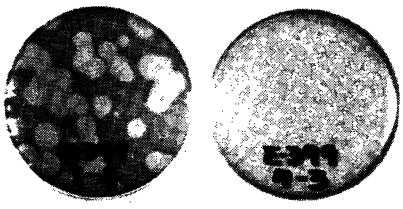

Fig. 2. Selection of MEF-1 *m* mutants in the course of the serial passage (E-399) of wild-type MEF-1 virus in HeLa cell cultures with medium YLA plus 20 to 40 percent NBS-137. Plate 1-4 was seeded with 10^{-4} dilution of first-passage fluid plaques of the MEF-1 wild-type virus. Plate 7-3 was seeded with 10^{-3} dilution of seventh-passage fluid plaques of MEF-1 *m* mutants. Both platings were made on the same lot of HeLa cell monolayers (55-mm petri dishes) with 10 percent horse serum agar overlay. The photograph was taken 5 days after the plates were seeded.

size by this particular mutant. This possibility is now being extensively studied, and the evidence thus far obtained seems to indicate clearly that *m* mutants have a unique sensitivity to the viral inhibitory activity of extract of agar overlay.

The *m* mutants have been compared with m^+ virus in virulence for mice by the intracerebral route of inoculation and have been found to be slightly less virulent than m^+ virus. The *m* mutants were stable through ten rapid serial passages in tissue cultures of both HeLa and monkey kidney cells. However it was found that a few larger plaques sometimes appeared on plates seeded with low dilutions (10^0 to 10^{-3}) of *m* mutants. Since the *m* mutants had been previously purified repeatedly by the limiting-dilution technique, it seems clear that the *m* virus gives rise to mutants of the m^+ type—that is, it reverts to wild type with respect to plaque size. Furthermore, the back mutant m^+ virus, after having been plaque-purified, was shown to have *i* character—that is, it is resistant to the inhibitory NBS used in the medium for serial transfers, and it produces large plaques on inhibitory NBS plates. Back mutations of other poliovirus mutants have also been noted by other investigators (*3, 4*).

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23 August 1957

Concentration of Ceruloplasmin in Plasma of Schizophrenic Patients

Akerfeldt has recently reported that N,N-dimethyl-*p*-phenylenediamine (DPP) is oxidized to red products more rapidly by serum from schizophrenic patients than by serum from healthy control subjects (*1, 2*). The oxidation of DPP is accelerated by ceruloplasmin (*1–4*), the plasma copper-protein, and inhibited by other substances in serum, including ascorbic acid (*1, 2*). Since measurement of the oxidation of DPP by serum may possibly have diagnostic value in mental disease (*1, 2*), and since ceruloplasmin is of prime importance in this oxidation, it is of interest to measure directly the concentration of ceruloplasmin in the serum, or plasma, of schizophrenic and healthy subjects. This report presents the results of such determinations in 20 schizophrenic and 21 control subjects.

The concentration of ceruloplasmin was measured spectrophotometrically (*3, 5, 6*). Blood was drawn from a fasting subject, and coagulation was prevented by the addition of 1.5 ml of 2.5-percent sodium citrate solution to each 10 ml of blood. The plasma was separated and clarified at 24,000 *g* in an International PR-2 centrifuge. After the optical density (O.D.) of the plasma had been measured at 610 mμ in a cuvette of 5-cm path length, the ceruloplasmin was decolorized by the addition of 0.1 ml of a 10-percent solution of sodium cyanide to the cuvette, which contained 2.7 ml of plasma. One hour later the optical density was again measured at 610 mμ. The difference in optical density

$$\Delta(\text{O.D.})^{5\text{ cm}}_{610\ m\mu}$$

was divided by 0.0034 to obtain the ceruloplasmin concentration in milligrams per 100 milliliters (*3*). Measurement of the recovery of added ceruloplasmin to plasma has shown that this method is accurate to about ±4 percent (*6*).

All the schizophrenic patients studied were free from significant somatic disease and were hospitalized in either the New York State Psychiatric Institute or the Bronx Municipal Hospital Center. The diagnosis of schizophrenia was made by staff psychiatrists without knowledge of the results of this study. Some of the patients were undergoing insulin-coma therapy at the time blood was drawn, as shown in Table 1. The control subjects were healthy medical students, house officers, and other medical school and hospital personnel.

The results of the determinations in the 20 schizophrenic patients and the 21 control subjects are shown in Table 1. The distributions of the plasma ceruloplasmin concentrations in the two groups show a considerable overlap. There is a range of 19 to 37 mg/100 ml in the schizophrenic patients, with a mean value of 27.8 mg/100 ml, and a range of 16 to 33 mg/100 ml in the control subjects, with a mean value of 23.7 mg/100 ml. Only three of the patients have concentrations greater than the highest value shown by a control subject. Four of the 20 patients have ceruloplasmin concentrations below the mean value of the control group, and five of the 21 control subjects have values above the mean of the schizophrenic group.

These findings indicate that it would be hazardous to classify an individual into one of the two groups on the basis of his ceruloplasmin concentration. Furthermore, it is relevant to emphasize that plasma ceruloplasmin concentrations which are significantly higher than normal by the *t* test occur in such apparently unrelated clinical conditions as a variety of neurological diseases (*6*), liver disease (*6*), and pregnancy (*7*), and marked increases in the concentration of this protein have been reported in infection (*8*) and myocardial infarction (*9*). Therefore, although the slight difference in the mean value of plasma ceruloplasmin in the schizophrenic and healthy groups, 27.8 and 23.7 mg/100 ml, respectively, is statistically significant (*10*), we be-

Table 1. Concentration of ceruloplasmin in the plasma of 20 schizophrenic patients and 21 control subjects.

Schizophrenic patients		Control subjects	
Sex and age	Plasma ceruloplasmin (mg/100 ml)	Sex and age	Plasma ceruloplasmin (mg/100 ml)
F, 19	25	M, 33	33
M, 25	27*	F, 22	25
M, 42	34*	M, 34	31
F, 42	28*	M, 32	27
M, 34	27	F, 40	23
M, 19	19*	F, 22	29
M, 23	21	M, 32	16
M, 22	24*	F, 32	18
M, 38	31*	F, 22	24
M, 18	36*	F, 27	20
F, 30	30*	M, 28	29
M, 19	22	M, 35	25
M, 29	30*	M, 23	17
F, 41	29*	M, 22	32
M, 22	28	M, 25	18
F, 41	30	M, 24	25
F, 27	30	M, 23	23
F, 56	37	M, 22	24
F, 27	29	M, 27	16
M, 15	20	M, 23	26
		M, 25	16
Mean	27.8	Mean	23.7

* These patients were in insulin coma when blood was drawn.

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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10. The value of *t*, calculated from the results in Table 1, is 2.58, indicating that differences of means as large or larger than those found here would not occur more than 1 to 2 percent of the time, if caused by chance alone.
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.

5 August 1957

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-

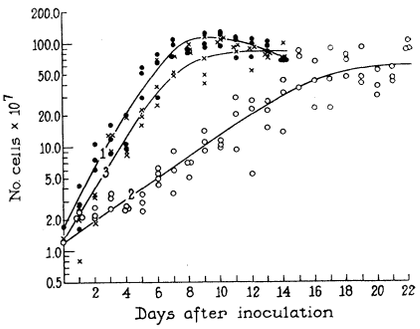

Fig. 1. Number of Ehrlich ascites tumor cells in the mouse peritoneum as a function of time. Curve 1 (●), N-methylformamide sensitive cells; no therapy; curve 2 (○), sensitive cells on N-methylformamide therapy; curve 3 (×), N-methylformamide resistant cells obtained after the 37th transfer generation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