considerable. It is surprising that the membrane in Fig. 1 represents a vaccinia virus content of 0.4×10^7 PkFU's/ml (extracted) when on the basis of gross pathology the membranes appeared entirely normal. The reduction in pathologic changes at the lower temperatures may be due to a reduced accumulation of virus per cell of the total number of cells infected.

The experiments of vaccinia growth in the 8- and 15-day-old chorioallantoic membrane suggest that a certain rather specific metabolic or physiologic state of these cells is required for maximal virus growth. The chorioallantoic membrane is in this optimal state for only a few days in the total embryonic life span. Some experiments were attempted whereby the 8- and 15-day eggs were incubated at sub-optimal temperatures but this resulted in a virus particle production too low to count. Unfortunately, particle count experiments are not possible (at present) when particle concentration is less than 1×10^7 particles/ml.

The mechanism of incomplete virus production is not known but with influenza virus it is produced by serial passage of virus in high concentrations. Since the nucleic acid content of incomplete influenza virus is low it has been theorized that the multiple cell infection strains the cells' ability to produce nucleic acid. No data are available as to whether cell incubation at low temperatures reduces or affects either cell or virus nucleic acid production.

Summary. A relative increase in non-infective or incomplete vaccinia virus occurs during virus growth in the chorioallantoic membrane of 12-day-old eggs incubated at 26°C and 30°C. Vaccinia growth in membranes of eggs 8 and 15 days of age is reduced but no increase in non-infective vaccinia was found. Serial passage of high concentrations of vaccinia virus in 12-day-old eggs likewise failed to increase the proportion of non-infective virus.

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Phosphorylase Activity in Denervated Skeletal Muscle.* (26898)

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The glycogen level of skeletal muscle decreases within 3 days following denervation (1) and returns to normal upon reinnervation(2). However, the changes in muscle phosphorylase activity, following denervation, are not in agreement. Enzyme activity in denervated muscle was reported to decrease slightly after several weeks and then decrease rapidly(3), or to decrease steadily from the day of denervation(4), or to increase up to 20 days after denervation(5). Furthermore, the increase in phosphorylase activity induced in normal skeletal muscle by epinephrine failed to occur in denervated muscle(5). The present study concerns an investigation of skeletal muscle phosphorylase activity at various periods after denervation,

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TABLE I. Phosphorylase Changes in Muscle after Denervation.

	Days after	P-lase *a* Fresh wt*	P-lase *a* NCPN†	P-lase *t* Fresh wt	P-lase *t* NCPN	Ratio *a/t* × 100‡
C§		125 ± 12	1.87 ± .22	180 ± 14	2.76 ± .25	69 ± 3.3
D§	5‖	140 ± 13	2.16 ± .12	194 ± 14	2.97 ± .19	73 ± 3.0
C		116 ± 13	1.68 ± .16	178 ± 14	2.58 ± .18	65 ± 2.8
D	10	61 ± 7	.96 ± .09	106 ± 7	1.64 ± .09	56 ± 3.3
C		130 ± 12	2.00 ± .18	190 ± 14	2.89 ± .21	69 ± 2.8
D	15	38 ± 4	.62 ± .04	74 ± 5	1.21 ± .11	51 ± 2.5
C		122 ± 11	1.78 ± .22	188 ± 11	2.72 ± .24	65 ± 3.0
D	20	25 ± 4	.41 ± .07	51 ± 6	.83 ± .11	49 ± 2.9
C		126 ± 6	1.78 ± .12	185 ± 8	2.62 ± .13	67 ± 2.2
D	30	43 ± 3	.70 ± .04	77 ± 5	1.34 ± .13	56 ± 1.3
C		156 ± 12	2.45 ± .20	218 ± 13	3.40 ± .22	71 ± 2.0
D	45	49 ± 4	.86 ± .07	88 ± 8	1.53 ± .15	57 ± 1.7
C		157 ± 9	2.26 ± .14	217 ± 12	3.12 ± .19	72 ± .9
D	60	55 ± 3	.88 ± .05	99 ± 5	1.60 ± .08	56 ± .7

* Activity = μg P liberated from glucose-1-PO_4 by 2.5 mg tissue, in 10 min. at 37°C, ± S.E. (7 rats/experiment).
† Activity = calculated as mg P liberated per mg of non-collagenous protein nitrogen ± S.E.
‡ Ratios based on fresh tissue wt.
§ C = control; D = denervated.
‖ Results at 5 days not significant, all others significant, $P < .05$.

and the effect of epinephrine on enzyme activity in denervated muscle, particularly after electrical stimulation.

Methods. Adult male Long-Evans rats (250-300 g) were anesthetized with ether and the right rectus femoris muscle was denervated by removal of 2 mm of the femoral nerve. In experiments concerned with enzyme changes at varying periods after denervation the left muscle served as control, and in other experiments bilateral denervation was performed. All subsequent manipulations of the muscles were carried out while the rats were under Nembutal anesthesia. L-Epinephrine-HCl (40 μg/100 g b.w.) was injected intraperitoneally 50 min before autopsy. Tetanic contraction of the rectus femoris muscle was produced by inserting needle electrodes into opposite ends of the exposed thigh muscles and stimulating for 10 or 20 sec from a square wave stimulator set at 60 cycles/min, 1 msec duration, at 20 volts. The muscles were frozen between blocks of dry ice after removal and stored at −20°C until used.

Active phosphorylase *a* and total phosphorylase *t* were assayed as previously reported(6) and the activity ratio (*a/t* × 100) was calculated. The activity is expressed as μg of P liberated from glucose-l-phosphate at 37°C in 10 minutes by 2.5 mg wet weight of tissue or, in some instances, per mg of non-collagenous protein nitrogen (NCPN) determined as described(7).

Results. Activity levels of phosphorylase *a*, *t*, and the *a/t* ratios based on wet weight of muscle, at 5 days following denervation were slightly but insignificantly increased over control values, ($P > .10$). At 10 days phosphorylase *a* and *t* activity decreased significantly in the denervated muscle, but the decrease in activity ratio was of borderline significance. At 15 days both phosphorylase activity and the ratios were significantly lower, and at 20 days these parameters were at their lowest point relative to the controls. From 30-60 days the level of enzyme activity and the activity ratios appeared to increase slightly, but were still much less than control values. No difference in these results was noted when they were based on NCPN (Table I). Attention is called to the increase in phosphorylase *a* and *t* in denervated muscle at 45 and 60 days above the 30-day level together with an increase in the control muscles. However, the activity ratios remained constant from 30-60 days post-denervation.

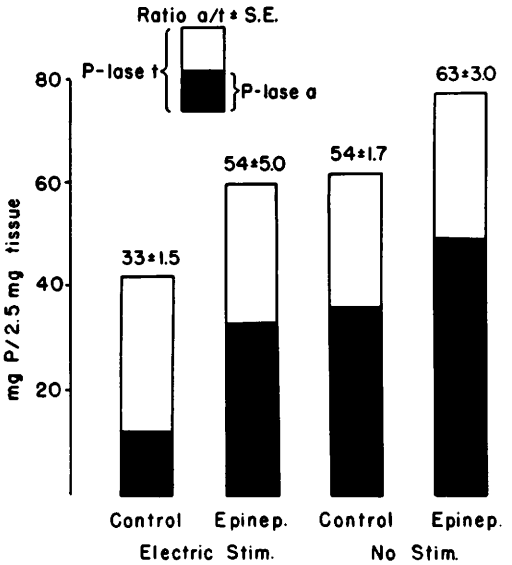

FIG. 1. Phosphorylase activity in denervated (15 days) muscle after electrical and epinephrine stimulation.

In an experiment to determine whether epinephrine increased phosphorylase activity in denervated muscle, rats were bilaterally denervated and after 15 days were divided into 2 groups, one of which received epinephrine injection. Since the effect of epinephrine on muscle phosphorylase is more readily demonstrable when the muscle is made to contract prior to removal for assay(8), in all animals the right leg was electrically stimulated for 20 sec before removal, while the left leg served as a non-stimulated control.

The results (Fig. 1) show that in denervated muscle, which was not electrically stimulated, epinephrine induced a statistically insignificant increase in the activity of both phosphorylase *a* and *t* but a significant increase in the activity ratio. In muscles which were stimulated electrically, epinephrine inhibited the decrease in activity which normally follows stimulation, so that the differences in the activity of phosphorylase *a*, *t* and *a/t* ratio, between epinephrine injected and non-injected animals, were significant.

A study was made of the effect of denervation on rate of recovery of enzyme activity following tetanic contraction, with and without epinephrine treatment. In both intact rats and in animals with muscles bilaterally denervated for 15 days the right leg muscle was stimulated for 10 sec and allowed to recover for varying periods of time before removal. The muscle of the left leg was stimulated for 10 sec and immediately removed. Another group of denervated rats received epinephrine 50 minutes prior to electrical stimulation.

Following electrical stimulation, the activity of phosphorylase *a* decreased in the normal muscle to an extent twice that of the decrease in phosphorylase *t* (Table II). The effect was also noted in a marked decrease in activity ratios. Stimulation of a 15-day denervated muscle produced a significant decrease in both phosphorylase *a* and *t* of approximately the same amount. Tetanic stimulation of the denervated muscle of rats pretreated with epinephrine lowered phosphorylase *a*, *t* and the *a/t* ratio, but only the decrease in the ratios was significant.

In normal muscles the recovery of phosphorylase *a* and the activity ratios occurred within 7.5 min, but the phosphorylase *t* returned in 5 min. In the denervated muscle recovery of phosphorylase *a* and *t* activity was not complete by 20 min, but the activity ratio returned to normal within 5 min. In the presence of epinephrine the return of the activity ratios to their control level in the denervated muscle occurred within 5 min, but since the decrease in phosporylase *a* and *t* themselves was not significant, it was difficult to demonstrate an effect of the hormone on these parameters.

Discussion. The slight increase in phosphorylase *a*, *t*, and ratios of *a/t* which occurred 5 days after denervation of the rat rectus femoris was of questionable significance, although others have reported a significant increase in these parameters as late as 20 days following denervation, using the rat gastrocnemius muscle(5). In another study on this muscle, phosphorylase activity was reported to decrease steadily from the day of denervation(4), while in denervated dog muscle, phosphorylase *b* only was reported and was found to be depressed slightly during the first 7 weeks, after which a marked decrease occurred(3). Such differences may be attributed to differences in the specific

TABLE II. Recovery of Phosphorylase Activity in Denervated Muscle after Electrical Stimulation.

Exp.*	Initial P-lase activ.†			Recovery of P-lase activ.†			
	a	*t*	*a/t* × 100	Min.	*a*	*t*	*a/t* × 100
A. Stim. normal m.	46 ± 2	143 ± 6	32 ± 4.2	2.5	57 ± 2	140 ± 2	41 ± 2.5
				5	90 ± 3	172 ± 6	53 ± 2.4
				7.5	109 ±11	163 ±14	65 ± .1
				10	118 ± 9	170 ±16	69 ± 1.0
(Ref. cont.–Tab. I)				15	122 ±26	168 ±34	72 ± 1.0
normal m.	130 ±13	190 ±14	69 ± 2.8	20	136 ±11	190 ± 2	72 ± 4.0
B. Stim. denerv. m.	25 ± 1	57 ± 2	45 ± 1.3	5	31 ± 2	62 ± 2	50 ± 1.5
				10	27 ± 2	52 ± 3	52 ± .7
(Ref. cont.–Tab. I)				15	30 ± 1	54 ± 2	55 ± .6
denerv. m.	38 ± 4	74 ± 5	51 ± 2.5	20	30 ± 1	54 ± 2	56 ± .0
C. Stim. denerv. m. + epineph.	36 ± 2	70 ± 4	51 ± 4.7	2.5	41 ± 1	75 ± 4	55 ± 3.0
				5.0	45 ± 1	72 ± 1	63 ± .9
				7.5	57 ± 2	86 ± 7	65 ± .6
(Ref. cont.–Fig. 1)				10	51 ± 7	75 ±11	68 ± .3
denerv. m. + epineph.	49 ± 6	77 ± 6	63 ± 3.0	15	46 ± 2	71 ± 6	67 ± 2.0

* Initial determinations (7 rats), recovery determinations (2-3 rats).
† Activity = μg P/2.5 mg tissue ± S.E., as in Table I.

muscle, methods of procuring the muscle, or the enzyme assay.

By the 10th day after denervation phosphorylase *a*, *t*, and the ratios were all lower than in the normal muscles. This condition persisted for 60 days. The decrease in enzyme activity observed agrees with some previous results(3,4) but not with others(5). Phosphorylase assays using tissue homogenates are highly variable at best and interpretations must take into account activity ratios as well as absolute values of phosphorylase *a* and *t*. Muscle atrophy produced by vit. E deficiency(9) and muscular dystrophy from other causes(10) are associated with decreased phosphorylase *a* and *t*, but under these conditions the ratios were similar to those in normal muscle. The decrease in enzyme activity was not necessarily associated with increases in amount of connective tissue relative to amount of muscle protein because the results calculated on the basis of NCPN for denervated muscle were similar.

In normal muscle epinephrine was reported to increase phosphorylase *a* and the activity ratios(11) and to inhibit the expected decrease in enzyme activity which ordinarily follows tetanic stimulation(8). In denervated muscle epinephrine increased only the activity ratios significantly, but prevented the expected decrease in phosporylase *a*, *t*, and the ratios which follow tetanic stimulation. Previous failure to demonstrate the effect of epinephrine on phosphorylase in denervated muscle may have been due to differences in procedures(5). Epinephrine induces glycogenolysis in denervated muscle(1) and it would seem likely that the phosphorylase activity would also increase.

In the experiments on recovery rates of enzyme activity from depressed levels 10 sec electrical stimulation was selected rather than 20 sec because the latter frequently overwhelmed the effect of epinephrine in preventing the decrease in phosphorylase activity. In normal muscles the return of enzyme activity occurred within 7.5 min. In the denervated muscle the return of activity ratios to their characteristic initial levels appeared slightly faster than in intact muscle, but phosphorylase *a* and *t* had not returned within a 20-min. period indicating some impairment in this instance. Tetanic contraction is reported to decrease only phosphorylase *a* by converting it into phosphorylase *b* which can be calculated by difference when phosphorylase *t* is determined(11,12). Although the decrease in phosphorylase *a* accounted in large part for the low ratios, the decrease in

phosphorylase *t* cannot be either ignored or explained.

Although others have shown that epinephrine increases rate of recovery of phosphorylase activity ratios in normal rat muscles(11,12), this was not observed in denervated muscle under these conditions. Perhaps if the denervated muscle had been stimulated to exhaustion, the effect of epinephrine on increasing the rate of recovery would have appeared as it did in normal muscle(11,12).

Summary. Following denervation of the rat rectus femoris muscle, phosphorylase *a* and *t* activity and the *a/t* ratio were maintained for 5 days, decreased by 10 days and remained low for 60 days. In muscles denervated for 15 days epinephrine increased the activity of phosphorylase *a* and *t* and the *a/t* ratio, and markedly inhibited the expected decrease in enzyme activity which follows tetanic contraction. Following electrical stimulation, the recovery of activity ratios in both intact and denervated muscles occurred at the same time. In denervated muscle the return of phosphorylase *a* and *t* was delayed as compared to that in normal muscle. Epinephrine showed no effect on the recovery of enzyme activity in the denervated muscle. Changes in muscle phosphorylase activity after denervation atrophy differ from those incurred from other causes.

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Influence of Adrenal Glucocorticoids on Distribution of Calcium and Phosphorus between Bone and its Surrounding Fluids. * (26899)

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Hypercalcemia has been reported in Addisonian crisis(1-3), following adrenalectomy in patients with Cushing's disease(4), and in adrenalectomized animals(5-8). In many of these instances the elevated serum calcium returned to normal levels following cortisone administration. Hypercalcemia accompanying sarcoidosis and hypervitaminosis D, and idiopathic hypercalcemia in children may be similarly reduced with cortisone(9-11).

Elevated serum phosphorus values have been observed in adrenalectomized rats(12) and monkeys(13), and in patients with Addison's disease(2). On the other hand, ACTH has been found to decrease serum phosphorus in the rat(14), and marked hypophosphatemia has been seen in patients following cortisone treatment(15).

Either a lack of such effects on serum Ca and P concentrations or conflicting results have been reported by other workers(11,14, 16). While these phenomena indicate effects

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