

TABLE I.

Exper. Condition	Days on Diet	Body wt. in gm.			Cc. O <sub>2</sub> used per M <sup>2</sup> of Surface Area per Hr.		
		Initial	Maxi-	Terminal	Initial	Final	Aver. all tests
			mum				
Controls	118	258	511	495			6278
Males, Diet 13	69	342	422	386	6897	6574	6314
Females, Diet 13	102	278	412	390	6193	6645	6433

The data (Table I) show that no significant difference exists between the metabolic rates, expressed as cc. of O<sub>2</sub> used per hour per square meter of surface area, of control and dystrophy animals. Furthermore, the rates of oxygen utilization by the animals in the early days of the experiments were essentially the same as that found in the terminal experiments on the same animals.

If an endocrine disturbance existed in such animals, reared under the above conditions, it was not of such a nature or degree as to be reflected in their rates of oxygen utilization. These observations on the intact animal offer no explanation as to the cause of the increased oxygen consumption by excised skeletal muscle from animals under similar dietary conditions.

*Summary.* Guinea pigs reared on a vitamin E deficient diet and exhibiting typical experimental muscular dystrophy show normal metabolic rates as determined by oxygen consumption measurements.

### 9383 P

#### Recovery of Influenza Virus from Chicago Epidemic.

J. MARION CLAMPIT AND F. B. GORDON. (Introduced by G. M. Dack.)

*From the Department of Bacteriology and Parasitology, University of Chicago.*

In the original isolation of the virus of human influenza, ferrets were used as the experimental animal.<sup>1</sup> Later it was found that after a few passages in ferrets the virus became pathogenic for young white mice.<sup>2</sup> Since some of the strains required only one or 2 ferret passages before becoming pathogenic for mice, numerous attempts have been made to infect mice by means of material taken directly from patients. Francis and Magill<sup>3</sup> have recently succeeded

<sup>1</sup> Smith, W., Andrews, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.

<sup>2</sup> Andrews, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2**, 859.

<sup>3</sup> Francis, T., and Magill, T. P., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 132.

in infecting mice with patients' nasal washings concentrated by ultracentrifugation. The disease in mice is characterized by loss of appetite, respiratory difficulty, malaise, and death usually by the fifth day. Lung consolidation with a mononuclear cell infiltration is the essential feature of the autopsy findings.

Using direct mouse inoculation we were able to recover a filterable infectious agent, identified as human influenza virus, from each of 9 specimens of sputum and nasal washings. These were obtained from 7 patients during a small epidemic of influenza in Chicago early in December, 1936. The material was collected 12 to 72 hours after onset, taken to the laboratory, ground with powdered glass and emulsified with veal infusion broth. After light centrifugation the supernatant material was used for inoculation of mice, according to the method originated by Shope<sup>4</sup> in his work with swine influenza. This consists of placing the nose of the anesthetized mouse under the surface of the inoculum for several breaths. Inoculations of passage mice were made in the same manner by means of a heavy emulsion of lung tissue. Four to 6 young mice were used for each of the original inoculations and 3 for each subsequent passage. The mice were sacrificed at 5-day intervals and the lungs examined for gross lesions. For the first passages pooled material from all of the lungs were used for inoculum. Later only consolidated portions were used. The strains varied in producing initial consolidation; the earliest appeared in the 3rd passage, the latest on the 8th. Five to 13 passages were necessary before consistent complete consolidation was produced, usually with death by the fifth day. After becoming adapted, all 9 strains produced identical results in mice. The gross and microscopic pathology were similar to that of lungs infected with a known influenza virus.

Evidence that this condition was caused by a filterable agent consists of the following: (1) Aerobic and anaerobic cultures of the lungs were usually negative after the first passage. A gram negative rod was cultured inconstantly. (2) Eight of the 9 strains have been filtered through a Berkefeld N candle, the filtrate producing consolidation in all cases. No aerobic or anaerobic bacteria could be cultured from the filtrate. (3) Two of the strains have been stored in 50% glycerin for 3 months without noticeable loss in virulence.

A control series was run exactly as in the original isolations except that an emulsion of a normal mouse lung was used as the first inoculum. No consolidation has occurred in 9 passages. Intraperitoneal injection of mice with an infectious lung emulsion had no effect,

---

<sup>4</sup> Shope, R. E., *J. Exp. Med.*, 1935, **62**, 561.

which indicates that the virus of ectromelia, an infectious disease of mice, is not present.

The circumstances of isolation and the pathology produced suggested that we were dealing with human influenza virus. Positive identification was accomplished by means of cross neutralization tests with a known human influenza virus, the PR8 strain. Antisera were obtained by intraperitoneal inoculation of the various strains into rabbits. For the test equal parts of a 2% emulsion of consolidated lungs and dilutions of antisera were mixed and incubated for 30 minutes at 37°C. Three mice were inoculated intranasally with each mixture. On the seventh day all survivors were sacrificed and the lung consolidation recorded. The results of the test are given in Table I. From these results our strains tested to date seem to be identical serologically with the PR8 strain.

TABLE I.  
Results of Cross Neutralization Tests with Strains of Influenza Virus from Chicago Epidemic.

Antisera		Viruses								
Strain	Dilution	PR8		W2		Pe				
PR8	1/1	0	0	0	0	0	0	0	0	0
	1/5	0	0	0	0	0	0	0	0	0
	1/25	0	0	0	1	2	2	2	2	2
	1/125	0	4	4	2	3	3	3	4	4
W2*	1/1	0	0	0	0	0	0			
	1/5	0	0	0	0	0	0			
	1/25	0	1	1	1	1	1			
	1/125	2	4	4	3	4	4			
Pe*	1/1	0	0	0				0	0	0
	1/5	0	0	0				0	0	0
	1/25	0	1	4				0	1	2
	1/125	4	4	4				3	4	4
V.I.B.		4	4	4	4	4	4	4	4	4
N.R.S.		4	4	4	4	4	4	4	4	4

Each number represents one mouse and refers to the degree of lung consolidation. 0 = no consolidation. 4 = total consolidation.

V.I.B. = veal infusion broth. N.R.S. = normal rabbit serum.

\*Strains isolated from Chicago epidemic.

*Summary.* A filterable agent has been recovered from sputum and nasal washings of patients in the acute stage of influenza. This was accomplished by direct intranasal inoculation of mice. Evidence has been produced which indicates that the strains are human influenza virus.