2. Petersen, O. H. and Poulsen, J. H. Experientia **22**, 821 (1966).

3. Fritz, M. E. and Botelho, S. Y., Program and Abstr. 46th Gen. Meeting Intern. Assoc. Dental Res., **1963**, 63.

4. Yoshimura, H. and Imai, Y., Japan. J. Physiol. **17**, 280 (1967).

5. Martinez, J. R., Holzgreve, H., and Frick, A., Arch. Ges. Physiol. **290**, 124 (1966).

6. Young, J. A. and Schögel, E., Arch. Ges. Physiol. **291**, 85 (1966).

7. Mangos, J. A., Braun, G., and Hamann, K. F., Arch. Ges. Physiol. **291**, 99 (1966).

8. Ichioka, M., Shimizu, H., and Shimizu, T., Z. Biol. **115**, 1 (1965).

9. Schneyer, L. H. and Schneyer, C. A., Am. J. Physiol. **209**, 1304 (1965).

10. Yoshida, Y., Sprecher, R. L., Schneyer, C. A., and Schneyer, L. H., Proc. Soc. Exptl. Biol. Med. **126**, 912 (1967).

11. Schneyer, L. H., Schneyer, C. A., and Yoshida, Y., Am. J. Physiol. **215**, 1146 (1968).

12. Strömblad, B. C. R. and Dresel, P. E., Can. J. Biochem. Physiol. **41**, 519 (1963).

13. Hisada, M. and Bothelho, S. Y., Am. J. Physiol. **214**, 1262 (1968).

14. Petersen, O. H., Poulsen, J. H., and Thorn, N. A., Acta Physiol. Scand. **71**, 203 (1967).

Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

Experimental Infection of Rhesus with Simian Virus 40 (SV40)* (33520)

K. V. Shah, S. Willard, R. E. Myers, D. M. Hess, and R. DiGiacomo
(Introduced by F. Bang)

Department of Pathobiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205; and Laboratory of Perinatal Physiology, National Institute of Neurological Diseases and Blindness, National Institutes of Health, USPHS, San Juan, Puerto Rico

Although simian virus 40 (SV40) is a common infection of rhesus (1) and a frequent contaminant of rhesus kidney cultures (2), little is known of the course of SV40 infection in rhesus and of the manner in which virus disseminates from an infected animals.

Meyer *et al.* (3) and Ashkenazi and Melnick (4) reported on some aspects of SV40 infection in African green monkeys. We studied experimental infection of juvenile nonimmune rhesus with SV40 by three routes of inoculation, and reported earlier the viremia and T antibody response in these animals (5). In the present communication, the remaining features of this infection and attempts to infect rhesus *in utero* are described.

Materials and Methods. All rhesus in this study were obtained from the free-ranging groups on Cayo Santiago, an islet off the east coast of Puerto Rico. This island is a part of the facilities of the Laboratory of Perinatal Physiology. These rhesus groups are free of active SV40 infection (6).

Strain A2895 of SV40, isolated originally from a hamster tumor produced by inoculation of rhesus kidney extracts (7), was obtained from Dr. B. Eddy. Prior to use in this study the virus had undergone at various times one additional passage in hamster tumor, one passage in HeLa cells, and 10 passages in *Cercopithecus* kidney cells, either primary cultures or continuous cell line BS-C-1.

Sixteen juvenile rhesus, 1–2 years old, were infected, six each by intranasal (i.n.) and subcutaneous (s.c.) routes and four by intragastric (i.g.) route. The virus dose for each animal in i.n. and s.c. groups was $10^{7.3}$ TCD_{50} contained in 0.4 ml of a $10^{-1.0}$ dilution of the virus. For i.g. inoculation, 1 ml of undiluted virus containing $10^{8.7}$ TCD_{50} was introduced into the stomach of each animal

* Supported by Grant E440 from American Cancer Society, Grant 5R07TW00141-07CIC from USPHS to The Johns Hopkins Center for Medical Research and Training, and by contract No. 43-63-25 with the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

by a stomach tube; the gastric acidity of two rhesus (nos. 13 and 14) was neutralized by 40 ml of 6%$NaHCO_3$ prior to virus administration.

The animals were caged individually in metabolic cages, and bled periodically for tests for viremia and antibodies. Throat and rectal swabs were expressed each in 1 ml of medium (199 with 2% inactivated fetal calf serum and 5000 units each of penicillin and streptomycin). Urine samples were collected either from urine naturally voided on a sheet of aluminum foil or by bladder puncture.

Specimens for virus isolation were stored at the laboratory at San Juan at $-10°$ for periods varying from a few days to 6 weeks, after which they were transported to Baltimore on dry ice and stored at $-70°$. Urine, throat swab and rectal swab specimens were prepared for virus isolation by addition of an equal volume of medium containing 25,000 units each of penicillin and streptomycin, followed by 1-hr centrifugation at 10,000 rpm in a refrigerated centrifuge.

Renal biopsies were taken at 29–31-weeks post inoculation. One kidney was exposed under intravenous Nembutal anaesthesia and a small piece of the renal cortex was removed. Kidney cells were grown as explants in 30-ml Falcon plastic flasks and as tryspinized cells in roller tubes, Leighton tubes, and Falcon plastic flasks. The growth medium was 199 with 10% inactivated fetal calf serum and antibiotics. Most of the cultured cells formed complete monolayers. They were harvested 21 days after planting by freezing and thawing the cell sheet and the medium three times. The minimum material tested from each animal consisted of cells from one flask and a pool of harvests from three or four roller tubes; these were tested separately for presence of SV40. The Leighton tube coverslips were examined for the presence of T and V antigens by indirect immunofluoresence tests (8).

Virus isolations and titrations were performed by inoculation of primary AGMK cells using two to four tubes per dilution. Each tube was inoculated with 0.2 ml. Cultures were observed for SV40 cytopathic effects for 21–28 days and all isolates were identified by neutralization tests. Neutralization tests were performed as described before (9). Complement fixation (CF) tests were carried out in microtiter plates using two units of complement and infectious virus as antigen. The T antibodies were identified in indirect immunofluorescence test with SV40 transformed hamster cells as described before (5).

In utero *infection.* Six pregnant female rhesus were obtained from the Cayo Santiago population. Their pregnancies were undated; therefore, the age of the fetus was estimated by vaginal and rectal palpation. The estimate of duration of pregnancy for the six fetuses was between 90 and 120 days.

The uterus was surgically exposed using full sterile precautions with the mother under barbiturate anesthesia. The fetal head was identified by palpation through the relaxed, thin uterine wall. Through a small scalpel incision in the uterine wall, away from the placental edges, the fetal head and upper portion of the fetal body were gently delivered. With the fetal head held firmly between thumb and forefinger, 0.1 ml of undiluted virus ($10^{7.0}$ TCD_{50}) was injected through a 25-gauge needle inserted approximately 1 cm into the cerebral substance through the soft calvarium. A further 1.0 ml of undiluted virus ($10^{8.0}$ TCD_{50}) was injected subcutaneously in the interscapular region of the fetus. Three of the six fetuses were also given the immunosuppressive drug, cyclophosphamide (Cytoxan), intramuscularly at a dose of 90 mg/kg of weight. Fetal weight in determining dose was estimated in relation to presumed fetal age. Mothers were bled 35 days after inoculation of fetuses for antibody tests.

Results. Infection of juvenile rhesus. All but one rhesus survived the observation period of 29–31 weeks without any illness. Visible tumors were not detected in any. The single fatality was an animal in the s.c. group which died 26 weeks after inoculation after a diarrheal disease; autopsy revealed no gross tumors.

Viremia. The patterns of viremia were described earlier (5) and are summarized in Table I. Viremia was demonstrated in 14 of

TABLE I. Viremia Patterns in Rhesus Inoculated with SV40 by Three Different Routes.

Group	Dose of virus (log TCD_{50})	No. in group	No. viremic	Viremia on days postinoculation	Peak titers/ml
i.n.	7.3	6	4	5, 6, 7, 8	$10^{1.2}$ to $10^{3.2}$
s.c.	7.3	6	6	1, 2, 3	$10^{1.7}$ to $10^{3.7}$
i.g.	8.7	4	4	5, 6[a]	$10^{1.4}$ to $10^{3.4}$

[a] Only 2 days when tests were made.

16 inoculated animals; it occurred earlier and reached higher titers in the s.c. group as compared to the i.n. group.

Virus in throat and rectal swabs. In tests of 98 throat swabs and an equal number of rectal swabs collected in the first 3 weeks after inoculation, virus was not recovered from any. The numbers and time of collection of throat and rectal swabs are given in Table II.

TABLE II. Number of Throat Swabs (or Rectal Swabs) Collected from Inoculated Groups and Tested for SV40 Virus.

Group	No. in group	No. of throat swabs (or rectal swabs) on days postinoculation				
		1–3	5–8	11–15	18–21	Total
i.n.	6	9	12	15	9	45
s.c.	6	9	12	15	9	45
i.g.	4		8			8
	Total	18	32	30	18	98

Viruria. Viruria was detected in three of six rhesus of the s.c. group but in none of six of the i.n. group (Table III). The few tests done for viruria in the i.g. group were negative.

The earliest viruria was demonstrable on day 13 after inoculation and the latest on day 60. All of the three rhesus which were viruric at any time, had virus in urine in the third week. The duration of viruria was variable. Animal 12 was viruric only between days 13 and 21 during which time all four urine specimens yielded virus. Animal 11 was viruric in the third and sixth week and animal 8 in the third and ninth week after inoculation. In the last two, viruria was detected intermittently. Virus titers in positive specimens ranged between $10^{1.5}$ TCD_{50}/ml and $10^{4.0}$ TCD_{50}/ml.

Virus and SV40 antigen in kidney cell cultures. Cells were grown from renal biopsies obtained 29–31 weeks postinoculation from 15 of 16 inoculated animals. Results of virus isolation are given in Table III. SV40 was recovered from cell cultures from a single animal, that of animal 12. Only one of three harvests from renal cell cultures of this animal was positive. This rhesus was viruric in the second and third week and not thereafter. Of the other two rhesus which were viruric at some time, no biopsy could be obtained from Animal 11 which had died earlier, and three harvests from cell cultures of animal 8 were negative.

Coverslip preparations of cell sheets grown from trypsinized renal cells of Animal 12 and 10 other rhesus were examined for SV40 V and T antigens in indirect FA tests; none were positive.

Virus neutralizing antibodies in serum. These appeared in all of 16 inoculated animals by 21 days and persisted through the observation period of 29–31 weeks. They were detected earliest in animals of the s.c. group, by day 5 in one animal, by day 8 in three, and in all six by day 12 (Table IV). This group was viremic on the first 3 postinoculation days. In the i.n. group, which was viremic between 5 and 8 postinoculation days, the time of first appearance of neutralizing antibodies was between 11 and 19 days. At 6 weeks, all rhesus in the s.c. group had serum antibody titers of 1:1024 or greater; titers in individuals of the i.n. group were lower, ranging between 1:128 and 1:1024. In the i.g. group, antibody titers at 4 weeks were between 1:256 and 1:1024. At 29–31 weeks, a majority of the animals maintained

TABLE III. Frequency of Viruria in Rhesus Experimentally Infected with SV40 Virus and Presence of Virus in Kidney Cell Cultures.

Group	No.	Titers[a] of viruria (weeks postinoculation)									Virus in kidney culture (29–31 weeks postinoculation)
		1	2	3	4	6	7	9	10–16	29–31	
i.n.	1		NN	NN	N	N	N	N	NN		N
	2	N	NN	N	N	N		N	NN	N	N
	3	NN	N	NN	N	N	N	N	N	N	N
	4	NN	N	NN	N	N	N	N	N	N	N
	5	NN	N	NN	N	N	N	N	NN	N	N
	6	N		NN	N	N	N	N	NN	N	N
s.c.	7	NN	N	NN	N	N	N	N	N	N	N
	8		NN	N2.5	N	N	N	1.5	N	N	N
	9		NN	NN	N	N		N	NN	N	N
	10	N		NNN	N	N	N	N	N	N	N
	11		NN	4.0, 3.5	N	3.5	N	N	NN	(Died at week 26)	
	12		3.0	3.5, 4.0, 3.5	N	N	N	N	N	N	+
i.g.	13							N	N	N	N
	14								N	N	N
	15									N	N
	16							N	N	N	N

[a] N indicates no virus isolated at 1:2 dilution of urine; values are positive logarithms of virus titer/ml.

the high titers of earlier bleedings, while in others there was a two- to four-fold reduction in antibody levels.

Virus neutralizing antibodies in urine. Ashkenazi and Melnick (4) have reported on the presence of neutralizing antibodies in urine of infected monkeys. In tests of 1:2 dilution of urine, neutralizing activity was not demonstrable in any of the first urine specimens of the 12 rhesus in i.n. and s.c. groups. Ten of these specimens were collected between 0 and 7 days postinoculation and the other two on days 8 and 12. Low level neutralization, as indicated by a delay in cytopathic effect of two to four days, was shown of 9 of 15 urines collected by bladder puncture at 29–31 weeks. These specimens were distributed as follows: 4 of 6 in i.n. group, 3 of 5 in s.c. group, and 2 of 4 in the i.g. group. The presence of neutralizing antibodies in the urine was independent of the serum neutralizing antibody titer.

Neutralizing activity did not appear in the urines until the nineth week; 33 specimens collected between 3 and 7 weeks from rhesus of i.n. and s.c. groups were all negative. Once neutralizing activity was evident in the urine of an animal, subsequent specimens until the end of the observation period also showed neutralization. Neutralizing activity appeared in the urine of both previously viruric animals that were examined as well as in urines from five of nine in i.n. and s.c. groups which were not demonstrated to be viruric.

Viral CF antibodies. The CF antibodies were first demonstrable on days 14 and 15 when three of six rhesus of the s.c. group but none of six in the i.n. group had antibodies; titers of positive sera were 1:64, 1:64, and 1:8. By 21 days, all inoculated animals had antibodies, with titers ranging from 1:8 to 1:128 (Table V). In subsequent bleedings at 13–16 and 29–31 weeks, there was a progressive fall in the mean geometric titers in all three groups. Antibody titers were somewhat higher for the i.g. group. At 29–31 weeks 5 of 15 inoculated animals had CF antibody titers of 1:4 or less.

TABLE IV. Development and Titers of Neutralizing Antibodies to SV40 Virus in Sera of Experimentally Infected Rhesus.

		Neutralizing antibody titers[a]																						
		(days postinoculation)																				(weeks postinoculation)			
Group	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	4	6	9–16	29–31
i.n.	1			—[b]			V[c]		—				+		+				(+)[d]			+		>1024	+	1024
	2			—			V		V				+		+				(+)			+		1024	+	256
	3			—			—		—				±		+				(+)			+		256	+	1024
	4		—			V		V				+		+		+				+		+		128	+	32
	5		—			—						—		—		—				+		+		128	+	64
	6		—			V		V				—		+		+				(+)		+		512	+	1024
s.c.	7	V		V			±						(+)		+				+			+		1024	+	>1024
	8	V		V					±				(+)		+							+		>1024	+	128
	9	V					±		+				(+)		+							+		>1024	+	1024
	10		V			—		—				(+)		+		+				+		+		>1024	+	1024
	11		V			+		+				(+)		+		+				+		+		>1024	>1024	
	12		V			—		+				+		(+)		+				+		+		>1024	+	128
i.g.	13					V	V															(+)	1024		+	512
	14					V	V															(+)	256		+	>1024
	15					V	V															(+)	>1024		+	>1024
	16					V	V															(+)	1024		+	

[a] Values represent reciprocals of neutralizing antibody titers.

[b] + = neutralization; and — = no neutralization by 1:5 dilution of serum.

[c] V denotes viremia.

[d] Parentheses indicates day of first detection of T antibodies in the individual animal.

TABLE V. Summary of CF Antibody Response to SV40 Virus in the Three Inoculated Groups.

Group		Time (weeks) after infection		
		3	13–16	29–31
i.n.	Prop. with antibodies	6/6	6/6	5/6
	Mean geometric titer	1:39	1:14	1:9
	Range of titer	1:8 to 1:128	1:8 to 1:64	<1:4 to 1:32
s.c.	Prop. with antibodies	6/6	5/6	3/5
	Mean geometric titer	1:45	1:14	1:5
	Range of titer	1:4 to 1:128	<1:4 to 1:64	<1:4 to 1:32
i.g.	Prop. with antibodies	4/4	4/4	4/4
	Mean geometric titer	1:68	1:38	1:22
	Range of titer	1:64 to 1:128	1:16 to 1:64	1:16 to 1:32

"Tumor" (T) antibodies. The details of T antibody response as measured by indirect FA tests with SV40 transformed hamster cells were described earlier (5). The time of first detection of T antibodies in individual animals is indicated in Table IV by parentheses. The T antibodies developed in all animals of the s.c. and i.g. groups and in four of six of the i.n. group. In the i.n. group, they were first detected on days 18 and 19, 4–6 days after the appearance of neutralizing antibodies. In the s.c. group, they were demonstrable 11 to 13 days after inoculation; neutralizing antibodies appeared on the same day as T antibodies in three rhesus, and in the other three, 4–6 days earlier.

In utero *infection.* Of the six fetuses inoculated, two died *in utero,* and one was stillborn. Two fetuses were delivered by Caesarian section 53 days after inoculation at what was thought to be full term, but they were premature and survived only a few hours. Neither of these two had received cyclophosphamide. They were without visible tumors in the subcutaneous tissue or in brain or other internal organs. Their cord bloods in dilution of 1:10 were negative for virus; their cord sera had titers of 1:512 and 1:1024 for virus neutralizing and 1:16 and 1:32 for T antibodies. These titers of neutralizing and T antibodies were comparable to those in sera of all mothers 35 days after fetal inoculation.

The sixth fetus was born naturally by vaginal delivery 74 days after inoculation. As the average duration of pregnancy in rhesus is 165 days, the age of this fetus at time of inoculation was estimated to be approximately 90 days. It had received, in addition to $10^{7.0}$ TCD_{50} of virus intracerebrally and $10^{8.0}$ of virus subcutaneously, 15 mg of cyclophophamide intramuscularly.

The newborn was separated from the

TABLE VI. Follow-up of Rhesus Infant Born 74 Days after *in Utero* Inoculation with SV40.

Item	Age of infant (days)				
Days after inoculation:	2 76	10 84	30 104	57 131	79 153
Viremia[a]	N	N			
Viruria[a]		N[b]	N	N	N
Neutralizing antibody, urine[c]			—	—	+
Neutralizing antibody, serum		1:1024			1:1024
T antibody		1:32			1:64

[a] N indicates negative at dilution of 1:5.

[b] Negative for SV40, adenovirus isolated.

[c] — = no neutralization; and + = neutralization by 1:2 dilution of urine.

mother immediately after birth and was reared separately with artificial feeding. Results of tests on this infant are given in Table VI. SV40 was not isolated in tests of a 1:10 dilution of blood on day 2 and of a 1:5 dilution of serum on day 10 after birth. Viruria was not detected in tests of four urine specimens collected between days 10 and 79 of the infant's life. Serum of day 10 had a neutralizing titer of 1:1024 and T antibody titer of 1:32; comparable titers for both antibodies were also found in serum of day 79. Neutralizing activity was first demonstrable in the urine specimen of day 79. The infant has remained free of signs of brain tumor or of subcutaneous tumor in the first 4 months of life.

Discussion. Juvenile rhesus were readily infected by a single exposure to SV40 by each of three routes of inoculation. The course of infection after s.c .inoculation differed from that after i.n. inoculation in that (a) viremia occurred earlier, in all animals, and in higher titers (b) viruria was demonstrable and (c) neutralizing, viral CF, and T antibodies appeared earlier and reached higher titers. In the more limited study after i.g. inoculation, viremia in all animals and antibody response were consistently demonstrated but urine specimens were collected too late to provide an adequate test for the occurrence of viruria. The ease with which infection was established in rhesus after i.g. inoculation constrasts sharply with the reported lack of antibody response in man after oral administration of SV40 (1, 10, 11).

Meyer *et al.* (3) have described experimental infection of two African green monkeys after i.n. inoculation of $10^{7.0}$ TCD_{50} of SV40. In this species, viremia was demonstrable for a period of 15 days, beginning on the second day after inoculation. In rhesus inoculated i.n. with a comparable dose of virus, the duration of viremia was only 4 days, beginning on day 5 after inoculation. Further, Meyer *et al.*, recovered small quantities of virus from the pharynx during the second week and less frequently from stool during the first week while we were not able to isolate virus from throat and rectal swabs of infected animals. It is possible that with our observation of inoculated tubes for no longer than 3–4 weeks, and storage of specimens at —10° for varying periods of time, minimal quantities of virus would have gone undetected.

Ashkenazi and Melnick (4) studied viruria and latent kidney infection in 10 African green monkeys after direct introduction of the virus into one or more of the following sites: kidney, brain, skin, subcutaneous tissue, and lung. They detected, in all 10, viruria at some time in the first 2 months and, in 4 of 8 monkeys, virus in kidney biopsy cultures as long as 6–8 months after inoculation. In rhesus, viruria was detected in only one half of those infected by the s.c. route (a group somewhat comparable to the above green monkeys) and in none inoculated intranasally, and at 29–31 weeks, virus was recovered from kidney biopsy cultures of a single animal. It is very likely that the frequency of virus recovery from kidneys would have been greater, had the biopsies been taken earlier or if whole kidneys were processed (3, 4). In rhesus, virus neutralizing activity in urine appeared at or after 9 weeks and was not confined to previously viruric animals. It is possible that these differences between the course of SV40 infection in rhesus and in African green monkeys, viz., longer viremia and higher frequency of viruria in African green monkeys, represent the higher virulence of SV40 for a host to which it is not adapted. On the other hand, these differences may represent variation among the virus strains tested.

The results of this investigation indicate that nonimmune rhesus can become infected by the oral or the respiratory route. However, the study did not identify the different possible modes of virus dissemination from an infected rhesus or clarify their relative importance.

The three fetuses, born alive after *in utero* injection of virus at about 90 days of gestation, did not display tumors at birth. The persistence of high antibody titers in the one surviving infant would suggest that the high levels of antibodies found in cord sera and in serum soon after birth were the result of an immunological response on the part of the fetus rather than of a passive antibody trans-

fer from the mother. Cyclophosphamide, given at the time of virus inoculation, and at the dose administered, did not prevent the immunological response.

Summary. Nonimmune rhesus were readily infected by administration of SV40 by intranasal (i.n.), subcutaneous (s.c.), or intragastric (i.g.) route. Viremia, demonstrable in animals of all three groups, occurred earlier and with higher titers in the s.c. group. Neutralizing and viral CF antibodies appeared in all inoculated rhesus and T antibodies in 14 of 16. Viruria was detected in three of six of the s.c. group, most commonly in the third week after inoculation. Virus neutralizing activity occurred in the urine at or after 9 weeks postinoculation and was not confined to previously viruric animals. Virus was isolated from kidney of one rhesus from biopsy cultures performed 29–31 weeks after inoculation. Three rhesus fetuses which were given SV40 intracerebrally and subcutaneously at about 90 days of gestation had, at or soon after birth, high titers of antibodies and no tumors. In the single surviving infant, antibody titers did not fall in the first 11 weeks of life.

1. Sweet, B. H. and Hilleman, M. R., Proc. Soc. Exptl. Biol. Med. **105**, 420 (1960).

2. Hsiung, G. D. and Atoynatan, T., Am. J. Epidemiol. **83**, 38 (1966).

3. Meyer, H., Hopps, H. E., Rogers, N. G., Brooks, B. E., Bernheim, B. C., Jones, W. P., Nisalak, A., and Douglas, R. D., J. Immunol. **88**, 796 (1962).

4. Ashkenazi, A. and Melnick, J. L., Proc. Soc. Exptl. Biol. Med. **111**, 367 (1962).

5. Shah, K. V. and Hess, D. M., Proc. Soc. Exptl. Biol. Med. **128**, 480 (1968).

6. Shah, K. V. and Morrison, J. A., Am. J. Epidemiol., March, 1969.

7. Eddy, B., Progr. Exptl. Tumor Res., **4**, 1 (1964).

8. Pope, J. H. and Rowe, W. P., J. Exptl. Med. **120**, 121 (1964).

9. Shah, K. V., Proc. Soc. Exptl. Biol. Med. **121**, 303 (1966).

10. Melnick, J. L. and Stinebaugh, S., Proc. Soc. Exptl. Biol. Med. **109**, 965 (1962).

11. Horvath, B. L. and Fornosi, F., Acta Microbiol. **11**, 271 (1964–65).

Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.