over a period of 5 hours. Deterioration of contractility is much more rapid in a medium containing phosphate rather than bicarbonate as a buffer(4,10).

Our experiments indicate that isotonically contracting preparations of the papillary muscle are quite sensitive to the load applied and that the speed with which failure ensues is a function of this load. On the other hand, failure in isometric preparations does not appear to be correlated with tension. If one is interested in the effects of drugs, *e.g.*, the cardiac glycosides, on mechanically produced failure in the papillary muscle the type of preparation and degree of loading perhaps are not critical as long as these variables are maintained constant. However, if one wishes to examine the effects of drugs on a preparation which approximates the normal or non-failing heart, it appears that resting tension is an important factor and should be taken into consideration. These experiments also show that tension alone can result in morphologic changes, ranging from slight alterations to severe degeneration. The mechanism of this degeneration is not clear but such changes are not unique in as much as they are observed in tissue post mortem if fixation is not carried out rather promptly.

Summary. Papillary muscles of the cat subjected to various resting tensions exhibited morphologic changes, the severity of which could be correlated with degree of loading with loads of 3 g or greater. These changes were similar to those which occur post mortem in cardiac muscle and appeared whether the muscle was stimulated to contract or subjected to static tension. Morphologic alterations appeared in both isotonic and isometric preparations but appeared more severe in isotonic preparations. Rate and degree of failure of isotonically contracting muscles could be correlated with size of the resting load but such a correlation was not observed with muscles contracting under isometric conditions.

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Thermal Sensitivity of Polioviruses Isolated during Oral Vaccine Field Trial: Comparison with Monkey Neurovirulence.* (25241)

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Recent observations by Dubes *et al.*(1,2), Lwoff and Lwoff(3), and Sabin and Lwoff(4), indicated that virulent strains of poliovirus grow readily at temperatures of 39° and 40°C, while multiplication of attenuated strains is greatly reduced at such relatively high temperatures. In the following experiments an effort was made to determine if this characteristic, here designated the thermal marker, T (for attenuated), I (for intermediate), and T^+ (for virulent), could be used for the fol-

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lowing purposes: 1) to detect any reversion to greater virulence of an attenuated poliovirus vaccine strain which might occur as a result of passage in the human intestinal tract; and 2) to determine whether the thermal marker could be applied as a screening test for differentiating poliovirus strains recovered in the field with respect to their neurovirulence, and thus might aid in differentiating the attenuated LSc strain of type I poliovirus from various other strains isolated during a field trial in which LSc had been introduced into the community as an oral vaccine. The results with the thermal marker were also compared with another *in vitro* marker, *viz.*, the capacity to multiply at low and high bicarbonate concentrations, the "d-d^{+}" character of Vogt, Dulbecco, and Wenner(5), and with neurovirulence as determined by intracerebral inoculation of rhesus monkeys.

Materials and methods. Virus strains. Strains of type I poliovirus isolated during an oral vaccine field trial using Sabin's LSc type I attenuated poliovirus were tested.‡ The trial was carried out in a village community near Phoenix, Ariz., and details concerning virus administration and collection and testing of specimens have been reported (7). Virus isolates tested were strains from the intestinal tracts of 3 children fed and infected with the LSc strain; one strain from the privy of an infected family; and 15 strains obtained from flies trapped at various points in and directly adjacent to the study area and also peripherally in the village. For comparison, 2 control poliovirus strains were used, i) virulent Mahoney type I strain, and ii) attenuated type I LSc vaccine strain from an aliquot of the same pool orally administered to children in the trial. In addition, examples of virulent and attenuated types II and III polioviruses were also tested for comparison. These were: —MEF 1 (virulent) and YSK (attenuated) for type II; Leon (virulent) and Leon KP_{34} (attenuated) for type III.§ Monkey kidney (MK) monolayer cultures were used throughout(8). Bottle cultures were overlaid with agar as described by Hsiung and Melnick(9). For determination of growth curves, of Mahoney and the LSc strains, tube cultures were used. *Tests for the thermal marker.* Second, or in some instances third, MK passage of strains to be tested were inoculated in 10 fold dilutions into 3 sets of MK bottle cultures, 2 bottles/dilution. After one hour's adsorption at 37°C, the cultures were overlaid with agar. One set was then incubated at 35°C, another at 37°C, and a third at 39°C. The 37°C incubator used was a walk-in type, employed routinely, while the 39°C and 35°C ones are specially constructed units each with a fan and thermostat (Fenwal thermoswitch) intrinsically sensitive to 0.1°F temperature changes. The temperatures in these 2 incubators had been recorded on a thermograph continuously over 3 weeks before experiments were begun, and at intervals thereafter; the daily variation was less than 0.5°C. During tests, the 35° and 39°C incubators were not opened until 4-5 days of incubation had been completed. At this time plaque titers/0.1 ml were determined. The efficiency of plating (EOP) at 37°C was taken as unity, the EOP of cultures grown at 35° and 39°C being related to this standard. In each test the attenuated LSc type I strain which had been fed to children in the field trial and the virulent Mahoney type I strain were included as controls. *Growth curves of type I strains.* Virulent Mahoney and attenuated LSc strains of type I virus were each inoculated into 3 sets of 24 MK tubes in doses of $2 \times 10^{5.5}$ TCD_{50}/ml. During adsorption period of 1 hour, the cultures were held at 37°C. Following this, cultures were washed 3 times with balanced salt solution, the medium was replaced and 24 tubes of each set were incubated at 35°, 37°, and 39°C respectively. Every 2 hours for the first 12 hours of incubation, and at 24 and 48 hours, 3 tubes held at each temperature were frozen and thawed once, the harvests pooled and titrated for total virus in MK tubes. At time of harvesting, cultures were examined for cytopathogenic effect (CPE). *Other tests for virulence and genetic stability.* In most instances, the same inocula were used both in testing for

‡ We are grateful to Dr. Albert B. Sabin for making the LSc type I oral vaccine(6) strain available to us.

§ Attenuated types II and III strains kindly provided by Dr. Albert B. Sabin(6).

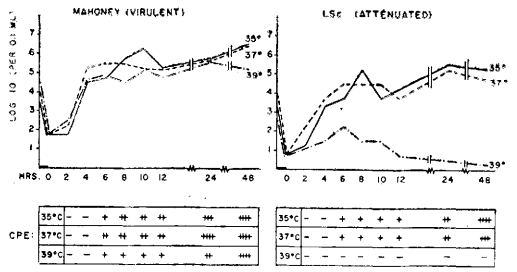

FIG. 1. Growth curves of virulent and attenuated type I poliovirus strains at different temperatures. Plus signs in panels at base of charts indicate degree of cytopathic effect (CPE).

thermal marker and for other virulence tests: in a few, the same passage level but a different preparation was employed. *The "d" marker* was determined in agar overlay cultures with low (0.08%) and high (0.5%) bicarbonate concentrations, as described by Hsiung and Melnick(10). *Monkey neurovirulence* was tested by inoculating a minimum of one million TCD_{50} of each strain intracerebrally into 2 rhesus monkeys. The dose was contained in 1 ml, of which 0.5 ml was introduced into each side of the thalamus. Animals were observed daily for 3-4 weeks. Those which developed signs of poliomyelitis were sacrificed at the height of paralysis, while the others were sacrificed at the end of the test period. The medulla and 3 levels of spinal cord were examined histologically for lesions of poliomyelitis.

Results. Growth curves of Mahoney and LSc type I strains at different temperatures. Fig. 1 indicates that 2 distinct patterns emerged, one for the virulent Mahoney strain, the other for attenuated LSc type I strain. Each point on curves represents a titration of harvests of 3 tube cultures. The Mahoney strain multiplied equally well at 35°, 37°, and 39°C, whereas multiplication of the LSc strain was greatly reduced at 39°C. Since all cultures were held at 37° for the adsorption period of 1 hour, the first cycle growth of LSc at 39° probably represents virus which adsorbed at 37°C and multiplied to a limited extent during the shift to 39°C.

Cytopathic effect (CPE) was observed at all 3 temperatures with the Mahoney strain, beginning at 6 hours (Fig. 1). With the LSc strain, no CPE was observed at 39°, although it appeared at 6 hours at both 35° and 37°C.

EOP of virulent and attenuated strains of polioviruses types I, II, and III at different temperatures. Virulent strains of all 3 types each had a high EOP at 39°C, while the attenuated strains all showed a low EOP at this temperature (Table I). The figures for EOP are expressed as log of the quotient when the titer (PFU/0.1 ml) at 39° (or 35°) is divided by the titer at 37°C. The results of several different tests are shown in Table I.

Thermal marker of type I strains isolated during LSc oral vaccine field trial. Comparison with other virulence tests. Results are shown in Table II. The virus isolated from 2 of the children who had received the oral vaccine, #23-8 and #20-8, exhibited the same pattern with respect to thermal marker as did the ingested LSc virus. The third child, #10-5, 4 days after LSc was orally administered, also excreted virus with a comparably low EOP at 39°C, but by 14th day, the excreted virus showed a shift to a slightly higher EOP at this temperature, and by the 29th post-feeding day virus isolated from this child had an EOP at 39°C characteristic of virulent strains. The d-d^+ marker of these same strains exhibited a less regular pattern, but the one T^+ strain was also d^+. Although all 7 strains isolated from the infected children were relatively avirulent for the monkey, a million or more TCD_{50} of

TABLE I. Growth of Virulent and Attenuated Polioviruses at Different Temperatures.

	Type	Strain	Titer in PFU per 0.1 ml at 37°	Efficiency of plating at 39°C EOP	Log	at 35°C EOP	Log
I	Virulent	Mahoney	8.5×10^6	.41	−.39	.64	−.2
	Attenuated	LSc	4.0×10^5	.000025	−4.6	.75	−.1
II	Virulent	MEF_1	2.0×10^6	.2	−.7	1.5	+.2
	Attenuated	Y-SK	4.5×10^4	.000011	−4.96	4.0	+.6
III	Virulent	Leon	8×10^6	.5	−.3	.7	−.2
	Attenuated	" KP_{34}	$>7.5 \times 10^5$	<.00013	−3.9	>7.3	+.9

TABLE II. Results of Virulence Tests on Type I Poliovirus Strains, Isolated during a Field Trial with Attenuated LSc Strain Type I Oral Vaccine. Comparison of 3 markers.

			Virulence tests						
			Thermal (T) marker† (EOP at 39°C)		"d" marker‡ (EOP at low bicarbonate conc.)		i-c monkey inoc.§ (dose 10^6-10^7 TCD_{50})		
Source of poliovirus	Strain No.	Date of collection* (1958)	Log EOP	Marker	Log EOP	Marker	Paralysis	Lesions	Marker
Excreted by infected children‖	23-8	17/II	−5.5	T	−3.2	d	0/2¶	0/2¶	M−
	"	24/II	−5.3	T	−3.0	i	"	"	"
	20-8	18/II	−6.0	T	−4.8	d	"	"	"
	"	20/II	−6.0	T	−1.9	i	"	"	"
	10-5	17/II	−6.1	T	−3.8	d	"	1/2	Mi
	"	27/II	−4.1	T	−1.9	i	"	"	"
	"	14/III	−1.3	T+	− .6	d+	"	"	"
Privy of infected family	20-A	20/II	−4.0	T	−4.3	d	"	"	"
Flies trapped in village	10-01	20/II	−4.8	T	−2.0	i	"	0/2	M−
	6-01	27/II	−3.5	I	−3.8	d	"	2/2	Mi
	1-26	20/III	−3.4	I	−2.2	i	2/2	"	M+
	"	19/II	−3.2	I	− .9	d+	1/2	"	"
	10-01	19/II	−2.8	I	−3.7	d	"	"	"
	13-03	25/II	−2.4	I	−1.5	i	2/2	"	"
	1-26	20/II	−2.4	I	−2.5	i	1/2	"	"
	"	27/II	−2.2	I	−2.0	i	2/2	"	"
	7-13	27/II	−1.9	T+	−2.0	i	"	"	"
	4-14	18/III	−1.7	"	−1.0	d+	"	"	"
	6-01	11/III	− .9	"	−1.7	i	"	"	"
	"	6/III	−1.8	"	−1.6	i	"	"	"
	3-06	20/III	− .7	"	−2.1	i	1/1	1/1	"
	4-17	18/III	− .4	"	−1.5	i	2/2	2/2	"
	3-06	11/III	+ .1	"	−1.5	i	"	"	"
Control virulent and	Mahoney (virulent)		− .9	"	− .5	d+	(10/10)**	(10/10)**	"
			− .3	"	− .3	"			
			− .6	"	− .2	"			
			− .3	"	− .5	"			
			− .4	"	− .4	"			
Attenuated type I strains	LSc (attenuated)				−4.2	d			
			−6.0	T	−4.0	d			
			−5.4	T	−5.8	d	0/9	0/9	M−
			−6.2	T	−5.0	d			
			−5.8	T	−4.2	d			

* LSc type I fed to 6 children in community 12/II/58.

† Designation "T" marker as follows: Log EOP ≤ -4.0 = T (attenuated).
-2.1 to -4.0 = I (intermediate).
> -2.0 = T+ (virulent).

‡ Designation "d" marker: Log EOP < -3.0 = d (attenuated).
-3.0 to -1.0 = i (intermediate).
≥ 1.0 = d+ (virulent).

§ Designation as to monkey neurovirulence: Neither lesions or paralysis = M− (attenuated). Lesions but no paralysis = Mi (intermediate). Lesions and paralysis = M+ (virulent).

‖ 23-8, 20-8, and 10-5 refer to 3 LSc fed children who became infected.

¶ Denominator indicates No. tested, numerator, No. positive.

** Tests performed earlier in this laboratory.

virus failing to produce signs of weakness or paralysis in any, CNS lesions were present in one of 2 animals inoculated with each of the 3 specimens isolated from child #10-5.

Strain isolated from privy of infected family. Strain #20-A (Table II), the only strain of poliovirus isolated from a fecal sample obtained from a privy (7), possessed the T and d markers, and induced no paralysis in 2 out of 2 inoculated monkeys, but one of the animals showed mild lesions.

Strains isolated from flies. Fifteen strains,

isolated during field trial from 7 different trapping sites, had thermal marker patterns ranging from the T, characteristic of attenuated strains, to the T^+ of virulent strains. Unfortunately, no flies were collected before the trial began, so that strains isolated prior to introduction of the LSc strain into the community were not available for comparison. Only one of the fly strains isolated approached the range of the LSc strain fed: this was #10-01, collected 20/II, which had an EOP of log —4.8, and failed to produce either paralysis or lesions in the 2 monkeys inoculated. The EOP of this strain at low bicarbonate concentrations placed it in the intermediate (i) range with respect to the d-d^+ marker. Seven other fly strains were characterized as intermediate in thermal sensitivity tests; all possessed the I thermal marker, and produced lesions in 14 animals inoculated, as well as some degree of muscle weakness in 9 of them. With respect to the d-d^+ marker, 2 of these 7 strains were classified as d, 4 as intermediate, and one as d^+ (Table II). Of the remaining 7 fly strains, all were T^+ with respect to the thermal marker, and all produced paralysis and lesions in 13 of 13 monkeys inoculated. Comparing the T-T^+ and d-d^+ markers, the 7 T^+ strains were characterized as d^+ in one instance, and as intermediate (i) in 6.

Discussion. Results of growth curves with virulent and attenuated type I poliovirus strains at different temperatures confirm the observations of others(1-4) that reduced multiplication at high (39°C) temperatures is a characteristic of attenuated poliovirus strains. This is further borne out by the EOP of plating at 39°C found for virulent and attenuated strains of types II and III polioviruses (Table I).

However, the results also indicate the difficulty in identifying a poliovirus strain isolated in the field as one which has been introduced into the community, even when 2 different *in vitro* and one *in vivo* "virulence" tests were employed. Nevertheless, our findings suggest that the thermal marker is probably a more reliable measurement than is the d-d^+ marker in this respect. It correlated better with monkey virulence tests in which the intracerebral route of inoculation and large doses were used, and gave more consistent results with strains isolated from persons *known* to be infected with a given strain, in this instance the LSc strain of attenuated type I virus.

As Table II shows, only one of the 15 fly strains behaved as did the LSc virus ingested. This strain, #10-01, 20/II, was isolated from flies trapped in the study area near a family in which a child had received the vaccine virus and become infected. It had a very low EOP at 39°, and failed to produce either paralysis or lesions in intracerebrally inoculated monkeys. It may have been a wild attenuated strain; or on the other hand, it may have been the LSc strain of virus which had been picked up by the local flies; this latter occurrence would not be surprising, considering that there were children in the area known to be infected with the LSc strain, and the level of sanitation (7) provided flies with ready access to fecal material.

Of the remaining 14 fly strains tested, 7 were intermediate (I) strains and 7 were T^+ with respect to the thermal marker. Many of these strains in all probability represent naturally occurring wild strains, some of lower and others of a higher degree of virulence both by *in vitro* and *in vivo* tests. Nevertheless, one cannot completely rule out the possibility that some strains might be the LSc, somewhat enhanced in virulence after human passage. That many strains were isolated from flies trapped early in the course of the trial, at widely separated points far from the 3 infected families, makes it seem more likely that they represent wild strains. Furthermore, the range of their T markers and monkey neurovirulence are similar to the patterns which we have found for *known* wild type III poliovirus strains isolated at the same time from the same traps.

From these results, one can conclude that the thermal marker is helpful in characterizing freshly isolated strains as relatively virulent or attenuated; further it is an aid in following the course of events after feeding attenuated poliovirus to humans, in determining whether enhancement of virulence occurred, and perhaps to some extent, whether the environment is contaminated with the vaccine

virus. Particularly is this true if the T marker of a given strain is considered in connection with other markers such as the d-d$^+$, and monkey neurovirulence. However, if the community under study is one in which poliovirus infections are highly endemic and naturally occurring strains of low virulence are prevalent, the problem of strain differentiation by means of above markers becomes complex. In such a situation thermal and other markers can suggest the identity of a given strain but cannot provide positive proof. It is possible that more clear cut results and better correlation of the T marker with monkey neurovirulence could be achieved if a level of 40°C rather than 39°C is used as the critical temperature. This possibility is currently under investigation.

Summary. 1. Virulent and attenuated strains of the 3 types of poliovirus have been shown to have different patterns of growth at high (39°C) temperatures when tested by means of agar overlay tissue culture technic. Attenuated strains (T marker) are inhibited and have a low EOP at this temperature, while virulent strains (T$^+$) grow equally well at 35°, 37°, and 39°C. Some strains give an intermediate (I) pattern. 2. The thermal marker T-T$^+$ was used to screen poliovirus strains isolated during a type I oral vaccine field trial, in an effort to determine: a) whether reversion to greater virulence had occurred as a result of human passage; and b) whether strains isolated from local flies were the vaccine virus which had been orally administered, or were naturally occurring wild strains. The results indicated that virus excreted by 2 of the 3 infected children consistently had thermal markers identical with that of the vaccine virus; one child, on the other hand, excreted virus which over a period of 29 days showed a shift to the thermal marker pattern of more virulent strains. Neurovirulence for monkeys was low for all strains isolated from children. One T strain isolated from flies had an EOP at 39°C in the same range as the LSc vaccine strain, while the 14 other fly strains tested all fell between this range and that of virulent strains. It was concluded that most of the fly strains recovered in the course of the field trial probably represented naturally occurring wild strains. 3. Comparison was made between the thermal (T-T$^+$) marker, the EOP at low and high bicarbonate concentrations (d-d$^+$ marker), and neurovirulence as tested by intracerebral inoculation of monkeys. The thermal marker correlated better with monkey virulence than did the d-d$^+$ marker. 4. Use of the thermal *in vitro* marker should be of some practical value in determining degree of spread of attenuated poliovirus strains introduced into a community, and in assessing degree of stability of vaccine strains on human passage.

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