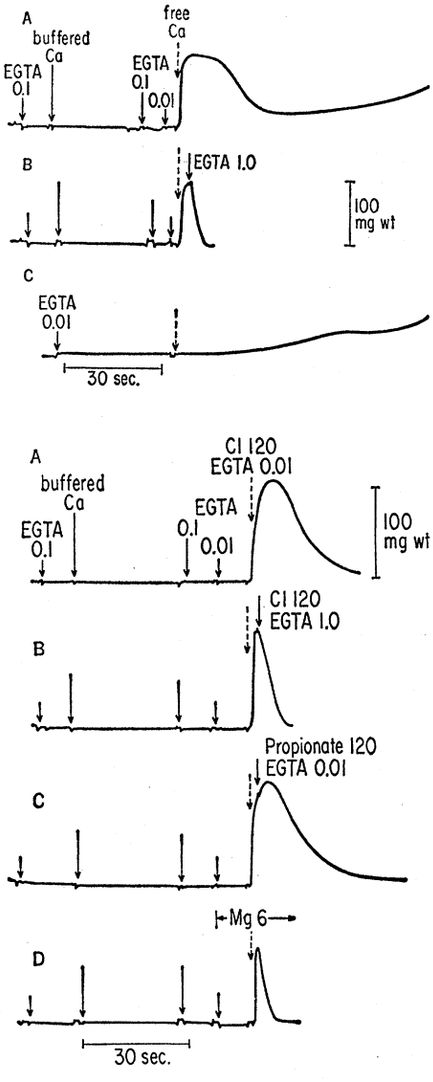

Fig. 1 (top left). Calcium-induced contractions of skinned muscle fibers. Arrows mark immersion in solutions containing buffered calcium, free calcium, or EGTA in the millimolar concentrations specified. (A) Quick contraction elicited by free calcium after loading. (B) Interruption of quick contraction by high concentration of EGTA. (C) Absence of quick contraction in unloaded fiber.

Fig. 2 (bottom left). Chloride-induced contractions of skinned muscle fibers. Arrows indicate solution changes; Cl 120 solutions contained 120 m*M* chloride in place of propionate, and Mg 6 solutions contained 6 m*M* $MgCl_2$ in place of 1 m*M* $MgCl_2$. All fibers were loaded with calcium before exposure to chloride. (A) Quick contraction elicited by chloride. (B) Interruption of contraction by high concentration of EGTA. (C) Course of contraction not influenced by subsequent removal of chloride. (D) Attenuated contraction obtained in the presence of 6 m*M* magnesium (1 m*M* ionized magnesium plus 5 m*M* MgATP).

duced when fibers that had been loaded in a buffered calcium solution containing propionate as the major anion were exposed to a high concentration of chloride (Fig. 2A). This contraction could also be curtailed by EGTA (Fig. 2B), but once initiated, it was not influenced by removing the chloride (Fig. 2C). Again, the response depended on the concentration of magnesium ion and required that the fibers be loaded in the buffered calcium. When the concentration of magnesium exceeded that of ATP, fibers generated only a brief twitch-like contraction (Fig. 2D). Fibers placed directly in the chloride solution without prior exposure to buffered calcium did not develop measurable tension.

Both contractions described above could be terminated by EGTA, could be induced only after exposure to buffered calcium, and were followed by relaxation without further change in the external medium. This suggests that both were due to a transient rise in the concentration of calcium in the space that contains the myofilaments, and that this calcium was derived from an internal source which had been preloaded. The contraction produced by a sudden rise in the concentration of chloride is believed to result from a change in electrical potential across the internal membranes (*10*). The contraction induced by calcium, on the other hand, seems to be initiated by a reaction of this ion with the internal membranes, for the response occurred in either chloride or propionate and was elicited by concentration changes of only $10^{-4}M$. Free calcium in the myofilament space therefore appears to trigger the release of internally stored calcium.

In the presence of free magnesium (1 m*M*), the contraction induced by calcium was abolished, whereas a brief twitch could still be induced by chloride. This suggests that a small quantity of calcium is released by a change in the electrical potential across the internal membranes, and when the concentration of magnesium ion is low, this calcium induces a larger liberation of calcium from internal stores. Thus the activation initiated by a change in electrical potential in this preparation appears to be amplified by a regenerative release of calcium.

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Simian Virus 40 in Polio Vaccine: Follow-Up of Newborn Recipients

Abstract. *Soon after birth, when susceptibility to carcinogens should be enhanced, a group of children received oral polio vaccine which was later found to contain significant amounts of simian virus 40. Eight years after the incident, no cancer deaths have been observed among the vaccinated children, but continued surveillance is needed before concluding that simian virus 40 is innocuous to man.*

The induction of cancer in laboratory animals by simian virus 40 (SV 40) (*1*) has had unusual public health implications. As an unrecognized contaminant of virus vaccines prepared in monkey kidney cell cultures prior to 1962 (*1*), SV 40 was given inadvertently with poliomyelitis and adenovirus vaccines to a substantial number of persons. The possibility that SV 40 is oncogenic in man was further suggested by its capacity to cause subclinical infection when administered with either attenuated (Sabin) or inactivated (Salk) polio vaccines (*2*) and by its capacity to produce cellular transformations suggestive of neoplastic growth in human

Table 1. Description of groups (total of 1077 subjects) receiving polio vaccine in neonatal period. Sabin I vaccine from original seed stock (Merck), Purivax from lot No. 67681 (M. Hilleman), Sabin II from lot No. 1031124-2 (Pfizer). $TCID_{50}$ (50 percent tissue culture infective dose in cercopithecus monkey kidney cells) measured by M. Hilleman, 1960.

Study group	No.	Age (day)	Date	Vaccine			
				Type	Amount (ml)	SV 40 titer ($TCID_{50}$ per milliliter)	Route
1	149	< 1	Jan. 1960	Sabin I	1	$10^{1.5}$	Oral
2	31	< 1	Aug.–Oct. 1960	Sabin I	1	$10^{1.5}$; 1:100 dilution	Oral
3	324	< 3	Nov. 1960	Sabin I	1	$10^{1.5}$; 1:100 to 1:1000 dilution	Oral
4	60	< 3	Dec. 1960	Sabin I	1	$10^{1.5}$; 1:10 dilution	Oral
5	152	< 3	Dec. 1960	Purivax	1–5	10	Intramuscular
6	361	< 1, 3, 10, & 12	Mar.–May 1962	Sabin II	1	1 particle per 5 ml	Oral

tissue culture (*3*). Thus, it was reassuring to find that children in the United States who were 6 to 8 years old in 1955 (early participants in the mass Salk vaccination program) had no subsequent variation in cancer mortality which could be attributed to SV 40 (*4*). In that study, however, the vaccinated children were possibly beyond the age of susceptibility, since newborn laboratory animals are especially prone to oncogenic viruses such as SV 40 (*1*). Furthermore, the dose of SV 40 in the inactivated or formalinized vaccines may have been too low, the interval of observation too brief (4 years), and the analytic methods too indirect. These limitations created the need for a long-term follow-up of children who, shortly after birth, received vaccine which was later found to contain SV 40.

From 1960 to 1962, polio vaccine in various forms and regimens was given to 1077 newborn infants at the Cleveland Metropolitan General Hospital, in a study to assess the feasibility of inducing active immunity to poliomyelitis in the presence of maternal antibodies. Normal term infants were assigned with parental consent to one of six study groups (Table 1). Attenuated poliovirus vaccine was given orally to 925 infants in five groups; some received very high titers of SV 40 within a few hours of birth. The remaining 152 children (group 5) were injected with large doses of inactivated poliovirus vaccine which, however, had a much smaller concentration of SV 40 than oral preparations. Later in infancy all the children received booster inoculations of attenuated or inactivated polio vaccine, or both, which presumably contained SV 40.

Table 2. Status of study groups, 31 December 1968.

Group	Children		
	Dead (No.)	Alive (No.)	Unknown (No.)
1	4	136	9
2	1	26	4
3	1	279	44
4	2	55	3
5	2	137	13
6	1	285	75
Total	11	918	148

Since 1964, we have made periodic efforts to determine the mortality among the vaccinated children, most of whom were 8 years old in December 1968 (*5*). In general, the children are from an urban, low socioeconomic, highly mobile, predominantly Negro population, and the follow-up of these children was often confounded by changes in name and residence (especially interstate migration).

Table 2 shows the status of the survey in December 1968 when 79 to 95 percent of the children in the various study groups were traced (*6*). Although it is possible that some deaths were missed by changes in name and residence, the overall mortality (11 deaths) was very similar to that of comparable infants who were not premature or deformed at birth (*7*). There were no deaths from cancer, as compared to an expected value of 0.6 computed by a modified life-table analysis made from published death rates for Ohio children. Furthermore, mortality did not differ significantly by vaccine category, and the specific causes of death (infections, accidents, and heritable disorders) suggested no relation to the administration of vaccine or its SV 40 content.

In sum, no effect on mortality could be detected among newborns ingesting SV 40 virus at a dose which is carcinogenic when given to hamsters parenterally (*1*). Although supplementing a previous report (*4*) in providing some reassurance regarding the hazard of SV 40 to human beings, our study has certain limitations. Whereas the attenuated (oral) vaccines contained much higher amounts of viable SV 40 than the formalinized (subcutaneous) vaccines, the neoplasms in laboratory animals were, nevertheless, induced by parenteral and not oral administration of the SV 40. In addition, we could not have detected neoplasms associated with a latent period which is longer than 8 years. Surveillance of these children is being maintained. Now scrupulously excluded from all vaccines, SV 40 is a reminder of the potential danger of "silent" adventitious agents in vaccines and the necessity for rigorous follow-up of persons receiving new forms of immunization.

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6. The higher proportion of untraced children in group 6 is attributed to their younger age, with many not yet listed on school censuses.
7. Expected figures were computed from unpublished data which J. Yerushalmy provided.
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