lieve that our results indicate that measurement of ceruloplasmin concentration in an individual is not useful in determining whether or not that individual is schizophrenic.

Akerfeldt concluded from his study that the difference between sera from schizophrenic and normal subjects "in their capacities to oxidize DPP is dependent mainly on the fact that the ceruloplasmin activity is higher and the ascorbic acid concentration lower" in the sera from schizophrenic patients than they are in sera from normal subjects (*1*). Our results indicate that the concentration of plasma ceruloplasmin is not a reliable criterion for distinguishing schizophrenic from healthy individuals. It appears, therefore, that if differences in the capacity to oxidize DPP can be consistently demonstrated between sera from schizophrenic and healthy individuals, these differences cannot be ascribed merely to variations in ceruloplasmin concentration (*11*).

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11. This work was supported in part by grants (A-572 and A-1059) from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service, and by a grant [NONR-1765(00)] from the Office of Naval Research. We are indebted to R. M. Simon for his assistance in the study of the schizophrenic patients.

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Development of a Strain of Ehrlich Ascites Tumor Cells Resistant to N-Methylformamide

Investigations of the genetic and nongenetic variability among homogeneous populations of mammalian cells have been hampered by the tendency of most mammalian cells to grow in aggregates *in vitro* as well as *in vivo*. This tendency, as well as the difficulty of obtaining variants subject to selective techniques, has to some extent discouraged the application of methods employed in microbial genetics to problems related to mammalian cell variability. As a preliminary attempt to investigate mutation and recombination among mammalian somatic cells, the applicability of selection for drug-resistant variants among populations of Ehrlich ascites tumor cells was explored. The relatively unaggregated clonal growth characteristics of Ehrlich ascites cells suspended in the mouse peritoneum (*1*) seemed to offer an opportunity for obtaining populations of homogeneous cells without the application of tissue culture techniques.

This report (*2*) is primarily concerned with the development of a population of Ehrlich ascites tumor cells resistant to N-methylformamide. The phenotype of N-methylformamide resistant cells with respect to growth rate and the survival of infected mice fed N-methylformamide is described.

A hypotetraploid Ehrlich ascites tumor originally obtained from T. Hauschka was used in this study. The tumor was maintained by injecting intraperitoneally 0.1 ml of ascitic fluid (approximately 1×10^7 cells) from a 7-day donor tumor into Swiss-Webster mice of either sex weighing between 18 and 20 g. N-Methylformamide was mixed in Purina Laboratory Chow meal at a concentration of 0.1 percent, and the mice were allowed to feed *ad libitum* from the day of inoculation until death. This concentration of the drug was optimal for tumor inhibition without toxic side effects (*3*). Determinations of the total cell populations were made by a method similar to that used by Klein and Revesz (*1*) and Lucké and Berwick (*4*).

A substantial increase in the mean survival time of mice infected with Ehrlich ascites tumor results from including N-methylformamide in the diet. The mean survival time of 237 infected mice that were fed the drug was 25.2 ± 2.7 (standard deviation) days, as compared with 13.1 ± 1.2 days for 322 mice in the absence of therapy.

A population of cells resistant to N-methylformamide was obtained by serial transfer of Ehrlich ascites tumor cells in mice fed N-methylformamide. As indicated in Table 1, a substantial shortening of the mean survival time of infected mice on N-methylformamide therapy occurred after the third serial transfer of tumor cells. The mean survival time after subsequent transfers was essentially the same for mice on therapy as for mice on the control diet and has remained between 14 and 19 days for over 80 transfer generations.

The shortened survival time was ac-

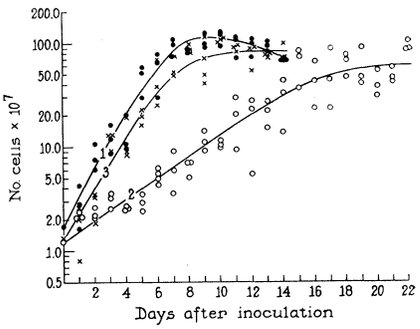

Fig. 1. Number of Ehrlich ascites tumor cells in the mouse peritoneum as a function of time. Curve 1 (●), N-methylformamide sensitive cells; no therapy; curve 2 (○), sensitive cells on N-methylformamide therapy; curve 3 (×), N-methylformamide resistant cells obtained after the 37th transfer generation in the presence of N-methylformamide on N-methylformamide therapy. Each point represents the total cell population of a single mouse peritoneum.

Table 1. Onset and maintenance of resistance of Ehrlich ascites cells to N-methylformamide (NMF). For the first 28 serial transfer generations cells were obtained from mice 14 days after inoculation. Subsequent to the 28th transfer generation, cells were obtained after 7 days' growth. While the data are too numerous to be presented *in toto,* the mean survival time of all mice on NMF from the third to the 80th transfer generation was 15.6 ± 1.7 days (718 animals). Although groups of animals fed an NMF-free diet were not employed regularly before the 11th transfer generation, the mean survival time of all animals so treated was 14.9 ± 2.2 days (615 animals).

Transfer generation	Drug	No. of mice	Mean survival time (days) ± S. D.*
1	NMF	8	24.3 ± 1.3
1		10	11.1 ± 1.9
2	NMF	7	23.1 ± 2.4
2		8	15.3 ± 2.1
3	NMF	7	16.4 ± 2.0
4	NMF	7	14.7 ± 2.9
5	NMF	6	14.5 ± 5.6
7	NMF	8	17.0 ± 5.4
9	NMF	9	14.6 ± 3.9
11	NMF	9	16.0 ± 2.8
11		7	16.6 ± 3.2
13	NMF	7	15.1 ± 3.1
13		4	14.5 ± 2.2
15	NMF	8	16.1 ± 3.6
15		10	14.7 ± 2.2
20	NMF	9	14.8 ± 3.6
20		6	14.3 ± 3.2
30	NMF	9	15.8 ± 2.5
30		9	16.5 ± 1.6
50	NMF	13	14.4 ± 3.5
50		9	14.4 ± 3.6
80	NMF	10	12.4 ± 2.1
80		9	9.9 ± 2.0

* S.D. = standard deviation.

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.

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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.).

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