TABLE II
γ of Phosphorus Liberated from the Substrate Containing 10 mg of Phosphorus in the Form of Sodium Phytate by the Phytase Present in 1 g of the Pulse Either Before or After Germination.

Pulses	Days of germination					
	0	1	2	3	4	5
Phaseolus mungo	39	46	66	82	91	91
Phaseolus radiatus	167	179	200	206	208	208
Cicer arietinum	39	43	47	50	50	50
Dolichos lablab	172	186	191	192	191	192

10 mg of phosphorus as sodium salt of phytin. The mixture was incubated at 35°C for 6 hours. The phosphorus liberated from phytin was estimated by the method of Fiske and Subbarow.[7]

Results. The total phosphorus content in mg per 100 g of *phaseolus mungo, phaseolus radiatus, cicer arietinum* and *dolichos lablab* was respectively 375, 302, 219 and 247. Phytin phosphorus values and phytase activities of the pulses are given in Tables I and II.

Discussion. Phytin phosphorus values of all the 4 varieties of pulses studied were found to decrease gradually during the process of germination and the maximum lowering of the value occurred either on the third or on the fourth day of germination. The phytase activity of the pulses was found to increase during the course of germination and when the phytin content was minimum the phytase activity was maximum. This suggests that the enzyme phytase which is formed during the process of germination acts upon the phytin phosphorus and partly destroys it. It has been observed by French *et al.*[6] that 48 hours after germination of peas there is a considerable increase in the inositol value of the pulse. Phytin being the calcium or magnesium salt of inositol hexaphosphoric acid, the increased inositol value of the pulse after germination might be due to the breaking down of phytin by the enzyme phytase. The germinated pulse is therefore nutritionally superior to the ungerminated one.

Summary. Phytin content and phytase activity of 4 pulses have been estimated both before and for varying periods after germination. Phytin content gradually diminished along with the increase in the phytase activity of the germinating pulse which was maximum either on the third or on the fourth day of germination.

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17266. Creatinine, Potassium, and Virus Content of the Muscles Following Infection with the "Coxsackie Virus."*

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A number of specimens of feces from children exhibiting symptoms of poliomyelitis have yielded viruses that produce degeneration of the skeletal muscles of suckling mice and hamsters.[1,2] The anatomic response in man is not known but the clinical records of patients from whose feces virus was isolated suggest that muscle weakness or paralysis is common and sometimes persists for months. While considering means of testing patients for muscle degeneration, it occurred to us that creatine losses probably accompany the destruction of the striated muscle cells and,

* This agent is being called "Coxsackie Virus" since the first recognized human cases were residents of that New York village.

1 Dalldorf, Gilbert, and Sickles, G. M., *Science*, 1948, **108**, 61.

2 Dalldorf, Gilbert, Sickles, G. M., Plager, Hildegard, and Gifford, Rebecca, *J. Exp. Med.*, 1949, **89**, 567.

TABLE I.
Concentration of Total Creatinine in Urine of Normal and Infected Suckling Mice.

		Total creatinine					
		Normal animals		Infected animals			
Group	Age (days)	No. in group	mg/ml	No. in group	Days following inoculation	Paralysis	mg/ml
1	6	22	0.45	22	1	—	0.3
	7	20	0.50	24	2	+	1.5
	8	20	0.50	18	3	++	2.7
	9	10	0.60	3	4	++++	2.0
2	6	22	0.30	23	1	—	0.33
	7	22	0.25	23	2	—	1.01
	7½	22	0.47	22	2½	+	3.00
	8	19	0.40	21	3	++	3.00
	8½	18	0.25	14	3½	+++	2.72
	9	17	0.26	8	4	++++	2.38
3	7	16	0.22	18	1	—	0.81
	7½	16	0.29	17	1½	—	1.05
	8	16	0.05	18	2	+	1.92
	8½	16	0.45	18	2½	++	3.50
	9	16	0.30	18	3	+++	3.50

further, that, since the muscle weakness in some patients was migratory and transient, serum potassium concentration should be tested. It has been impossible to secure suitable serum specimens from immature mice but the potassium and total creatinine values of the muscles have been determined, as well as urinary creatinine. The present report of these results includes, in addition, certain observations on the infectivity of the muscles.

Methods. The muscle samples for chemical analyses taken from immature mice at various times following intraperitoneal injection of brain suspensions of the T. T. strain, were placed in tared and sealed containers and weighed within 2 hours. The urine samples were obtained by lightly pressing on the lower abdomen with a wooden applicator. Since suckling mice do not urinate without external stimulus, small urine specimens were readily procured. In order to avoid possible differences between litters, the mice were first pooled and redistributed at random[3] before inoculation.

Potassium was determined by the phosphotungstate method of Van Slyke and Rieben.[4]

The samples for muscle creatinine determinations were prepared according to the directions of Rose, Helmer, and Chanutin,[5] and creatinine was determined by the colorimetric method of Folin and Wu.[6]

Excised muscle fragments were initially used for infectivity tests. Subsequently, preparations consisting of pools of the extremities, including bone and fascia, were used.

Potassium and Creatinine Determinations. Potassium determinations were made of samples of muscle from 24 normal and 23 paralyzed suckling mice. The mean for the first group was 2.96 ± 0.16 mg per g of tissue, and that of the experimental group was 2.08 ± 0.28 mg per g. The number following the ± sign is the mean deviation. The greater value for the experimental group may be due to the uneven distribution of the muscle lesions. A striking feature of this experience was that all but one of the potassium values of the paralyzed animals were less than the least value of the controls.

Creatinine determinations were made of 8 samples of each of the above groups. The

[3] Thompson, W. R., *Bact. Rev.*, 1947, **11**, 115.

[4] Van Slyke, D. D., and Rieben, W. K., *J. Biol. Chem.*, 1944, **156**, 743.

[5] Rose, W. C., Helmer, O. M., and Chanutin, Alfred, *J. Biol. Chem.*, 1927, **75**, 543.

[6] Folin, Otto, and Wu, Hsien, *J. Biol. Chem.*, 1919, **38**, 81.

TABLE II.
Infectivity of Pools of Legs and of Brains of Paralyzed Mice.

Source of virus	Test animals	Dilutions of virus								
		10-1	10-2	10-3	10-4	10-5	10-6	10-7	10-8	10-9
Suckling mice legs	Suckling mice 4-5 days				8/8	8/8	8/8	7/8	0/7	1/7
" " "	Weanling mice 7-8 g	9/9	8/10	3/10	1/10					
" " "	" " 8-10 g	0/10	0/10	0/10	0/10					
" " "	Mice 10-12 g	0/5								
" " "	" 14 g	0/5								
Suckling mice brains	Suckling mice 4-5 days	8/8	8/8	4/8	0/7	0/7				
" " "	Weanling mice 7-8 g	1/10	1/10	1/10	0/10	1/10				
Weanling mice legs	Suckling mice 7 days				7/7	8/8	7/7	4/8		
" " "	Weanling mice 7-8 g	7/7	4/7	2/7	2/7					
Weanling mice brains	Suckling mice 7 days	7/7	8/8	2/7	0/7					
" " "	Weanling mice 7-8 g	0/7	0/7	0/7	0/7					

Note: No. of mice paralyzed or dead/No. of mice inoculated.

mean concentration of total creatine in the control group was 1.95 ± 0.15 mg per g and for the experimental group, 0.96 ± 0.23 mg per g.

The concentration of total creatinine (creatine plus creatinine) in the urine of various normal and infected suckling mice is shown in Table I. The mean for the normal animals was 0.35 mg per ml, while the concentration in diseased animals was frequently ten times as great. Increased excretion of creatinine occurred in two instances before the onset of symptoms. It should be noted that the samples from the sick mice were smaller. This is thought to have been due to the smaller size of the affected animals, since a comparison of their urinary pigments did not indicate that their urine was more concentrated. Stunting is characteristic of suckling mice infected with the virus. Whether this is a result of intrinsic changes or inferior nursing we do not know.

Infectivity of muscle. The muscles of paralyzed mice are more infectious than the tissues of the central nervous system. Suspensions prepared from pooled legs are roughly ten thousand times as infectious for suckling mice as those prepared from brains (TableII). When 10- to 12-g mice are inoculated with brain suspensions the organs contain little or no virus and while an occasional focus of muscle degeneration may sometimes be found, the mice exhibit no recognizable signs of infection. On the other hand, 10- to 12-g mice inoculated with muscle suspensions are sometimes paralyzed and weanling mice (7 to 8 g) are usually paralyzed. These observations prompted us to attempt to adapt the virus to 10- to 12-g mice by alternate passage[7-10] through suckling mice. Six alternate passages have shown no increase in infectivity and the brains and muscles of the older mice remain less infectious than those of suckling animals. Serial passages in older mice have also failed.

The greater infectivity of muscle suspensions provided effective antisera from recovered hamsters and a rhesus monkey. The monkey showed no signs of infection following intramuscular inoculation but developed humoral antibodies after a series of intraperitoneal injections. Paralysis and muscle lesions were induced in one of 6 new-born guinea pigs inoculated intraperitoneally with 0.5 ml of a 10% suspension of hamster legs. The paralyzed extremity was infective for suckling mice, while the brain was not.

Virus has been found in the feces of 7- to 8-g mice on the day of paralysis but not in the feces of 18-day-old hamsters that had been paralyzed for 2 days.

Limited tests with a few of the strains of

[7] Baker, J. A., *Amer. J. Vet. Res.*, 1946, **7**, 179.

[8] Baker, J. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 183.

[9] Koprowski, Hilary, James, T. R., and Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 178.

[10] Dean, D. J., and Dalldorf, Gilbert, *J. Exp. Med.*, 1948, **88**, 645.

virus that affect both central nervous system and muscle have shown that suspensions of legs are either less virulent or about as virulent as suspensions of brains. This conforms with the signs of infection, with the histologic findings, and with the degree of creatinuria associated with the two types of disease.

Discussion. Whether or not these observations have application in clinical medicine is unknown. Creatinuria follows paralytic poliomyelitis[11-13] but has been assumed to be a sequel of neurone destruction. Since there is some evidence that muscle degeneration may occur in the initial stages of the disease,[14] the subject should be re-examined. Hassin described a case of poliomyelitis of the Landry type in which the lesions were restricted to the muscles.[15] Clawson is reported to have found no muscle lesions in a series of 22 cases of acute poliomyelitis[16] but Dr. Kornel Terplan, Buffalo General Hospital Laboratory, provided us with histologic preparations from a case of fatal bulbar poliomyelitis in which extensive muscle lesions similar to those which occur in suckling mice were seen.

The infectivity of the muscles is consistent with the morphologic changes and reaffirms the peripheral nature of the paralysis in the mice. It is interesting that the higher titer of the muscle preparations makes it possible to induce paralysis in mice of an age-group that is resistant to infection with brain suspension. While these findings modify, they also confirm the original observation of the remarkable natural immunity of mice and hamsters following weaning. This is further supported by failure to adapt the T.T. strain to weaned mice by alternate passage through susceptible animals.

Summary. Infection of unweaned mice with certain strains of "Coxsackie virus" is followed by loss of muscle potassium and creatinine and by creatinuria.

The muscles of paralyzed mice are highly infectious.

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12 Magers, E. J., *J. Biol. Chem.*, 1934, **105**, lvi. *(Sci. Proc.)*.

13 Wang, Erling, *Acta Med. Scand.*, 1939, supp. 105, p. 1.

14 Carey, E. J., Massopust, L. C., Zeit, W., and Haushalter, E., *J. Neuropath. and Exp. Neurol.*, 1944, **3**, 121.

15 Hassin, G. B., *J. Neuropath. and Exp. Neurol.*, 1943, **2**, 293.

16 Bell, E. T., The Progressive Pathology of Poliomyelitis. In: Poliomyelitis. Papers and Discussions presented at the First International Poliomyelitis Conference. J. B. Lippincott, Philadelphia, 1949, p. 135.

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17267. The Role of Complement in the Lysis of Leucocytes by Tuberculoprotein.*

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The nature of the mechanism underlying delayed or tuberculin-type hypersensitivity has long interested students of immunology. Whereas many workers have attempted to demonstrate an antibody in tuberculin allergy which was similar in properties and mode of action to antibodies known to be responsible for anaphylactic or Arthus type of hypersensitivity, the preponderance of such studies has led to the assumption that cells, rather than serum antibodies, were the mediators of tuberculin hypersensitivity. Holst[1] first noted

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1 Holst, P. M., *Tubercle*, 1922, **3**, 337.