companied by a very obvious visible increase in the rate of tumor growth. Very little ascitic fluid could be obtained from mice on N-methylformamide therapy when the mice were infected with sensitive tumor cells before 14 days of growth. Under the same circumstances, infection with resistant cells resulted in extremely large yields of ascitic fluid by the seventh day after inoculation. This difference in tumor growth rate was examined by direct count of ascitic cells present as a function of time. As shown in Fig. 1, the generation times of sensitive cells in the absence of therapy and of resistant cells in mice fed N-methylformamide were 24 and 26 hours, respectively. The generation time of sensitive cells in the treated host was approximately 66 hours. The growth rate of resistant cells in the absence of therapy is indistinguishable from that of sensitive cells under the same circumstances.

To test whether resistance to N-methylformamide was due to some adaptive mechanism depending on the presence of N-methylformamide for its maintenance, mice fed an N-methylformamide-free diet were infected with resistant tumor cells. At 7-day intervals mice were sacrificed, and the cells obtained were transferred to another group of mice fed the N-methylformamide-free diet. At each transfer generation a group of inoculated mice was fed N-methylformamide. The data obtained for 50 serial transfer generations were quite homogeneous and quite similar to those already presented in Table 1 (transfer generations 11 to 80). This indicates that resistance is maintained in the absence of therapy and that the resistant cell population displays a degree of stability indicative of a genetic alteration.

It seems probable, in view of the above, that N-methylformamide resistance arose as a consequence of mutation and selection in a manner analogous to the appearance of resistance in bacterial populations suggested by Law (*5*). Direct tests of this notion are not easily performed with this material. However, it can be indirectly examined by determining whether the appearance of resistance during the third transfer generation in the presence of the drug (Table 1) was consistent with a reasonable mutation rate (about 10^{-6} or less). As was pointed out previously, the generation time of the sensitive cells in the presence of N-methylformamide is 66 hours, while that of the resistant cells is 26 hours. Since enough time is available between transfers (14 days) to allow more than five sensitive cell generations per transfer, one resistant cell per 10^5 to 10^6 sensitive cells in the original inoculum would have time to become the predominant member of the total cell population within three tumor transfer generations. Thus the observed rapidity of the onset of resistance is not inconsistent with a mutation rate of about 10^{-6}.

Preliminary experiments using artificial mixtures of 10^7 sensitive cells and 10, 10^2, and 10^3 resistant cells have yielded resistant cell populations within the intervals expected on the basis of the generation times of the two cell types in the presence of the drug.

A recent report by Potter and Law (*6*) of the development of resistance to azaserine by an ascitic form of a plasma cell neoplasm and its stability in the absence of the drug suggests the general efficacy of the selection of drug-resistant ascitic cells *in vivo*. Cell lines resistant to N-methylformamide and azaserine would seem eminently suitable for biochemical as well as for more refined genetic analysis. However, these analyses would probably best be done in tissue culture under more defined conditions than those afforded by the mouse peritoneum.

HUDI S. GROSS
ARTHUR FURST
Cancer Chemotherapy Laboratory and
Department of Pharmacology, Stanford University, Stanford, California
S. R. GROSS*
Department of Biological Sciences, Stanford University

References and Notes

1. G. Klein and L. Revesz, *J. Natl. Cancer Inst.* 14, 229 (1953).
2. This work was supported in part by an Institutional Grant from the American Cancer Society to Stanford University and research grants C2798(C) and C2167(C6) from the National Cancer Institute, U.S. Public Health Service.
3. A. Furst, W. C. Cutting, H. Gross, *Cancer Research* 15, 294 (1955).
4. B. Lucké and M. Berwick, *J. Natl. Cancer Inst.* 15, 99 (1954).
5. L. W. Law, *Nature* 169, 628 (1952).
6. M. Potter and L. W. Law, *J. Natl. Cancer Inst.* 18, 413 (1957).

* Present address: Rockefeller Institute for Medical Research, New York, N.Y.

29 August 1957

Discovery of Cholesterol in Some Red Algae

There have been some reports on the isolation of algal sterols, including fucosterol (*1*), sitosterols (*2*), chalinasterol (*3*), and sargasterol (*4*), but there has been no report on the isolation of cholesterol from the vegetable kingdom. This report (*5*) describes the discovery of cholesterol in some red sea weeds (Rhodophyceae).

Dry powder of *Rhodoglossum pulcherum* (Kützing) Setchell et Gardner (*6*) was extracted three times with boiling benzene and with stirring, and the resultant dark brown oil (1.1 percent) was saponified with 4-percent methanolic alkali. Subsequent extraction with benzene afforded an unsaponifiable matter (12 to 13 percent from crude oil). Standing a methanolic solution of it in a refrigerator overnight yielded yellow-colored crystals (55 percent from unsaponifiable matter). A few recrystallizations from methanol gave a sample of melting point 142° to 145°C (*7*), which was precipitated with digitonin and which was positive with Liebermann-Burchard's color test.

Purification of the sterol (mp 142° to 145°C) twice through its dibromoacetate, which is precipitable in a solution of dry ether and glacial acetic acid, gave a pure sample of mp 147° to 148°C; $[\alpha]_D$ −40.0 (*8*). Perbenzoic acid titration, bromination, and catalytic hydrogenation of the steryl acetate indicated that the sterol possesses just one double bond. The following derivatives were made from the pure sterol: (i) Acetate, mp 114° to 115.5°C; $[\alpha]_D$ −44. (ii) Benzoate, mp 144° to 145.5°C; $[\alpha]_D$ −14. (iii) Stenone, mp 84° to 86°C; $[\alpha]_D$ +88; $\lambda_{max.}^{EtOH}$ 241 mμ (ε, 17,800, calculated as cholestenone). (iv) Stanol, mp 142° to 143°C; $[\alpha]_D$ +23.5. (v) Dibromosterylacetate, mp 113° to 114°C; $[\alpha]_D$ −47.8. (vi) Dibromide, mp 112° to 114°C; $[\alpha]_D$ −44.3.

All these derivatives of the sterol were identified with the corresponding derivatives of authentic cholesterol by mixed melting points and infrared spectra. Furthermore, the result of x-ray diffraction analysis of the algal stenone was the same in all respects as that of authentic cholestenone within an error of 1 percent, including experimental errors. We are therefore convinced that the sterol is cholesterol.

In addition, we also isolated cholesterol from easily soluble fractions of the sterols obtained from some other red algae, all of which (*9*) belong to the family Gelidiceae: *Gelidium amansii* (Iam), *Gelidium subcostatum* (Okam.), *Pterocladia tenuis* (Okam.), *Gelidium japonicum* (Okam.), and *Acanthopeltis japonica* (Okam.).

KYOSUKE TSUDA
SABURO AKAGI
YUKICHI KISHIDA
Institute of Applied Microbiology, University of Tokyo, and
Takamine Research Laboratory, Sankyo Company, Tokyo, Japan

References and Notes

1. I. M. Heilbron, R. F. Phipers, H. R. Wright, *Nature* 133, 419 (1934); *J. Chem. Soc.* 1934, 1572 (1934).
2. P. W. Carter, I. M. Heilbron, B. Lythgoe, *Proc. Roy. Soc. (London)* B128, 82 (1939).
3. S. Ito, K. Nagashima, T. Matsumoto, *J. Chem. Soc. Japan* 77, 1119 (1956).
4. K. Tsuda *et al.*, *Pharm. Bull. Tokyo* 5, 85 (1957); a final report is in preparation.
5. A final report is in preparation. We thank Y. Yamada, T. Uchida, Y. Nakamura, and M. Yamada of Hokkaido University for their kind advice, which helped us in collecting and identifying the algae. We are also indebted to S. Sudo of the Tokai Fishery Research Institute and to the members of the Ito Fishery Research Section in Shizuoka Prefecture for their kind help in collecting the algae.

6. This alga was collected at Akkeshi in Hokkaido in June and was carefully selected to avoid contamination with any traces from animal sources.
7. All melting points listed are uncorrected.
8. All optical rotations were measured in chloroform at 25°C.
9. The algae were collected at Shirahama in Shizuoka Prefecture.

15 July 1957

Prenatal Protection of Mice by Yeast Antibiotic (Malucidin)

We prepared a complex protein with antibiotic properties from brewer's and baker's yeasts. This material, when injected into animals in doses of 1 to 10 mg/kg of body weight, protected them against infections caused by a number of organisms, including several species of Gram-positive and Gram-negative bacteria, fungi (including *Candida albicans*), and *Shigella* endotoxin (*1*). In many respects this material is different from other antibiotics; it has a very wide spectrum of activity and a long-lasting effect. Mice injected with larger doses of this new agent were refractory to inoculation with *Proteus* OX19 for at least 1 mo. This observation stimulated our interest in investigating the effect on their offspring of treatment of pregnant mice with Malucidin.

The mice received injections of Malucidin in the later stage of pregnancy and 2 to 3 days later gave birth to litters. Injections of Malucidin were given intravenously or intraperitoneally; since there was no difference in the results after injection of Malucidin by either route, the data were combined (Table 1). Young mice were tested for resistance to *Proteus* infection when they reached the age of 2 to 3 weeks, in which stage they continued to be suckling. Results are summarized in Table 1.

Table 1. Protection of mice by prenatal injection of Malucidin. Numerators indicate the number of survivors; denominators indicate the number of mice used.

Group No. and treatment of mother	No. of Proteus organisms injected into suckling mice			
	250 M	750 M	1.5 B	3 B
Experiment with mice 3 wk old (av. body weight, 7 g)				
1. Control—no treatment	3/4	0/7*	0/2	0/2
2. Injected with 5 mg of Malucidin on 2 consecutive days	2/2	9/12*	2/4	0/6
Experiment with mice 2 wk old (av. body weight, 5 g)				
3. Control—no treatment	0/5	0/6		
4. Injected once with 10 mg of Malucidin	3/4	0/6		
5. Injected twice with 10 mg of Malucidin 24 and 48 hr after birth of offspring	0/5	0/6		

* The difference between these two groups was statistically significant: $P < 0.01$.

As can be seen from the table: (i) pregnant females treated with Malucidin produced progeny more resistant to *Proteus* infection than those of normal, untreated mice; and (ii) as group 5 indicates, the resistance was not transmitted with the milk, since the offspring of mice treated with Malucidin after delivery were not more resistant than normal, untreated mice.

I. A. PARFENTJEV
Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut

References

1. I. A. Parfentjev, *Yale J. Biol. and Med.* 26, No. 1, 75 (1953); ———, *Am. Brewer* 87, No. 7, 39 (1954); ———, *Federation Proc.* 14, 474 (1955); ———, *ibid.* 16, 428 (1957); L. F. Whitney and R. Arch, *Vet. Med.* 52, No. 5, 247 (1957).

28 June 1957

Volcanic Activity and Alaskan Spruce Growth in A.D. 1783

In the absence of historical accounts, tree-ring chronologies have provided considerable data for reconstructing climate of the past. This report is an attempt to explore further the association between a specific series of climatic phenomena, volcanic eruptions, and anomalies in Alaskan tree-ring patterns for the year A.D. 1783. The effects of major volcanic activity upon world climate were amply dramatized by the significant drop in world temperatures following the eruption of Tomboro in 1815, of Krakatoa in 1883, and of Katmai in 1913 (*1*). It now seems possible that, under certain conditions, previously unrecorded volcanic eruptions can be detected by their effect on the annual ring records of white spruce [*Picea glauca* (Moench.)] in western Alaska.

When J. L. Giddings began his northern Alaskan tree-ring studies, he noted that the final growth cells for the year A.D. 1783 were obscure, particularly in ring records of spruce growing at tree line and at the biological limit of the species. At the time the ring sealed off for that year, a distinctive layer of thin, faintly visible cells was added, rather than the customary dark late cells; this layer has been designated "faint latewood" (*2*). The 1783 faint latewood is common to many, but not to all, northern Alaskan white spruce which have been sampled and which are of sufficient age to contain it. The unique ring occurs sporadically in tree-line spruce of the Copper and Kuskokwim rivers and is common in the Yukon River spruce (*3*).

On the basis of recent inquiries it appears probable that the unique characteristics of the 1783 ring bear a direct relationship to certain widespread natural phenomena that occurred during the summer of 1783 in Europe, Japan, and the United States. In the eastern United States, at least, this was a year without a summer. Benjamin Franklin (*4*) commented upon the climate for this particular year and noted:

"During several of the summer months of the year 1783, when the effects of the sun's rays to heat the earth in these northern regions should have been the greatest, there existed a constant fog over all Europe, and great part of North America. This fog was of a permanent nature; it was dry, and the rays of the sun seemed to have little effect toward dissipating it, as they easily do a moist fog, arising from water. They were indeed rendered so faint in passing through it that, when collected in the focus of a burning-glass, they would scarce kindle brown paper. Of course, their summer effect in heating the earth was exceedingly diminished.

"Hence the surface was early frozen.

"Hence the first snows remained on it unmelted, and received continual additions.

"Hence perhaps the winter of 1783–4 was more severe than any that happened for many years."

Franklin further stated that smoke from a volcanic eruption in Iceland might have been carried by winds to various parts of the world, which would explain the abnormally cold summer. The Skaptar Jokull eruption in Iceland was the one to which he referred, and it was most active on 8 and 18 June of 1783. Symons (*5*) noted that a "dry fog" appeared over all of Europe on 17 June 1783 and that it was world-wide in its distribution.

In a recent study of summer temperature and Scandinavian tree growth, Schove (*6*) remarks that Finland had a bad harvest during 1783, while central Europe had a great deal of heat and excessive south and southeasterly winds. He states further that the narrowness of the tree-rings in northern Europe for that year may have been due to the dust-haze that followed the volcanic eruptions, and he also comments upon the peculiar nature of this ring in Alaska.

In addition to the major volcanic activity in Iceland, there was also the eruption of Asama in Japan, on 4 Aug. 1783, which has been termed "the most frightful eruption on record" (*5, 7*).

Spruce increment borings were taken recently by the writer in western Alaska during the growing season, and these may serve as a gross index of the period of growth in western Alaskan spruce.