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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References and Notes

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 10^5. 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*).

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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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1. J. W. Beams, *Proc. Am. Phil. Soc.* **101** (1957).
2. T. Svedberg and K. O. Pedersen, *The Ultracentrifuge* (Oxford Univ. Press, London, 1940).
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.

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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),

Table 1. Associations of smoking with physique and serum cholesterol among 167 Italian-American factory workers.

Characteristic	*P* value (χ^2)	Biserial coefficient of correlation	
		Nonsmokers versus smokers*	Nonsmokers and light smokers versus heavy smokers†
Bi-iliac breadth	.10	−.05	−.23
Serum cholesterol	.10	.32	.23
Bi-iliac breadth/biachromial breadth	.05	.04	−.26
Subscapular skinfold	.05	−.19	−.16
Ectomorphy	.05	.25	.29
Weight	.01	−.25	−.37
Height/(cube root of weight)	.01	.25	.29
Endomorphy	.01	−.21	−.28

* Standard errors for all values in column 3, ± 0.11.
† Standard errors for all values in column 4, ± 0.09–0.10.

Neapolitan parentage, and American birth or upbringing, 213 had volunteered for a long-range study of coronary heart disease. Thirteen were eliminated from the study in 1956 for various technical reasons. Thirty-three more dropped out between 1956 and 1958: 17 declined to participate further, 13 were no longer employed by the firm, 2 were ill, and 1 had died. While nothing is known about those who did not volunteer initially, it is known that the 33 men dropped between 1956 and 1958 did not differ in smoking habits ($p < .70$) from the 167 who remained in the study: 11 men aged 20 to 29 yr, 63 aged 30 to 39, 77 aged 40 to 49, and 16 aged 50 to 59.

Smoking was graded in five categories: 0, if the subject was not currently smoking and had never been a regular smoker; 1, rare or occasional smoking (for example, one to two cigars or pipes a day or one to five cigarettes a week, but no combination of these) 2, regular smoking, less than 20 cigarettes or five cigars or pipes a day; 3, 20 to 30 cigarettes or 6 to 10 cigars or pipes a day; and 4, more than this. The 12 men who had stopped smoking were arbitrarily placed in grade 2. There were 30 nonsmokers (18 percent); of the 137 smokers, 8 smoked cigars only, 3 smoked pipes only, 1 smoked cigars and pipes, 1 smoked cigars and cigarettes, and 124 smoked cigarettes only. Grade 1 contained 9 men; grade 2, 35; grade 3, 78; and grade 4, 15.

Standard techniques were followed for anthropometry (*7*), for somatotyping (*8*), and for serum cholesterol (*9*) and blood pressure determinations. Dietary data were obtained through a 45-minute interview, a modified Burke diet history (*10*), and a detailed questionnaire filled out by whoever prepared the subject's food at home. Activity was graded, by a physician, in five categories based on estimated exertion both on and off the job. The anthropometric observations included data of several kinds descriptive of body form.

1) Standard dimensions [height, weight, and biacromial (shoulder) and bi-iliac (hip) breadths] and certain ratios derived from them (height/cube root of weight), biacromial breadth/height, bi-iliac breadth/height, and biacromial plus bi-iliac breadth/height], reflecting leanness-stockiness and linearity-laterality of build.

2) Data on the thickness of skinfolds in the arm (triceps) and back (scapular) areas, which measure subcutaneous fat.

3) Data on somatotype, comprising photographically based ratings of endomorphy (fat), mesomorphy (bone and muscle), and ectomorphy (linearity); dysplasia (a measure of lack of correspondence among five body regions in respect to the three foregoing somatotype components); and gynandromorphy (a rating of morphological masculinity). Another index developed in an attempt to grade morphological masculinity is Tanner's "index of androgyny" (*11*) (3 times the biacromial breadth minus the bi-iliac breadth).

By the chi-square test, the following factors showed no significant association with smoking ($p > .10$): birthplace; age; height; biacromial breadth; biacromial breadth/height; bi-iliac breadth/height; biacromial plus bi-iliac breadth/height; 3 times the biacromial breadth minus the bi-iliac breadth; triceps skinfold; triceps plus subscapular skinfolds; mesomorphy; gynandromorphy; dysplasia; systolic and diastolic blood pressure; daily caloric intake; and percentages of daily calories contributed by total fat, saturated fatty acids, polyunsaturated fatty acids, protein, carbohydrate, and alcohol. Smoking was associated at the 10-percent probability level with bi-iliac breadth and serum cholesterol; at the 5-percent level, with bi-iliac breadth/biacromial breadth, subscapular skinfold, ectomorphy, and physical activity; and at the 1-percent level with weight, height/(cube root of weight), endomorphy, and somatotype group.

The direction and strength of the associations below the 10-percent probability level were determined by computing biserial coefficients of correlation (*12*), except for somatotype group and activity, which are not amenable to such statistical treatment. Somatotype group consists of 13 combinations of somatotypes grouped by component dominance, primary and secondary. As for activity, inspection of the contingency table showed no regression of activity on smoking—that is, smokers of all grades had very similar activity levels. On the other hand, the most active and the least active men smoked more than those of average activity—a finding which reflects a curvilinear regression of smoking on activity.

For characteristics associated with smoking at or below the 10-percent probability level, Table 1 presents biserial coefficients of correlation computed in two ways: (i) by comparing nonsmokers with all smokers, and (ii) by comparing nonsmokers plus light smokers (grades 0, 1, and 2) with heavier smokers (grades 3 and 4). The latter comparison afforded generally higher coefficients.

The results show a consistent and statistically significant tendency—though not a particularly close one, certainly not close enough to serve as a basis of prediction for individuals—for lean men to smoke more than stout or fat (but not muscular) men. This association, disclosed by all three anthropometric techniques, was not the result of different diets, insofar as can be judged from the dietary constituents considered here, since smoking and caloric intake—total caloric intake as well as intake of component foods—were independent of each other. Absence of the commonly observed increase in smoking with age probably reflects the concentration of ages in the 30's and 40's. The present findings confirm previous reports (*2, 4, 13*) of slightly higher serum cholesterol among smokers; the 30 nonsmokers had a cholesterol level of 212 ± 8.8 mg. (S.E.) per 100 ml of serum; the 137 smokers, a level of 237 ± 3.7 mg (S.E.) ($p < 0.01$). Contrary to findings previously reported, smokers in this series were no less masculine in physique (*3*), were no more active (*4, 14*) and consumed no more alcohol (*14*) than nonsmokers (*15*).

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15. This work was supported by the Tobacco Industries Research Council. It is part of a continuing study by the Department of Nutrition, Harvard School of Public Health. F. J. Stare and M. F. Trulson of that department kindly gave me access to their data.

10 April 1961

Fluorine Substitution Affects Decarboxylation of 2,4-Dichlorophenoxyacetic Acid in Apple

Abstract. Marked differences exist in the rate at which 2,4-dichlorophenoxyacetic acid (2,4-D) is broken down by different plant species and varieties. This activity has been measured by collecting carbon dioxide from leaves treated with carboxyl-C^{14} labeled compounds. The substitution of fluorine for chlorine in the 4-position of 2,4-D inhibits this decarboxylation in leaves which are able to break down 2,4-D rapidly.

It has been reported by Luckwill and Loyd-Jones (*1*) and Edgerton (*2*) that 2,4-dichlorophenoxyacetic acid (2,4-D) is decarboxylated in the leaves of several varieties of apple. The rate of breakdown or decarboxylation was found to be different in several varieties and would appear to be the basis for the resistance of some varieties to the regulatory effects of this compound on growth. With the McIntosh variety, on which sprays of 2,4-D are ineffective in delaying fruit and leaf petiole abscission, the compound is rapidly decarboxylated in the leaf. In Stayman and Winesap varieties, which show a pronounced response to 2,4-D sprays, decarboxylation occurs at a much slower rate (*2*).

In some studies on petiole abscission and fruit drop in 1959 with several growth regulators, it was found that 2-chloro,4-fluorophenoxyacetic acid (2,4-F) had a marked effect on preventing abscission in McIntosh as well as in several other varieties. The formative effects of 2,4-F on shoot and leaf growth of McIntosh were similar to those produced by 2,4-D on a susceptible variety such as Stayman (Fig. 1). Thus the substitution of fluorine for chlorine in the 4-position of the phenoxy ring appeared to block the decarboxylation of 2,4-F in McIntosh leaves.

To investigate this possibility, tests were conducted with 2,4-F (*3*) similar to those previously carried out on 2,4-D. Carboxyl-labeled 2,4-F was prepared having a specific activity of 1 mc/mmole identical with the carboxyl-labeled 2,4-D which had been used in the earlier decarboxylation studies and with which it was compared. Equipment was assembled to supply apple shoots with dilute solutions of the two carboxyl-labeled compounds and to collect the $C^{14}O_2$ given off by the leaves. The shoots were cut from trees of the desired varieties and transferred immediately to small flasks containing the carboxyl-labeled solutions. These were then placed in large jars through which air could be drawn and the CO_2 absorbed in NaOH solutions. Light was excluded during the respiration periods in order to collect the maximum amount of CO_2 from the leaves. The carbonate was precipitated from the NaOH solution, and the precipitate was collected on filter paper for counting.

It was found that the decarboxylation of the 2,4-F-1-C^{14} occurs at a much slower rate in McIntosh leaves than does the decarboxylation of the 2,4-D-1-C^{14} (Table 1). In 24 hr only 4 percent of the C^{14} was recovered as $C^{14}O_2$ from the McIntosh shoots supplied with 2,4-F-1-C^{14}, while 33 percent of the C^{14} was recovered from the shoots supplied with 2,4-D-1-C^{14}. Even in Stayman, where decarboxylation of 2,4-D is extremely slow, the breakdown of the compound is further retarded by the substitution of the fluorine. Bioassays have shown that the decarboxylated form of 2,4-D is comparatively inactive.

Autoradiographs have been made of McIntosh and Stayman leaves treated with these carboxyl-labeled compounds. They show a rapid disappearance of C^{14} from McIntosh leaves supplied with 2,4-D-1-C^{14}. Similar leaves supplied with an equal amount of 2,4-F-1-C^{14} show much higher C^{14} activity. Thus it is apparent that 2,4-F may be absorbed by McIntosh leaves and move through the leaves to abscission sites without being inactivated.

Table 1. Production of $C^{14}O_2$ by apple leaves on detached shoots treated with 2,4-D-1-C^{14} and 2,4-F-1-C^{14}.

Treatment	Amount absorbed* (μg)	Absorbed C^{14} recovered as $C^{14}O_2$ (%)	
		4 hr	24 hr
	McIntosh		
2,4-D-1-C^{14}	40	3.25	33.04
2,4-F-1-C^{14}	45	0.43	4.13
	Stayman		
2,4-D-1-C^{14}	40	0.04	0.56
2,4-F-1-C^{14}	50	0.03	0.14

* After the 4-hr absorption period the shoots (8 leaves per shoot) were transferred to distilled water and the respiration run continued for an additional 24 hr.

Further evidence of the stability and effectiveness of 2,4-F in delaying abscission is its effect on the flowers. The application of 2,4-F either as dilute aqueous sprays or in a lanolin paste to flowers of several varieties including McIntosh was found to inhibit their abscission. In fact, it was observed in 1960 that nearly all of the flowers persisted throughout the summer on the McIntosh trees treated the previous fall with 2,4-F for control of preharvest drop, even though the proportion of flowers that formed mature fruits was reduced by the treatment (Fig. 1). This is similar to the stability of 2,4-D in Stayman. It has been reported (*4*) that Stayman trees sprayed with 2,4-D for delay of drop one year may show significant reduction in preharvest drop

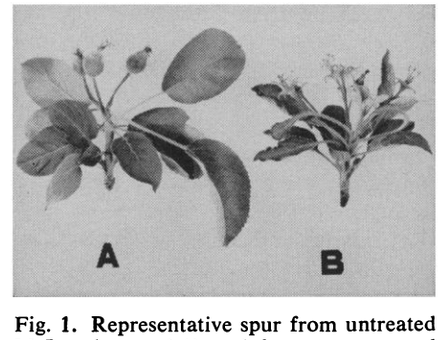

Fig. 1. Representative spur from untreated McIntosh tree (*A*) and from tree sprayed with 20 ppm 2,4-F (*B*). The spray was applied Sept. 1959; spurs were photographed 13 June 1960. Note the leaf distortions and persistent flowers on spur from treated tree. The leaf symptoms include enlargement of leaf veins, shortening and narrowing of the leaves, and development of marginal ruffles similar to the distortions produced by 2,4-D on susceptible varieties and species.