg/kg) with aseptic precautions and using stereotaxic technique, each dialytrode is implanted within the brain through a 3 mm burr hole and it is fixed in place by dental cement [for further details see Delgado (5, 6)]. In the external dialytrode, the Winchester socket protrudes through the open scalp. In the transdermal dialytrode, the reservoirs are placed subcutaneously being invisible after closing the wound. After healing, 2–3 weeks later, the reservoirs are easily localized by palpation, being accessible by piercing scalp and reservoir with a #28 needle (Fig. 2). By injection into one side and collecting from the other, a circulation can be established through the intracerebral dialytrode bag. For electrical recording or stimulation, an insulated wire with exposed tip is introduced inside one of the subcutaneous reservoirs by means of a hypodermic needle.

Experimental animals.

A total of ten rhesus monkeys, weighing 3.0–4.8 kg were used in the present experiments. In 6 animals, two dialytrodes were implanted into the head of the caudate nucleus (one in each side), for periods of 4 to 16 months. In 2 other monkeys dialytrodes were implanted bilaterally in the head of the caudate nucleus and in the amygdaloid nucleus. In 2 more animals transdermal dialytrodes were implanted bilaterally in the head of the caudate nucleus for a period of 6 months.

Instruments and Procedures.

Most perfusion experiments were performed with a Harvard pump, equipped with 2 ml syringes. In a few occasions injections were performed with a 50 μl Hamilton syringe. Hydraulic connections between syringes, dialytrodes and collecting tubes, were made with #20 polyethylene tubing.

Electrical stimulations were provided by the instrument designed in our laboratory, and they were constant current, monopolar, cathodal, square pulses 0.5 msec pulse duration, 100 Hz, monitored in voltage and amperage by a 2 beam oscilloscope. Electrical recordings were obtained with a Grass 8 channel electroencephalograph.

Perfusates from the brain were collected on ice. The standard perfusion fluid was sterile physiological Ringer (Cutter Laboratories). Analysis of labelled substances was performed with a Packard model 3375 liquid scintillation spectrometer. Analysis of perfusates containing ^{14}C-D-glucose and newly formed amino acids were made by automated amino acid analysis and scintillation counter, as previously described (3, 4).

Sugar components of glycoproteins were determined by gas chromatography using 50 μl samples of the perfusate from the caudate nucleus. Samples of solvent and of standard chemicals were treated in the same way as controls. A 2 μl sample, prepared and concentrated from the 50 μl initial volume, was

introduced into the gas chromatograph equipped with electron capture detector (ECD). For further details, previous publications may be consulted (21, 22).

Results

A. — *In vitro studies*

The dialytrode bag was totally immersed in a bath, formed by a small plastic centrifuge tube filled with 0.5 ml of liquid. Circulation of fluids were then established through the dialytrode by means of the Harvard pump, at a rate of

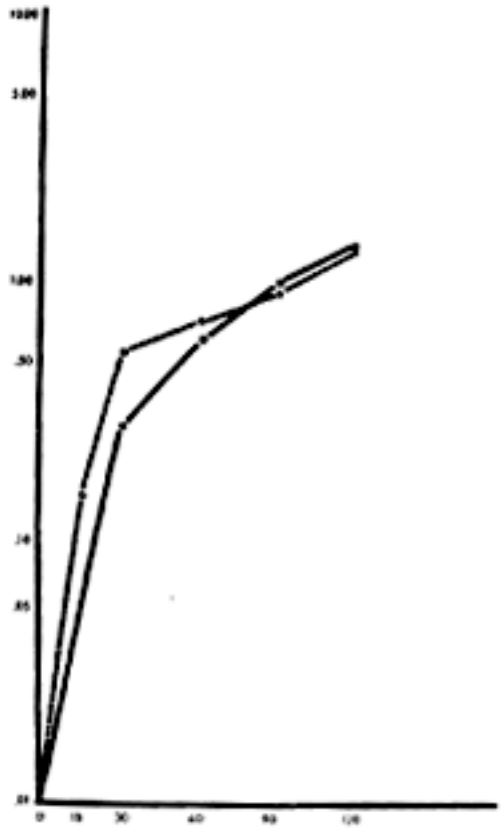

FIG. 3

In vitro experiments showing the passage of ^{3}H tyrosine from the dialytrode to the bath (solid line), and from the bath to the dialytrode (interrupted line).

Abscissa: Time in minutes. Ordinate: Percentage of activity (cpm) in one medium with respect to the other (see text).

4.0 μl/min. Results were similar with different bags and one example is shown in Fig. 3.

1. *Outward passage.* To determine the rate of diffusion from the inside to the outside of the dialytrode membrane, ^{3}H-tyrosine (1 μC/ml, New England Nuclear Corp.) was added to the perfused Ringer, providing a total amount of about 400,000 counts per 0.5 ml of fluid. This tyrosine Ringer was circulated through the dialytrode and the bath volume was changed at intervals of 15, 30, 60, 90 and 120 minutes, to analyze its content of ^{3}H-tyrosine. The outside of the bag was washed off twice in each change. Results shown in Fig. 3 are expressed as percentage of activity detected in the bath, with respect to the activity existing inside the dialytrode bag. (For example 0.5 ‰, mean that 2,000 counts were detected in 0.5 ml bath, when the perfusion fluid had 400,000 counts per 0.5 ml. At a speed of 4 μl/min, about 0.5 ml were circulated in 2 hours.)

Results show an outward passage of about 0.5 ‰ of ^{3}H-tyrosine per hour, which is a considerable amount considering that the capacity of the dialytrode bag is only 3 μl and the surface of the exchange membrane only 15 mm^2.

2. *Inward passage.* To determine the rate of diffusion from outside to inside of the dialytrode, a similar experiment was performed, placing the ^{3}H-tyrosine in the bath, passing Ringer through the dialytrode and analyzing the radioactivity of the perfusion fluid. The possible collection of intracerebral chemicals was thus estimated. As shown in Fig. 3 there was a detection of ^{3}H-tyrosine in the perfusate within 15 minutes of the start of the experiment, with a peak of about 1 ‰, after 2 hours of perfusion. These values were similar to those obtained in the previous study of outward passage.

Additional experiments performed with ^{3}H-DL-norepinephrine (0.5 μC/ml), showed similar diffusion rate of 1 ‰ per 2 hours.

B. — *In vivo studies*

1. *Injection into the brain.* Evidence of outward passage of chemicals from inside of the dialytrode to the brain tissue was obtained by recording neuronal activity. In previous experiments with chemitrodes we have demonstrated that injection of amounts as small as 3 μl of L-glutamate (1 M) into the amygdala, produced a typical high voltage fast activity lasting for several minutes and followed by a rebound of depressed voltage (9).

The present experiments were performed with two monkeys with dialytrodes in the amygdala, in which L-glutamate (1 M) was perfused at a rate of 4 μl/min for 30 minutes. About 10 minutes later after perfusion was begun, the spontaneous electrical activity of the amygdala increased in amplitude and in frequency, and at the end of 30 minutes there occurred typical glutamate induced seizure activity, as shown in Fig. 4, which lasted for 2-6 minutes and was followed

by another 10–15 minutes of bursts of irregular high voltage fast waves, and ended with a few hours of depressed activity. On the next day the spontaneous activity of the amygdala was normal. The phenomenon was repeated in experiments performed on different days and it was localized to the perfused side.

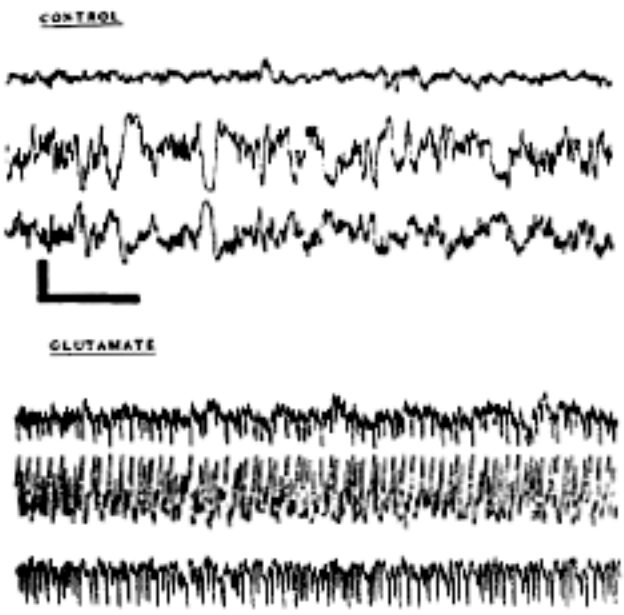

FIG. 4

Demonstration of the passage of glutamate from the dialytrode to the brain. Typical glutamic seizures are evoked in the amygdala when L-glutamate 1 M is circulated through the dialytrode.

Linkages: 1st channel: Putamen, 2nd: Amygdala. 3rd: Amygdala. Calibration: Horizontal: 1 second. Vertical: 100 μv.

2. *Collection from the brain.* Evidence of inward passage of metabolites from the brain to the Ringer circulating inside the dialytrode bag was established in the following manner.

(*a*) Amino acids: In one monkey using a dialytrode the left amygdala was perfused with sodium chloride 0.154 M at a rate of 1.3 μl/min for 12 hours, and the collected fluid was subjected to automatic amino acid analysis. Results shown in Fig. 5 demonstrated the collection of rather large amounts of glutamine-plus asparagine-plus serine, glutamate, glycine and α-alanine, while no measurable amount of GABA was present. In another case, perfusion during 12 hr of sleep resulted in a small GABA peak. The experiment was repeated on four different days with similar results (Fig. 5). Control analysis of the fluid used

in these perfusions gave trace amounts of "glutamine" without any other detectable amino acids.

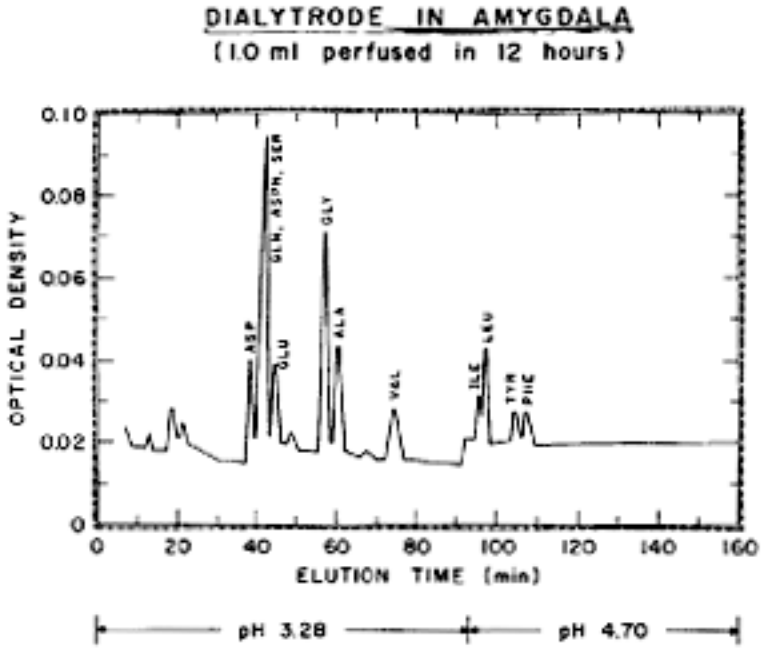

FIG. 5

Automatic amino acid analysis of perfusate from the left amygdala demonstrating presence and collectability of amino acids.

(*ii*) Glycoproteins: In two other monkeys the left caudate nucleus was perfused with Ringer solution at a speed of 4 μl/min for one hour, and 0.2 ml sample was analyzed by gas chromatography for sugar and amino-sugar compounds of glycoproteins in order to determine the presence of ribose, deoxyribose, xylose, glucose, glucosamine, galactosamine, glutamic acid and γ-aminobutyric acid. The peaks of the perfusate from the caudate nucleus, as shown in Fig. 6, demonstrated the presence of the following substances listed from greater to lower concentration: Fucose, mannose, glucose, galactosamine, galactose. These compounds were identified by using standard chemicals under similar gas chromatographic conditions.

3. *Two way exchange.* (to and from the brain) Evidence of diffusion of the same chemical first into the brain and then back inside the dialytrode bag was obtained by two sets of experiments.

(*i*) Washout and secondary collection. In three monkeys the dialytrode implanted in the left caudate nucleus was perfused first with Ringer, then with

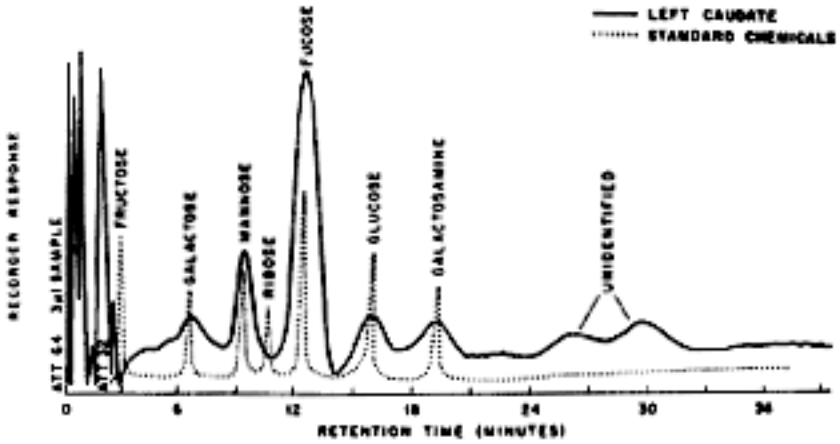

FIG. 6

Gas chromatographic analysis of the perfusate from the left caudate nucleus demonstrating presence and collectability of glycoproteins. The solid line shows actual chromatogram. The broken line indicates the chromatogram of standard chemicals. Gas chromatographic conditions were as follows:

Column: 15 %, Carbowax on chromosorb W; Stainless steel column 6' long, 1/8 outer diameter; temperature 115° C.

Detector: Electron capture, temperature 150° C.

Injector: Temperature 175° C.

Carrier gas: N_2.

^{3}H-tyrosine, and then with Ringer again. Results are shown in Fig. 7. Control Ringer perfused at 4 μl/min for 30 minutes had only a few background counts. The perfusate with ^{3}H-tyrosine circulated also for 30 minutes had counts far beyond the scale of the graph. Then Ringer was perfused at a speed of 80 μl/min for 5 minutes, repeating this washout 3 times. The last of these samples indicated a low count, below 100. When the perfusion speed was slowed to 4 μl/min for 30 minutes the 4 samples obtained showed an initial peak, above 1,000 with a new curve of slow washout which had a final value below 500 cpm. Perfusion speed was then reduced to 0.8 μl/min and continued overnight with the appearance of another peak of counts above 1,600. Three more samples were taken of 30 minutes each at speed of 4 μl/min and the final counts were near background activity.

This result seemed to indicate the passage of ^{3}H-tyrosine to the brain with a slow efflux of radioactivity back into the dialytrode.

(*ii*) Biosynthesis of amino acids. In one monkey a solution in Ringer of U-^{14}C-D-glucose (5 μC/ml) was perfused through the left amygdala dialytrode at a rate of 1.2 μl/min for one hour. After a quick washout Ringer solution was perfused at the same rate for 12 hours, and the perfusate analyzed in the automatic amino acid analyzer. Traces of label appeared in aspartate and

citrulline, but not in other substances. This result indicated that the injected glucose had diffused to the brain where it was converted into the two labelled amino acids in sufficient amount to diffuse back to the dialytrode.

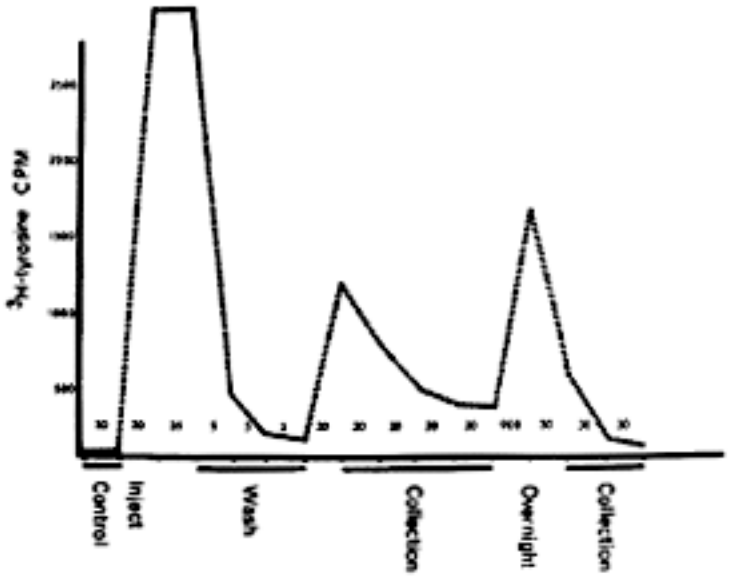

FIG. 7

Washout and secondary collection from a dialytrode implanted in the left caudate nucleus, indicating the *in vivo* two way exchange of ³H-tyrosine (see text).

4. *Experiments with labelled DOPA.* Experiments were performed in three monkeys, perfusing the head of the caudate nucleus with labelled DOPA (^{14}C, Nuclear Chicago, 31 mc/mole) in an attempt to demonstrate its local conversion into dopamine and release into the perfusate as we have demonstrated earlier using chemitrodes (29). In these monkeys, washout curves were obtained similar to the one shown in Fig. 7, without the detection of labelled dopamine or deaminated metabolites in the perfusion fluid. This result was interpreted as an indication of very slow passage of the released catecholamines into the dialytrode.

C. — *Histological study*

After termination of the experiments, each animal was anesthetized with Diabutal and perfused through the heart with saline and with formaline 10 %. After removing the calvarium with the implanted block of dialytrodes, the head was placed in the stereotaxic instrument and coronal sections of the brain were made at 10 cm intervals. The block with dialytrode tracts was placed in a

Tatrander microtome and frozen with a Histofreeze. The whole 10 cm piece of brain was sectioned at 50 μ, taking high contrast pictures every 250 μ. Selected cuts were mounted and stained with hematoxilin-eosine to be investigated microscopically.

In three monkeys final experiments had been performed to test the effects of Triton X-100, a powerful surfactant agent. As it will be reported elsewhere this substance is unsuitable for biological use because large reactions were found in all 3 animals, with considerable brain destruction, probably related with recorded signs of electrophysiological irritation.

The other monkeys had a well formed capsule of glia, limiting the implanted dialytrodes, as shown in Fig. 8. Neurons were well preserved beyond the glia reaction.

Subcutaneous reservoirs in the two animals with transdermal dialytrodes, were well tolerated, being encapsulated by a smooth, uniform fibrotic tissue, without signs of infection or rejection after 6 months of implantation and frequent use.

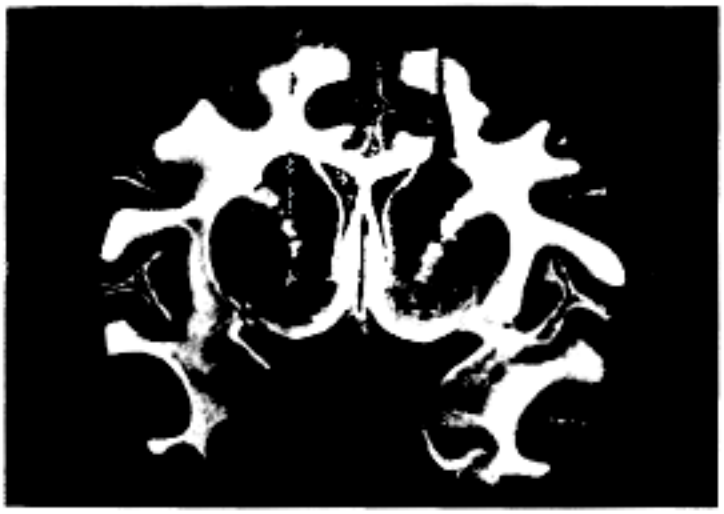

FIG. 8
Dialytrode tract after 8 months of implantation

Discussion

The methodology described in the present paper permits long term studies of electrical and chemical phenomena inside the brain of awake animals. Placing a permeable membrane at the tip of a push-pull cannula avoids the main handicap of intracerebral perfusions, i.e., the poor recovery of perfusates due

to blocking of the tubes. The dialytrode system, being closed inside of the brain, also prevents possible infection.

With the added modification of subcutaneous reservoirs, the system is foolproof, unobtrusive when not in use and readily available when desired. This transdermal device seems to be suitable for clinical applications to man when medication should be delivered to a specific target of the brain, for example for long term functional blocking of specific structures. As shown in previous studies (11), long term slow injection of local anesthetics into the amygdala and hippocampus of monkeys, resulted in a long lasting block of excitability accompanied by a decrease in aggressiveness, without producing other behavioral manifestations.

The possibility of repeated sampling of chemicals from the same structure, in the same subject offers considerable scientific interest. In previous studies with chemitrodes in monkeys we have shown that large amounts of amino acids considered to be possible CNS transmitters, such as GABA, glutamate, aspartate, glycine and cystathionine can be detected in various cerebral structures (4). Using transdermal dialytrodes it would be possible to make repeated sampling of local brain chemicals for diagnostic as well as for therapeutical purposes.

In comparing dialytrodes and chemitrodes, the presence of the permeable membrane has both advantages and disadvantages. The main handicap is the obstacle represented by the membrane which slows down the exchange of fluids. This is probably the reason why in our study we were unable to detect dopamine after perfusion with labelled DOPA. The main advantage is that dialytrodes ensure good recoveries and that fluids always circulate smoothly and without problems.

The capacity of the dialysis bag is rather small, only 3 μl, therefore the surface for chemical exchange of 15 mm^2 should be appropriate for the establishment of equilibrium within a reasonable period of time. The "*in vitro*" study showed that 1 % of 0.5 ml (= 5 μl) of ^{3}H-tyrosine passed through the membrane in 2 hours. By applying positive or negative pressure this amount may be changed manyfold. Preliminary studies indicate that with 30 mm Hg., exchange rates of 2-4 μl/min may easily be obtained. Application of gas chromatographic technique for analysis of minute amounts of chemicals in brain perfusates seems to be very promising. The collected sugar components of glycoproteins were in the nanogram range, as determined by comparing chromatographic peaks with standard chemicals. At this time, however, no effort was made to estimate the exact concentration of each component. It should be expected that local changes of sugars, amino-sugars, amino acids and other substances in specific brain structures may have a relation to different neurophysiological and behavioral conditions such as epileptogenic activity, sleep-wakefulness, rage-placidity, and this is the orientation of our ongoing research.

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