penicillin used throughout the study and were sterile. In six animals, all withdrawn samples were sterile; in one, severely contaminated. Four of the foregoing six animals with sterile dialysis fluids were subsequently nephrectomized. One developed an intussusception. All withdrawn dialysis fluids initially were contaminated but subsequently could be made sterile by the intramuscular injection of penicillin. Nephrectomy appears to lower the resistance of the animal to bacterial contamination (*10*). Since the nature of the contaminant was not investigated, no definite statement concerning the source of such contamination can be made at this time.

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Received March 5, 1954.

A Method of Making Prefilled Microelectrodes

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In making microelectrodes for intracellular recordings, the greatest difficulty encountered is in the step of filling the tip with electrolyte. The method described by Ling and Gerard (*1*) uses fairly vigorous boiling to drive out the air, but this process destroys a large percentage of the microtips. Nastuk (*2*) has recently described another method in which boiling is avoided, but it requires several delicate manipulations.

The difficulty of filling the microelectrode can be circumvented by drawing the tips from capillary tubes that have been previously filled and maintained in a closed electrolyte system during the pulling. A specific application in connection with the Gamma pipette puller will be described. In this instrument, which is operated by gravitational force, the capillary is in a horizontal position and lengthens as heat is applied to it by a hot wire. For drawing prefilled microelectrodes, lucite blocks carrying deep wells filled with the electrolyte solution in use are attached to the jaw elements of the puller (Fig. 1). Capillary tubing of

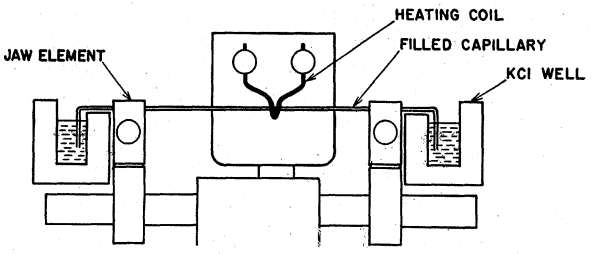

Fig. 1. Diagrammatic representation of the prefilled glass capillary in the Gamma pipette puller modified by adding a lucite well at each of the jaw elements.

appropriate lengths is bent at both ends. The bent tubing is first filled with the electrolyte solution and then is inserted into the jaw elements of the puller with the ends dipped in the reservoirs.

When heat is applied to the middle of the filled capillary, thermal expansion expels the fluid from the heated region into the wells. As the capillary is pulled apart to form the microtips, these are again filled by the electrolyte drawn from the reservoir. The filled electrodes are then immediately removed from the clamps and are hung vertically with the tips downward. This allows any large air pockets to rise toward the coarse end of the capillary where they can be dealt with easily.

In about 40 percent of the microelectrodes made in this manner, small air bubbles remain in the shaftlet or in the tip. However, when these electrodes are stored for a few days on a rack in a container filled with the electrolyte, most of the small air bubbles disappear. Thus most of the microelectrodes become usable without further manipulation.

Several other variants of this technique have been used successfully with the Gamma puller and other instruments. Microelectrodes with 1.0 to 2 μ tips are made with the greatest of ease from capillary tubes of 0.7 mm outside diameter. For tips smaller than 1 μ,

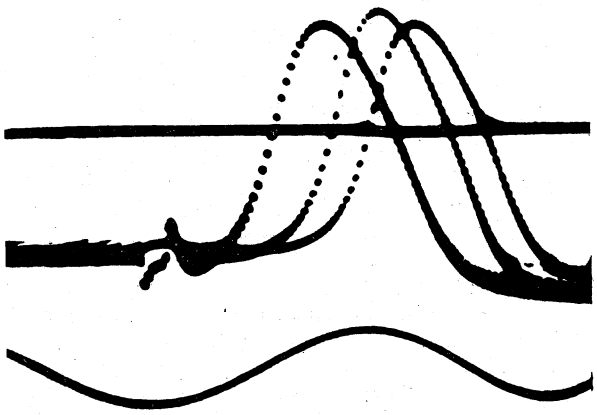

Fig. 2. Simultaneous records of activity at three microelectrodes inserted into a squid giant axon of diameter 480 μ. The distances between the electrodes (from left to right) were 3.7 and 2.5 mm. The resting potential (50 mv) is indicated by the displacement of the three electrode traces from the base line. Spike heights, 94 to 100 mv. Time curve, 1000 cy/sec.

prefilled capillary tubes are first narrowed on the puller to 0.3 to 0.4 mm for a short segment which is allowed to cool and refill with the electrolyte before being drawn to form microtips. This method of preparing microelectrodes has been employed continuously during the past 2 yr in connection with investigations of intracellular potentials in the squid giant axon (*3*), in the electroplaques of the electric eel (*4*), and in cardiac muscle fibers and spinal ganglion cells grown in tissue culture (*5, 6*). As many as three such microelectrodes have been inserted at one time into a region of the squid giant axon as small as 3 mm without damage to the spike height (Fig. 2).

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Received January 6, 1954.

Differences in the Rate of Reduction-Oxidation Potentials in Salivary Samples of Certain Groups of Individuals

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Salivary redox potentials as a function of time have been reported in patients with dental caries (*1*), in patients with coronary heart disease (*2*), and in putatively normal individuals (*2*). This paper pertains primarily to a study of redox potential patterns in a group of mentally defective individuals within a single state institution (*3*). The salivary redox pattern of these individuals as a group differed from the normal and from the coronary heart disease group when examined statistically.

Intensities of electron transfer were observed in saliva in a group of 57 mentally defective males whose chronological ages varied from 10 to 37 yr. Their mental ages varied from 4 to 10 yr, and the IQ average was 41. Four young adults (two males aged 24 and 29 yr, and two females aged 24 and 26 yr) and a boy of 11 had been tested repeatedly to determine duplicability and reliability of the procedure. These are not included in any of the three groups of the main study reported here.

In a previous publication (*2*), we reported intensities of electron transfer in saliva for a group of 66 men who had had at least 6 mo activity after having experienced myocardial infarction prior to the age of 40, and in a group of 73 healthy working males comparable to the infarction group by being of roughly similar age and ethnic origin.

About 1.5 ml of saliva was collected from each individual directly into a 5-ml beaker without physical or chemical stimulation. At least 2 hr had elapsed after eating, drinking, or smoking prior to the collection of the salivary sample. This freshly collected sample was immediately tested in a Beckman potentiometer (laboratory model G) with standard calomel-platinum electrodes. The results are shown in Table 1.

From the data plotted in Fig. 1, several characteristics are apparent. The trend is downward (negative direction) from a mean of approximately +280 mv as a starting potential for all three groups. Over a period of 30 min, the mean potential reaches −132 mv for the normal healthy males, −246 mv for the coronary heart disease group, and +11 for the mentally defective group.

It is also apparent from Fig. 1 that in the coronary heart disease group the electron transfer shows at all intervals greater negativity than either the control group or the mentally defective group. The difference is systematic, and the absolute differences between the groups increase with time.

Table 1. Salivary redox potentials in 73 control males, 66 male patients with coronary heart disease, and 57 mentally defective male individuals.

Time (min)	Control			Coronary heart disease			Mentally defective		
	No.*	$\bar{X}$ (mv)	S.E.	No.*	$\bar{X}$ (mv)	S.E.	No.	$\bar{X}$ (mv)	S.E.
¼	73	280.0	5.14	66	270.5	5.93	57	275	5.71
1	70	253.0	5.11	59	239.0	8.18	57	255	7.03
2	69	243.0	6.19	60	213.0	9.67	57	233	10.44
3	67	202.0	6.76	57	179.5	12.40	57	216	14.43
4	63	195.0	8.11	61	157.0	13.90	57	209	12.02
5	73	171.0	9.23	63	133.5	16.72	57	196	13.44
10	73	93.5	16.49	60	0.5	28.77	57	145	22.05
15	71	23.5	22.71	62	− 98.5	33.76	57	110	24.77
20	71	− 35.5	28.09	61	−156.5	36.80	57	70	26.62
25	69	− 85.5	29.39	61	−180.5	35.89	57	38	28.41
30	70	−132.5	30.23	62	−246.5	36.70	57	11	29.54

* The numbers in these two columns vary because readings for certain intervals were occasionally missed.