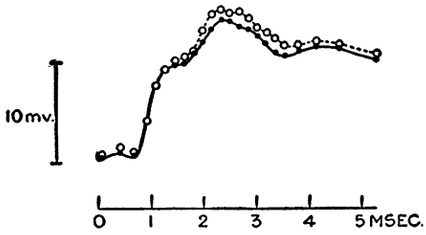

Fig. 2. Effect of conditioning volley in nerve to gastrocnemius-soleus on subthreshold excitatory postsynaptic potential evoked in a deep peroneal motoneuron by stimulation of L6 DR and the upper half of L7 DR. Conditioning-test interval, 20 msec.

their time course. Such an increase in excitatory postsynaptic potential (EPSP) is shown in Fig. 2. The deep peroneal motoneuron concerned initially had a monosynaptic firing index of 0, and a polysynaptic firing index of 100. The firing index of the polysynaptic response, which latter, judging by its latency, was disynaptic, could be modified by recurrent facilitation; at two different levels of orthodromic drive it was increased from 0 to 26 and from 33 to 90. In order to measure the effect of recurrent conditioning on the EPSP, groups of control and conditioned responses were alternated and photographed on moving film. Changes in the subthreshold EPSP were then determined by measuring the amplitude of these conditioned and unconditioned EPSP's at each of 23 different intervals following the stimulus artifact. The second beam of the oscilloscope was used as an arbitrary reference for measurement of EPSP amplitude. The solid line (dots) in Fig. 2 represents the average control EPSP; the dashed line (circles), the average conditioned EPSP. Tracings of conditioned and unconditioned membrane potentials revealed little or no shift in resting potential as a result of conditioning. There was also no difference between the early parts of the two groups of EPSP's. However, most of the later part of the potential was significantly increased by recurrent conditioning ($P < 0.02$). Changes of this type have been seen in a number of motoneurons, and increases in EPSP's have generally been more marked in the later part of the potential.

Intracellular stimulation of motoneurons by means of a bridge circuit similar to that used by Frank and Fuortes (*6*) has shown that an intracellular stimulus delivered through the micropipette is generally made more effective by recurrent conditioning. In the case of the cell whose EPSP is illustrated in Fig. 2, at three different strengths of intracellular stimulation, namely wth current pulses 7.5 msec in duration and ranging from 2.25 to 2.5 $\times 10^{-8}$ A, conditioning increased the firing index from 0 to 19, from 8 to 32, and from 28 to 69. This increase in intracellular firing index is of the same order of magnitude as that of the disynaptic firing index described above.

These observations made by means of intracellular recording are consistent with the hypothesis that recurrent facilitation is due to a reduction of background inhibition. Reduction of tonic inhibitory action and the consequent slight decrease in membrane conductance could lead to both the effects on membrane potential and changes in excitability which have been seen. However, other interpretations of the findings available so far may also be possible and further experiments designed to clarify the meaning of the membrane changes are under way at this time.

Facilitation has been looked for so far in 93 deep peroneal cells and 10 quadriceps motoneurons, and has been found, as evidenced by the criteria listed above, in 59 of the former and 8 of the latter. Eccles, Iggo and Ito (*7*) have found that in cats lightly anesthetized with sodium pentobarbitol, and with the spinal cord sectioned at L2, recurrent facilitation, as judged by the presence of a small depolarization, is recorded very infrequently. And Granit and Rutledge (*8*) have interpreted the findings of these workers as showing that "recurrent facilitating effects were rare and very small." As shown above, the changes in membrane potential that accompany recurrent facilitation are indeed small. However, the fact that facilitation has been found in 63 percent of the deep peroneal neurons and in most of the quadriceps cells that have been studied shows that in the unanesthetized high spinal cat this phenomenon is certainly not rare. Furthermore, while the membrane changes recorded have been small, pronounced changes in firing index have been seen in a number of cells. Clearly, recurrent facilitation is a widespread phenomenon in certain motoneuron nuclei and appears capable of bringing about significant changes in motoneuron firing (*9*).

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20 April 1961

Recovery of the Living Fossil Mollusk, Neopilina, from the Slope of the Cedros Trench, Mexico

Fourteen small monoplacophoran mollusks, relict animals with a discontinuous geologic range between the Paleozoic and the Recent, have been recovered from a biological trawl sample taken 31 December 1960 from the slope of the Cedros Trench, Baja California, Mexico (*1*). Specimens of *Neopilina* previously have been reported from off Costa Rica, off Peru, and off Cape San Lucas, Mexico (*2*). This collection represents the most northern record for the Monoplacophora as well as the largest single collection.

The specimens (Fig. 1) are from the Allan Hancock Foundation station No. 7230–60, located 30 mi off Natividad light, Baja California, Mexico, between 27°52′25″ to 27°51′30″N and 115°44′30″ to 115°45′15″W (dead-reckoning position). The depth varies between 1514 fathoms at the start of the station to 1493 at its completion. The specimens range in size between 1.3 mm and 2.37 mm long.

The minute animals collected have six pairs of diminutive gills and a segmental musculature similar to that described by Lemche and Wingstrand (*3*)

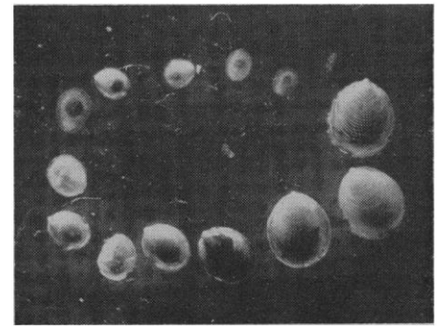

Fig. 1. Specimens of Neopilina.

for *Neopilina* (*N.*) *galatheae* Lemche. The six pairs of gills suggest that the animals belong to *Neopilina* (*Vema*) rather than to *Neopilina* (*Neopilina*), but the exact assignment of the specimens to one of the two known species remains uncertain. This uncertainty is due to the fact that the specimens are apparently different in shell sculpture from both *Neopilina* (*Neopilina*) *galatheae* Lemche and *Neopilina* (*Vema*) *ewingi* Clarke and Menzies (*4*).

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1. The net of the biological trawl was nylon with a mesh diameter of 0.5 mm. The position and correction of sonic fathoms was provided by Elizar Uchupi. The cruise was supported by National Science Foundation grant No. 12329 to K. O. Emery of the Geology Department of the University of Southern California.
2. Data regarding the positions of other captures of *Neopilina* are given in a paper by Menzies *et al.* [*Oikos* **10**, 168-182 (1959)]. We understand that a description of the capture of *Neopilina* by the Scripps Institution of Oceanography from the slope off Cape San Lucas, Mexico, is scheduled for publication (information courtesy of Robert Parker, Scripps Institution of Oceanography, La Jolla, Calif.).
3. H. Lemche and K. G. Wingstrand, *Galathea* Rept. **3**, 9–72 (1959).
4. A detailed study of the shell sculpture is in progress. A report is in preparation on the ecological conditions and the fauna associated with the monoplacophorans with the hope that such information will aid our understanding of the ecology of these unusual animals and with the hope that the data will assist in their future capture.

20 April 1961

Molecular Weight Determinations

Abstract. A Beams magnetically suspended equilibrium ultracentrifuge was used to determine the molecular weight of sucrose, ribonuclease, and insulin. Both long- and short-column ultracentifuge cells were used. The longer cells gave greater precision, but required a longer time for equilibrium to occur.

The Beams type magnetically supported equilibrium ultracentrifuge (*1*) has been used to determine the molecular weights of a number of substances, including sucrose, ribonuclease, and insulin. The measurements were made to test the reliability of the apparatus as well as to determine directly the molecular weight values.

For a monodisperse substance in a dilute solution, the molecular weight (M) is given by the relation (*2*):

$$M = \frac{2RT \ln \frac{f_2 c_2}{f_1 c_1}}{(1 - Vd)\ 4\pi^2 N^2\ (r_2^2 - r_1^2)}$$

where N is the rotor speed in revolutions per second, T is the temperature, c_1 and c_2 are the concentrations at the radial distances r_1 and r_2, respectively, f_1 and f_2 are the activity coefficients, and V is the partial specific volume. The rotor speed is determined with a precision of 1 part in 10^5, the temperature is measured to at least 1 part in 10^4, and the ratio c_1/c_2 is determined to 1 part in 10^3. The quantities $(1 - Vd)$ and the activity coefficients are measured outside the centrifuge and are the least precisely known of the factors in the equation.

The sucrose was obtained from National Bureau of Standards lot No. 5706 with the solvent triply distilled water. The concentration was determined with a microbalance. The specific refractive increment was measured in this laboratory and is in agreement with the value obtained by interference methods. Chromatographically pure crystalline bovine ribonuclease was obtained from the Sigma Chemical Company. The solvent used was a solution of 0.1M NaCl, 0.035M K_2HPO_4, and 0.004M KH_2PO_4, having a pH of 7.7. A quantity of crystalline zinc-insulin was kindly furnished by Merck, Sharp and Dohme Research Laboratories. The solvent was 0.1M KH_2PO_4 and 0.0033M H_3PO_4 with a pH of 2.8.

Typical results obtained are listed in Table 1. It should be noted that greater accuracy is obtained with the longer cells, but more time is required to reach equilibrium. Consequently, short cells should be used if denaturation occurs during a relatively long experiment (*3*).

Table 1. Typical results of molecular weight determinations. M_o is the formula weight; M_{obs}, observed molecular weight; t, time required for the experiment, in hours; L, length of the ultracentrifuge cell, in millimeters; c, concentration, in g/100 ml; and N, frequency of the rotor, in rev/sec.

t	L	c	N	M_o	M_{obs}	$(1 - Vd)$
				Sucrose		
22	8	2.990	398.95	342.3	341.9 ± 0.58	0.3761
4	3	2.988	262.10	342.3	343.5 ± 1.40	.3762
				Ribonuclease		
62	8	0.281	174.00	13,663	13,650 ± 23	.3016
14	3	.241	235.96	13,663	13,696 ± 58	.3016
				Insulin		
35	5	.152	306.02		11,427 ± 31	.2606
12	3	.365	267.44		11,517 ± 46	.2606

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3. We should like to thank J. W. Beams for his help and invaluable advice on this project. The work was supported by a grant from the National Institutes of Health.

10 March 1961

Constitution and Smoking

Abstract. Among 167 adult male factory workers of Neapolitan parentage but of American birth or upbringing, the lean men smoked significantly more than the fat ones. Smoking was positively correlated with serum cholesterol but was not associated with morphological masculinity, blood pressure, diet, or consumption of alcohol.

The detection of determinants of tobacco smoking would help in understanding and possibly preventing diseases associated with smoking, notably lung cancer, emphysema, chronic bronchitis, and cardiovascular disease. The search for constitutional correlates of smoking is being conducted chiefly along psychological lines (*1*). A few investigators (*2–4*) have reported associations of smoking habits with physique and blood pressure, but at borderline levels of significance or with inconsistent direction.

Since the use of tobacco may vary from one cultural group to another, it is desirable to study subjects with a common culture. If, in addition, the subjects have similar biological backgrounds and thus constitute a relatively homogeneous group, any associations found between smoking and other personal characteristics take on added meaning.

Such a group has been under investigation since 1956 (*5*). In 1958 it comprised 167 male factory workers whose parents were born within 75 miles of Naples, Italy; of the men themselves, 151 were born and raised near Boston, Mass., and the other 16 near Naples. Seven of the Italian-born men had been brought to the United States before the age of 10, and nine men had come to this country when they were 10 or older. Of 300 males employed in a single factory (*6*) who met the criteria of age (20 to 59 yr),