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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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.

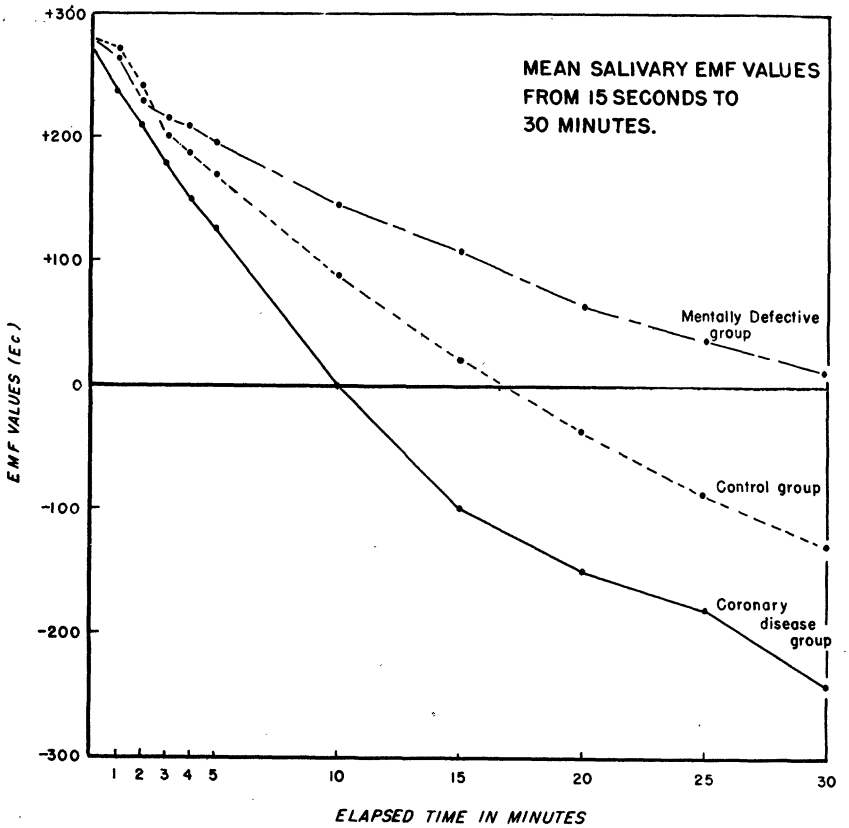

Fig. 1. Reduction-oxidation potentials as a function of time for three groups of individuals: 57 mentally defective males; 66 men who had experienced myocardial infarction; and 77 putatively normal males.

It should be noted that the mean chronological age of the mentally defective group is lower than that of the other two groups. To be certain that the difference in electron-transfer pattern was not a factor of this age difference, a small group of normal control males of mean age 21 was tested. For this group, an average drop at 30 min within 20 mv of the healthy control group, whose mean age was 39, was obtained.

Sharp differences in the rate of declining potential distinguish the groups between 4 and 15 min (Table 2).

In such a study, one would not be justified in disregarding the bacterial content of the saliva, since it is known that saliva contains bacteria and since bacteria in suitable media have been reported to yield negative oxidation-reduction potentials with time (*4*). However, there is one important difference between the bacterial reducing pattern and the salivary reducing pattern. Bacteria in media are commonly reported to start the rapid drop toward negativity later than the 30-min period considered in the present experiments, and commonly in the period from 2 to 24 hr. Nevertheless, it seemed worth while to perform salivary bacterial counts on a sampling of our subjects. These did not reveal any definite association between the bacterial counts and the reducing intensity of saliva.

Table 2. Distribution of cases with respect to salivary oxidation-reduction potentials from 4 to 15 min, inclusive, in coronary heart disease group, normal group, and mentally defective group.

Salivary redox drop from 4 to 15 min, inclusive	Coronary heart disease group (%)	Normal group (%)	Mentally defective group (%)
1–100	25	52	73
101–300	46	25	19
301–500	9	20	7
Over 500	20	3	1

How may the gross differences between the groups in their mean values for intensity of electron transfer in saliva be explained? Since there may be many oxidation-reduction systems in saliva, it would be unreasonable to attribute the entire change to a single oxidation-reduction system. Saliva contains such inorganic ions as I^- and CNS^-. It contains glutathione. Evidence is accumulating for the presence of oxidation-reduction enzymes in saliva. To distinguish and to determine quantitatively the units responsible for the differences between the salivary oxidation-reduction potentials of the mentally defective group and the group subject to coronary infarct prior to the age of 40 yr should be useful in elucidating their basic biochemical differences.

After about 3 min, the electron-transfer patterns (drop in oxidation-reduction potentials) for the three groups studied are consistently and significantly dis-

tinguishable. The drop toward negativity is faster, and the negativity obtained is greater on the average for the group who had experienced myocardial infarction prior to the age of 40 than for their comparable "normal" control group. The drop toward negative values is slower, and negativity is not obtained on the average for a group known to be mentally defective.

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3. We are indebted to the following for access to subject material: the Coronary Research Project, Massachusetts General Hospital; the W. E. Fernald State School; and Lever Brothers Company. We also thank Dr. Paul Dunston, for data concerning age, IQ, and clinical diagnosis of the mentally defective group; Dr. Howard Dexter of the Forsyth Dental Infirmary staff, for the salivary bacterial counts; and Dr. Hugo Muench of the Harvard School of Public Health, for his evaluation of the statistical methods employed herein. Dr. M. E. Cohen and Dr. Irwin Sizer offered many helpful suggestions.

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Communications

Preliminary Studies on the Structure of Angiotonin

Angiotonin of a purity 2 to 3 times that of Edman (*1*) has been obtained by utilizing sodium chloride fractionation, adsorption on and elution from Amberlite IRC-50, and finally partitioning on a Celite column using a butanol-propanol : sodium chloride-acid solvent system (*2*). By partitioning in a countercurrent apparatus between butanol-propanol and 0.1*N* hydrochloric acid, an angiotonin was obtained of even greater purity. Only one active pressor principle was evident after 100 transfers. Angiotonin prepared in the afore-described manner was used in the following analyses.

A two-dimensional chromatogram (Fig. 1) of a hydrochloric acid hydrolysate shows 13 different amino acids to be present in the angiotonin molecule. The leucine spot was later shown, by a Dowex-50 column (*3*), to contain both leucine and isoleucine, making a total of 14 different amino acids.

The amino end-group was determined by the 1:2:4-fluorodinitrobenzene (DNP) method of Sanger (*4*). The ether soluble fraction from the acid hydrolysis of DNP angiotonin was chromatographed by the method of Biserte and Osteux (*5*) and found to contain only DNP aspartic and some dinitroaniline. Only N^5 DNP lysine was observed in the water-soluble fraction. The fact that only one amino derivative was found is evidence that the angiotonin used here is mainly one entity.

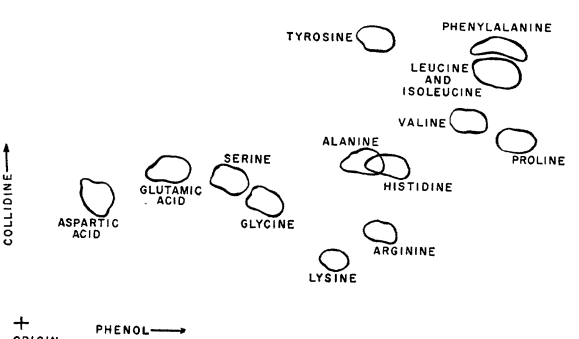

Fig. 1. Two-dimensional chromatogram of an acid hydrolysate of angiotonin, using phenol and collidine as the developing solvents. The amino acids were visualized by spraying with ninhydrin and, after steaming, with diazotized sulfanilic acid.

By hydrazinolysis (*6*) of angiotonin, the amino acid on the carboxyl end could be isolated as the free amino acid. It was then chromatographed (two-dimension) by the same procedure as is illustrated in Fig. 1 and shown to be either leucine or isoleucine.

Chromatographic analysis of the hydrolysate, using a Dowex-50 column (*3*), gave the amino acids shown in Fig. 1 in the following molecular ratio: 2 aspartic acid, 1 serine, 1 glutamic acid, 2 proline, 1 glycine, 1 alanine, 1 valine, 1 isoleucine, 1 tyrosine, 1 phenylalanine, 2 leucine, 2 histidine, 1 lysine, 2 arginine.

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The Use of Electrically Conducting Glass for Counting Lesions*

A device has been constructed that greatly facilitates counting the local lesions on *Nicotiana glutinosa* which appear after viral infection. The novel feature of this counter is the electric conducting glass employed. Because the glass is frosted and translucent, its entire area can be illuminated softly and evenly

* This work was facilitated by research funds provided by the U.S. Atomic Energy Commission. A sample of EC glass for experimental purposes was supplied by the Corning Glass Works, Corning, N.Y.