7. *The Case of Periodic Data.*—In a recent paper[5] I introduced the method of trigonometric spline interpolation. The discussion of sections 4 and 5 carries over to the periodic case and need not be elaborated. The analogue of Problem 1 is as follows: assuming $2m + 2 \leqq n$, $\epsilon > 0$, we are seeking the function $f(x)$, of period 2π, having a square integrable $(2m + 1)$st derivative and which solves the problem

$$\epsilon \int (\Delta_m f)^2 dx + \sum_1^n (f(x_\nu) - y_\nu)^2 = \text{minimum}, \quad [\Delta_m = D(D^2 + 1^2) \ldots (D^2 + m^2)],$$

the integration being over an entire period while the x_ν are increasing with $x_n - x_1 < 2\pi$. The unique solution is a trigonometric spline function $S(x, \epsilon)$ having properties analogous to those stated in Theorems 1, 2, and 3. Naturally, the role of $Q(x)$ is now played by the trigonometric polynomial $T(x)$, of order m, which solves the problem

$$\sum_1^n (T(x_\nu) - y_\nu)^2 = \text{minimum}.$$

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[1] For references, see *Proc. Roy. Netherl. Acad.*, **A67**, 155–163 (1964). A recent paper is by T. N.E. Greville (Math. Res. Center Tech. Report No. 450, Madison, Wis., January 1964).

[2] We denote by π_k the class of real polynomials of degree not exceeding k.

[3] Whittaker, E. T., *Proc. Edinburgh Math. Soc.*, **41**, 63–75 (1923).

[4] See these PROCEEDINGS, **51**, 28 (1964), formula (15), for a simplification occurring in the case when the x_ν are in arithmetic progression.

[5] To appear in the November 1964 issue of *J. Math. Mech.*

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*OCCURRENCE OF SOLUBLE ANTIGEN IN THE PLASMA OF MICE WITH VIRUS-INDUCED LEUKEMIA**

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The cells of leukemias induced in mice by several different viruses possess specific antigens that can be demonstrated by serological methods.[1–8] Leukemias induced by Friend, Moloney, and Rauscher viruses share antigenic determinants that are not present in leukemias induced by Gross virus.[6, 8] It has recently been shown that the antigen characteristic of leukemias induced by Rauscher virus may be acquired by the cells of unrelated transplanted leukemias during passage in mice infected with Rauscher virus, a phenomenon which has been named "antigenic conversion."[9] These converted cells are susceptible to the cytotoxic activity of specific Rauscher antiserum, and this sensitivity persists indefinitely on serial transplantation of converted lines. Permanent antigenic conversion by Rauscher virus has now been shown to occur *in vitro* in an established tissue culture line of the leukemia EL4.[10] Thus it is clear that leukemia cells can support the continued multiplication of an

unrelated leukemogenic virus and that persistence of the specific antigen in converted cells could be accounted for in this way. However, the appearance of Rauscher antigen as a result of virus replication, in cells either induced or converted by Rauscher virus, is not the only way in which sensitivity to Rauscher antiserum may be acquired. We have now observed that the plasma of mice infected with Rauscher virus contains a soluble antigen, separable from infective virus, that carries the specificity of Rauscher leukemia cells. When certain lines of leukemia cells that do not possess Rauscher antigen are incubated in infective plasma, adsorption of antigen takes place and the cells become sensitized to Rauscher antiserum.

Materials and Methods.—Mice: The strains used in these studies were obtained from our own inbred colonies.

Leukemias: The majority of these arose spontaneously or were induced in our own stocks by Gross,[11] Friend,[12] Moloney,[13] or Rauscher[14] viruses, or by X radiation. Methods of induction and designation of these leukemias are described in previous publications.[2, 6, 15] Friend disease was induced by intraperitoneal (IP) inoculation of homogenates of infected spleen. The early splenomegalic phase of Rauscher disease was induced in BALB/c by IP inoculation of 0.1 ml of heparinized plasma from BALB/c mice similarly infected with this virus. The indicator cell used in most of this work was the C57BL/6 radiation-induced leukemia ERLD,[15] which arose in 1962 and is now in its 61st transplant generation. Several BALB/c mice with primary leukemia induced by Moloney virus (LT(v)402), used as a source of plasma in adsorption experiments, were kindly provided by Dr. J. Glynn, National Cancer Institute. R/EL4 was derived from the long-transplanted C57BL ascites leukemia EL4 by a single passage in a BALB/c mouse infected with Rauscher virus, during which it acquired the antigenic specificity of Rauscher leukemia cells (antigenic conversion[9]).

Ascites sarcomas: BP8 (C3H) and Meth A (BALB/c) were originally induced by benzpyrene and methylcholanthrene, respectively (see ref. 16).

Antisera: These were prepared by immunization of mice with 5–10 inoculations of viable leukemia cells from spleen or ascites over a period of 2–5 months. Sera were obtained by tail-bleeding under ether anesthesia and were stored at −70°C. References to the descriptions of these typing sera appear in Table 2. The Rauscher and Moloney typing sera were prepared by immunization of C57BL/6 mice with highly antigenic C57BL/6 Rauscher or Moloney leukemias that regress on initial subcutaneous inoculation.[6] These sera contain no isoantibodies and therefore can be used in cytotoxic tests with leukemias of all genotypes.

Direct cytotoxic and absorption tests: The cytotoxic test is based on the technique of Gorer and O'Gorman.[16, 20] Equal volumes (0.03 ml) of cells (2×10^6/ml), antiserum dilution, and pooled absorbed guinea pig serum diluted $1/2$ as a source of complement were incubated for 45 min at 37°C. Viability per cent was determined microscopically after the addition of trypan blue. The diluent was medium 199. In each test, controls were included in which the cells were incubated with either guinea pig serum or a $1/3$ dilution of antiserum alone. These controls contained no more than 10% stained cells and are therefore omitted from the tables.

For absorption tests, antisera were diluted to 2 doubling dilutions below the end-point (50% of test cells stained by trypan blue), mixed with an equal volume of packed washed cells, and incubated for 45 min at room temperature followed by 45 min at 4°C. The serum was recovered by centrifugation in the cold.

Adsorption procedure: Heparinized plasma was collected by cardiac puncture, under ether anesthesia, from BALB/c mice infected 10–14 days previously with Rauscher virus. Cell suspensions were prepared from normal or leukemic spleens, or from ascites fluid, and washed in medium 199. 5×10^6 viable packed cells were suspended in 0.5 ml of infective plasma and left at 4°C for 60 min with occasional shaking. The cells were washed twice in the cold, each time in 12 ml medium 199, and were then resuspended to a concentration of 2×10^6/ml for the cytotoxic test. For controls, cells were prepared under identical conditions with plasma from *normal* mice of the same strain and sex.

Results.—Titration of Rauscher virus: The infectivity of pooled BALB/c plasma,

17 days after infection with Rauscher virus, was assayed in mice of the same strain. Plasma dilutions to 10^{-3} produced massive splenomegaly by 21 days, and death of these mice by 90 days (Table 1). Parallel titration of the same plasma pool was performed in resistant C57BL/6 mice, with production of cytotoxic antibody as the end-point. By 26 days after infection, antibody titers of $^1/_4$ or more were found in mice inoculated with 10^0–10^{-5} dilutions of infective plasma (Table 1). No antibody was detected in the serum of the BALB/c mice that were inoculated with higher dilutions of infective plasma and failed to develop Rauscher disease.

TABLE 1

TITRATION OF RAUSCHER VIRUS BY TWO METHODS*

Dilution of infective plasma (0.1 ml IP)	BALB/c ♀ Mice		C57BL/6 ♀ Mice			
	Splenomegaly at 21 days	Deaths at 90 days	Titer of Cytotoxic Antibody† at 14 days	26 days	52 days	110 days
10^0	4/4	4/4	1/2	1/128	1/128	<1/2
10^{-1}	4/4	4/4	1/8	1/256	1/128	<1/2
10^{-2}	4/4	4/4	1/4	1/64	1/64	<1/2
10^{-3}	4/4	4/4	1/1	1/16	1/64	<1/2
10^{-4}	1/4	0/4‡	1/1	1/4	1/32	
10^{-5}	0/4	0/4	<1/1	1/4	1/4	
10^{-6}	0/4	0/4	<1/1	<1/1	<1/1	
10^{-7}	0/4	0/4	<1/1	<1/1	<1/1	
10^{-8}	0/4	0/4	0	0	0	

* By development of Rauscher disease in susceptible BALB/c mice, and by antibody formation in resistant C57BL/6 mice.

† Expressed as highest dilution of serum killing 50% of the Rauscher typing leukemia cells (C57BL/6 ♀ Rauscher 2) in cytotoxic test: each value represents result obtained with pooled sera of 4 animals.

‡ Splenomegaly in 3/4 mice: no splenomegaly at higher dilutions of virus.

Cellular adsorption of Rauscher antigen: Table 2 shows that the transplanted radiation-induced leukemia ERLD (C57BL/6) became sensitive to Rauscher antiserum after the cells had been incubated in the infective plasma of BALB/c mice with Rauscher disease. ERLD cells with adsorbed Rauscher antigen were sensitive also to antiserum against Friend virus leukemias, and in several tests the Moloney antiserum consistently showed a low level of cytotoxic activity. Antisera with Gross or ML specificity had no activity on the sensitized cells. The possibility that either the H-2 or the TL surface antigens are receptors for Rauscher antigen is not supported by the finding that adsorption of antigen does not influence sensitivity to H-2 or TL antisera (Table 2). Since the antigen is obtained from BALB/c mice, and may incorporate material from the membranes of infected cells, it is possible that the antigen carries some of the H-2d isoantigenic specificity of BALB/c normal cells. However, high-titer H-2d antiserum was not cytotoxic for ERLD cells with adsorbed Rauscher antigen.

Properties of Rauscher antigen: The property of sensitizing ERLD indicator cells to lysis by Rauscher antiserum is present in serum as well as in plasma. This activity of infective BALB/c plasma was markedly reduced by heating to 56°C for 30 min and was completely abolished in 30 min at 65°C. The Rauscher typing serum neutralized the sensitizing activity of undiluted infective BALB/c plasma to a titer of $^1/_{16}$ (equal volumes of diluted antiserum and infective plasma). Table 3 shows the infectivity and sensitizing activity of fractions obtained by centrifugation of infective BALB/c plasma. The antigen is not sedimented under conditions where all infective activity is confined to the pellet. Sedimentation of the antigen does occur on centrifugation for 16 hr at 105,000 × g.

TABLE 2

MODIFICATION IN ANTIGENIC PROPERTIES OF TRANSPLANTED C57BL/6 RADIATION-INDUCED LEUKEMIA ERLD AFTER EXPOSURE *in vitro* TO THE PLASMA OF BALB/c ♀ MICE INFECTED WITH RAUSCHER VIRUS

Cytotoxic antiserum	Test cell	Dilution of Antiserum: 1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192
		per Cent Dead ERLD Cells (Stained by Trypan Blue)												
C57BL/6 ♀ anti-C57BL/6 ♀ Rauscher 2 (Rauscher typing serum)[6]‡	ERLD-N*	<10	<10	<10	<10									
	ERLD-R†	>95	91	86	85	82	60	38	12	<10				
C57BL/6 ♀ anti-Friend virus leukemia (Friend typing serum)[2, 6]	ERLD-N	<10	<10	<10	<10									
	ERLD-R	>95	88	72	44	12	<10							
C57BL/6 ♂ anti-C57BL/6 ♀ Moloney 2 (Moloney typing serum)[6]	ERLD-N	<10	<10	<10	<10									
	ERLD-R	29	11	<10	<10									
C57BL/6 ♀ anti-AKR ♀ K36 (Gross typing serum)[17]	ERLD-N	<10	<10	<10	<10									
	ERLD-R	<10	<10	<10	<10									
C57BL/6 ♂ anti-DBA/2 ♂SL2 (ML typing serum)[18]	ERLD-N	<10	<10	<10	<10									
	ERLD-R	<10	<10	<10	<10									
C57BL/6 ♂ anti-ASL1 (A strain) (TL typing serum)[15, 19]	ERLD-N	>95	>95	>95	>95	>95	81	68	62	54	35	<10		
	ERLD-R	>95	>95	>95	>95	82	77	69	64	59	22	<10		
BALB/c ♀ anti-EL4 (C57BL) (H-2d anti H-2b)	ERLD-N	>95	>95	>95	>95	>95	>95	>95	>95	>95	82	55	38	<10
	ERLD-R	>95	>95	>95	>95	>95	>95	>95	>95	>95	79	60	26	<10
C57BL/6 ♀ anti-BALB/c ♀ Meth A (H-2b anti H-2d)	ERLD-N	<10	<10	<10	<10									
	ERLD-R	<10	<10	<10	<10									

* ERLD-N: ERLD cells incubated in normal BALB/c plasma, and twice washed (control).
† ERLD-R: ERLD cells incubated in the plasma of BALB/c infected with Rauscher virus, and twice washed.
‡ This serum neutralized Rauscher virus to a titer of 1/5 (0.1 ml equal vol of undiluted infective BALB/c plasma and antiserum dilution IP in BALB/c test mice): no neutralization with control antiserum *C57BL/6 anti-BALB/c ascites sarcoma Meth A*.

TABLE 3

SEPARATION OF INFECTIVE VIRUS AND SOLUBLE ANTIGEN BY CENTRIFUGATION OF PLASMA FROM BALB/c MICE INFECTED WITH RAUSCHER VIRUS*

Fraction used to sensitize ERLD cells	Period of centrifugation (hr)	Dilution of Rauscher Typing Serum: 1/2	1/4	1/8	1/16	1/32	1/64	Infectivity
		Per Cent Dead ERLD Cells (Stained by Trypan Blue)						
Plasma pool	0	86	>95	>95	>95	82	25	3/3†
Supernate	2	83	>95	>95	>95	68	18	0/3
Pellet	2	42	42	32	23	22	<10	3/3
Supernate	4	>95	>95	>95	76	47	<10	0/3
Pellet	4	69	76	54	25	<10	...	3/3
Supernate	16	24	32	<10	<10	<10	...	0/4
Pellet	16	89	93	>95	>95	68	38	4/4

* Centrifugation at 40,000 rpm in no. 40 rotor of Spinco model L ultracentrifuge (105,000 × *g*): pellet resuspended to original volume in normal BALB/c plasma. Identical fractions prepared by centrifugation of normal BALB/c plasma showed no sensitizing activity for ERLD cells.
† BALB/c ♀ mice with splenomegaly 32 days after inoculation of 0.1 ml undiluted material.

Capacity of cells other than ERLD to adsorb Rauscher antigen: Many transplanted leukemias were tested for the capacity to adsorb Rauscher antigen. Two further examples of leukemias that acquired marked sensitivity are shown in Table 4. Other leukemia cells, the cells of two ascites sarcomas, and all normal spleen cells of the strains examined, were not lysed by Rauscher antiserum after incubation in infective plasma. However, antigen was adsorbed by certain of these cells, since they became capable of absorbing cytotoxic activity from Rauscher antiserum, after exposure to infective plasma. According to this criterion, spleen cells of strains C57BL/6, DBA/2, and 129 adsorbed antigen, whereas spleen cells of strains BALB/-c, C3H/An, AKR, I, and C57L did not.

TABLE 4

ACQUISITION OF SENSITIVITY TO CYTOTOXIC RAUSCHER TYPING SERUM BY VARIOUS CELLS EXPOSED TO INFECTIVE PLASMA

Test cells (previously incubated with plasma of BALB/c mice infected with Rauscher virus*)	Dilution of Rauscher Typing Serum: 1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
	Per Cent Dead Cells (Stained by Trypan Blue)							
Leukemia cells:								
ERLD (C57BL/6)	91	94	96	88	83	47	33	11
EL4 (C57BL)	81	82	78	75	62	30	23	<10
BALB/c ♂ Gross 2	91	94	89	93	89	44	33	<10
Individual tests of C57BL/6 leukemias E♀SL2, E♀RL3, E♂RL4, and E♂RL9	<10	<10	<10	<10				
Sarcoma cells:								
Meth A (BALB/c)	<10	11	12	<10	<10			
BP8 (C3H)	<10	<10	<10	<10				
Normal spleen cells: Individual tests of strains C57BL/6, BALB/c, DBA/2, 129, AKR, C3H/An, and I	<10	<10	<10	<10				

* Every test included controls in which the test cells were incubated in normal BALB/c plasma; these were invariably negative in cytotoxic tests with Rauscher typing serum.

Relation of the soluble antigen to the cellular antigen of Rauscher leukemias: Absorption of Rauscher antiserum with ERLD cells sensitized by adsorbed Rauscher antigen removed all cytotoxic activity both for antigen-sensitized ERLD cells and for leukemias induced by Rauscher virus. This indicates that the specificity of the soluble antigen is the same as that of the antigen detected on Rauscher leukemia cells by the cytotoxic test.

TABLE 5

ACQUISITION OF SENSITIVITY TO CYTOTOXIC RAUSCHER TYPING SERUM BY ERLD CELLS AFTER EXPOSURE TO THE PLASMA OF MICE BEARING VARIOUS PRIMARY OR TRANSPLANTED LEUKEMIAS

ERLD incubated with plasma of mouse with leukemia:	Transplant generation	Dilution of Rauscher Antiserum: 1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
		Per Cent Dead ERLD Cells (Stained by Trypan Blue)							
C57BL/6 ♀ Rauscher 2*	28	>95	>95	>95	93	77	62	18	<10
EL4 (C57BL/6)	>100	<10	<10	<10	<10				
R/EL4*	19†	94	>95	>95	>95	55	22	<10	<10
C3Hf/Bi ♂ Gross 1	3	<10	<10	<10	<10				
BALB/c ♂ Moloney	Primary	81	38	<10	<10				
BALB/c ♂ Friend	"	>95	>95	94	87	80	32	14	<10
C3Hf/Bi ♂ Gross	"	<10	<10	<10	<10				
C57BL/6 ♀ Gross	"	<10	<10	<10	<10				
None (normal plasma from C57BL/6, BALB/c or C3Hf/Bi)		<10	<10	<10	<10				

* The plasma of the C57BL/6 mice bearing these transplanted leukemias contains infective Rauscher virus as well as sensitizing antigen.
† 19th transplant generation in C57BL/6 of a line of EL4 after antigenic conversion by Rauscher virus.[9]

Occurrence of antigen in the plasma of mice with various primary and transplanted leukemias: An antigen which renders ERLD cells sensitive to lysis by Rauscher antiserum is present in the plasma of mice with primary leukemia induced by Friend or Moloney viruses. Antigen is present also in the plasma of C57BL/6 mice with transplanted Rauscher leukemias, and in the plasma of C57BL/6 mice with a leukemia line (R/EL4) that has been purposely infected with Rauscher virus.[9] The plasma of mice with either primary or transplanted Gross leukemias contained no antigen reactive with Rauscher antiserum. Table 5 shows the results of these tests.

Reactions of Friend and Moloney typing sera with ERLD cells sensitized by the homologous antigen: ERLD cells incubated in the plasma of mice with Moloney leukemia were sensitized to lysis by the Moloney typing serum, as well as to lysis by Rauscher antiserum. Similarly, both Friend and Rauscher antisera were cytotoxic for ERLD cells incubated in the plasma of mice infected with Friend virus. The antiserum with Gross specificity was not active against ERLD cells that had been incubated in the plasma of mice with leukemias induced by Gross, Friend, Moloney, or Rauscher viruses.

Discussion.—There are marked differences in the responses of various inbred mouse strains to infection with Rauscher leukemia virus. Susceptible BALB/c hosts develop the characteristic early disease in which massive splenomegaly is a prominent feature; lymphatic leukemia may occur in late survivors.[14] Adult C57BL mice are highly resistant to leukemogenesis by Rauscher leukemia virus, although lymphatic leukemias may be induced by the inoculation of newborn recipients.[14] In contrast to the sustained viremia that occurs in BALB/c mice,[21] C57BL mice respond to infection by forming antibody with specific cytotoxic activity against Rauscher leukemia cells. Both the production of disease in susceptible strains and the production of cytotoxic antibody in resistant strains can be used to titrate infective virus. These two methods of titration are of approximately equal sensitivity.

The plasma of infected BALB/c mice contains an antigen, separable from infective virus, that has the specificity of Rauscher leukemia cells. The antigen is

present also in the plasma of mice bearing either transplanted Rauscher leukemias or leukemia lines that have undergone antigenic conversion as a result of infection with Rauscher leukemia virus. The antigen is demonstrable by its property of adsorption to certain leukemia cells, which then become sensitive to lysis by Rauscher antiserum. This component of infective plasma qualifies as a soluble antigen since it is not deposited by centrifugation at 105,000 $\times$ g for two hr, a procedure that removes infective virus. The plasma of mice infected with Friend virus, and of mice with primary or transplanted leukemias induced by Moloney virus, contain antigens which similarly sensitize the indicator cells to lysis by the relevant specific typing sera. The observation that indicator cells sensitized by adsorbed Friend or Moloney antigen are lysed by Rauscher antiserum provides further evidence that the leukemias induced by these three viruses are immunologically related (FMR system[6]). No soluble antigen associated with either primary or transplanted Gross leukemias has yet been demonstrated, nor does the plasma of animals with Gross leukemia sensitize indicator cells to lysis by FMR antisera. This accords with other evidence that Gross leukemias are antigenically distinct from the FMR group.[2, 3, 5, 6, 8] Possibly future technical modifications may permit the demonstration of soluble antigens associated with Gross leukemias.

It seems likely that the soluble antigen described in this report is a product or subunit of the leukemogenic virus, in line with the occurrence of soluble viral antigens in the course of infection with viruses of other types. Huebner and his colleagues have demonstrated soluble complement-fixing antigens in extracts of tumors induced by various oncogenic viruses, and in cultured cells infected by these viruses *in vitro*.[22] They have obtained considerable evidence that these antigens are determined by the genome of the virus, rather than by the genome of the infected cell. Recently, soluble complement-fixing antigen has been found in cultures of chick embryo cells infected with viruses of the avian leukosis group.[23]

The cytotoxic activity of Rauscher antisera against Rauscher leukemia cells is completely removed by absorption with indicator cells coated with soluble antigen. Since the soluble antigen is probably a component of the virus and carries the specificity of the Rauscher leukemia cell, it seems that both the antigenicity of the leukemia cells and their sensitivity to cytotoxic antibody can be adequately explained by the incorporation of viral antigen into the cell membrane. Thus, on evidence obtained with the cytotoxic test it appears unnecessary to postulate the occurrence of cellular antigens other than those directly determined by the virus. The finding that rat leukemias induced by Moloney virus absorb all specific cytotoxic activity from sera prepared against mouse Moloney leukemias[24] may be taken to support this view. It is unlikely that the antigen would be the same in two species if it were not induced by the viral genome.[22]

Among the various cell types examined, the capacity to adsorb soluble antigen and thus become sensitive to lysis by specific antisera and complement has so far been restricted to certain transplanted leukemias.[25] Other cells may adsorb antigen without becoming sensitive to lysis by specific antisera; in this case the presence of antigen can be demonstrated by absorption of Rauscher antibody. The normal spleen cells of certain mouse strains adsorb antigen whereas those of other strains do not, suggesting that specific receptor sites are concerned in the process of adsorption. However, there is no obvious relationship between this property

and the susceptibility of various inbred strains to the development of Rauscher disease.

Summary.—The plasma of mice infected with Rauscher virus contains, in addition to infective virus, a soluble antigen that has the same specificity as the cellular antigen of leukemias induced by Rauscher virus. Virus can be titrated in C57BL mice by measuring the production of cytotoxic antibody in response to the inoculation of serially diluted infective plasma. This method is as sensitive as the production of Rauscher disease in susceptible BALB/c mice. The soluble antigen is demonstrated by its capacity to adsorb onto certain cells and thus render them susceptible to lysis by specific cytotoxic Rauscher antiserum. An antigen with the same specificity appears in the plasma of mice with transplanted Rauscher leukemias of lymphatic type. The plasma of mice with primary leukemias induced by Moloney or Friend viruses similarly sensitizes indicator cells to the cytotoxic activity of the homologous typing sera, and also to Rauscher antiserum. These cross reactions provide further evidence that leukemias induced by the Friend, Moloney, and Rauscher viruses are immunologically related (FMR system). Soluble antigen has not yet been found in the plasma of mice with leukemias induced by Gross virus.

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*UNIQUENESS OF BACTERIAL RIBOSOMES**

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Ribosomes can be identified during the ultracentrifugation of cell extracts with boundaries having sedimentation coefficients from 20 to 100 Svedberg units (S). The 70–80S class, which is required for protein synthesis, predominates at Mg^{++} concentrations between 0.01 *M* and 0.001 *M*.

Many S determinations have been made on this class of ribonucleoprotein particles from several species of microorganisms, plants, and animals. The reported values do not always agree, even for the same species, often because the physicochemical conditions differed. Since sedimentation velocities vary with concentration, viscosity, and temperature, corrections must be applied in order to obtain values which can be compared. Although only a few such values are available, it is generally assumed that bacterial ribosomes belong to the 70S class whereas all others belong to the 80S class.

In the present work the corrected S values of ribosomes from 25 species of bacteria and 26 of fungi have been determined. Statistical evidence for the existence of two distinct classes has been obtained. It is suggested that the uniqueness of the sedimentation coefficient of the ribosomes from bacteria should be used to define this group of organisms.

Materials and Methods.—Selection of organisms: Mycelial and yeast forms belonging to the following classes were analyzed: Zygomycetes, Ascomycetes, Deuteromycetes, and Phycomycetes. Bacteria were selected so as to cover a range of 34–80% guanine plus cytosine (GC) content of their DNA. Within each group, the organisms were chosen on the basis of their availability in pure form, their ability to grow rapidly in a liquid medium, and their nonpathogenicity. All organisms were grown under physicochemical conditions eliciting a maximum rate of growth and harvested during the growth phase.

Preparation of extracts: Cells and filaments were washed with and resuspended in Tris buffer (10^{-2} *M*), pH 7.4, containing 10 μmoles per ml of $MgCl_2$ (Tris-Mg). All yeasts and a few molds were disrupted with glass beads in a Nossal disintegrator. Most molds were ground with washed, 50-mesh sea sand and bacteria with levigated alumina. The pH was readjusted to 7.4, and beads, sand, or alumina were removed by centrifugation. The extract was clarified by two centrifugations at 25,000 × *g* and 4°C, and the supernatant was used for ultracentrifugal analysis.

For blue-green algae, it was necessary to eliminate the pigments from this supernatant. It was therefore centrifuged through a 3–20% linear gradient of sucrose in Tris-Mg, at 25,000 rpm for 255 min in the SW25 rotor of the Spinco model L preparative ultracentrifuge. One-ml fractions were collected after piercing the bottom of the tube. The optical density at 260 mμ (OD_{260}) of each fraction was measured, and those fractions corresponding to the ribosomal peak were pooled and washed by two centrifugations at 40,000 rpm for 120 min. The ribosomal pellets were resuspended in Tris-Mg.