Hodgkin's diseased glands were reported as negative in 3 cases by Fox and Farley.[3]

Here we are further recording the negative results obtained in 11 patients with Hodgkin's disease treated by Roentgen ray in whom the diagnosis was assured by biopsy. All but one patient showed a skin reaction of only a few millimeters, similar to that seen in normal persons. In this one the control, saline and 0.3% tricresol measured 0.5 cm. and the unheated extract measured 1.0 by 1.5 cm. at 24 hours, but after 48 hours measured 1-2 mm.

Extracts were made from the lymph nodes of an untreated case of Hodgkin's in the third month of the disease and also from a case of lymphosarcoma. The clinical course of these cases has verified the histological diagnosis. The fresh nodes were extracted with saline, centrifuged and 0.3% tricresol added to the cloudy, supernatant fluid. One-half of this was heated for an hour at 60°C. on 2 successive days. The final extract was not filtered.

7226 P

Etiology of Whooping Cough.*

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Three beliefs exist in regard to the etiology of whooping cough: That the disease is caused by *H. pertussis,* that the agent is a filtrable virus, or that both play a part. *H. pertussis* is practically always present in the disease and absent in health.[1] That the organism plays a most important rôle has been shown by occasional successful transmission experiments[2, 3, 4] and by reports of effective protection with suitable *H. pertussis* vaccine.[1, 4, 5] The recent description of intranuclear inclusion bodies[6] taken together with certain other considerations has led some[3, 7] to believe that a virus plays either a

[3] Fox, H., and Farley, D. L., *Am. J. Med. Sci.*, 1922, **163**, 313.

* Aided by a grant from the Rockefeller Foundation.

[1] Miller, J. J., *J. Am. Med. Ass'n.*, 1933, **100**, 681; excellent summary.

[2] Sauer, L. W., and Hambrecht, L., *Am. J. Dis. Child.*, 1929, **37**, 732.

[3] Rich, A. R., Long, P. H., Brown, J. H., Bliss, E. A., and Holt, L. E., *Science*, 1932, **76**, 332.

specific etiological rôle or, accompanied by *H. pertussis,* acts as primary infective agent. Attempted transmission experiments with filtrates have been equivocal or negative.[3, 4] Because of too recent isolation of the organism or of inoculation with filtrate shortly before[4] the few successful transmission experiments with *H. pertussis* are open to the criticism that a virus may have been present. It seemed necessary to devise experiments in which *H. pertussis* or virus effects were clearly separated. In this communication we are reporting successful whooping cought transmission by means of what we believe to be virus-free *H. pertussis.*

The newer knowledge of the phases of *H. pertussis*[8, 9] and their precise differentiation by electrophoresis[10] provided the desired approach. When first isolated *H. pertussis* differs culturally, morphologically, serologically, and electrophoretically from older laboratory cultures. Leslie and Gardner call the former, Phase I, and the variations rapidly appearing on subculture, Phases II, III, IV. It is possible[9, 10] that this change represents the well-known virulent ("S") to avirulent ("R") transition of other bacteria.

Our plan was to study, identify, and maintain this theoretically infective phase† for months, and then, when this had been done long enough to preclude the presence of a virus, to attempt transmission. Accordingly, we selected several fresh strains of *H. pertussis* of our own and from other laboratories and during several months checked them periodically for Phase I persistence. Our experimental animals were chimpanzees. In inoculating animals we attempted to reproduce natural, droplet, inhalation infection by spraying the organisms into the air about the animals' faces. Also, we added a few cc. of living cultures to their milk. This was done 3 successive days. During experiments animals were placed in rigorous isolation.

While doing the preliminary work referred to, we attempted transmission of whooping cough with a Phase IV, theoretically avirulent, strain ("A" No. 61 N. Y. B. of H.) After 2 weeks' isolation the animal was inoculated. No infection resulted. This conforms

[4] Macdonald, H., and Macdonald, E. J., *J. Inf. Dis.*, 1933, **53**, 528.

[5] Sauer, L. W., *J. Am. Med. Assn.*, 1933, **101**, 1449.

[6] McCordock, H. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1288.

[7] Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1932, **51**, 346.

[8] Leslie, P. H., and Gardner, A. D., *J. Hyg.*, 1931, **33**, 424.

[9] Lawson, G. McL., Thesis, 1932, Harvard School of Public Health.

[10] Shibley, G. S., and Hoelscher, H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 31.

† These studies will be the subject of another communication.

with Toomey's similar recently reported findings.[11] Six weeks later with the same animal we attempted transmission with a recently isolated Phase I strain ("No. 13," subcultured 4 times). Fifteen days later a respiratory infection developed characterized by slight nasal discharge and short hacking, occasionally paroxysmal cough. It lasted 14 days. The leucocyte count remained within normal limits. *H. pertussis* was not recovered. Whether this represented a virus cold, mild pertussis or a modified attack due to low virulence of the organism or to previous inoculation with Phase IV cultures, we cannot decide.

By October a second ape was tame enough for use. From strains under observation, we selected an organism seeming to possess all Phase I criteria (strain "Z" obtained from Dr. Hans Zinsser). The strain isolated April 20th and received May 20th, had been subcultured by us over 60 times.

After 5 weeks' isolation, the ape was inoculated with the theoretically infective and virus-free strain. On the 9th day the animal developed slight cough. Five days later it became paroxysmal, mounting to a series of 25-35 successive explosive coughs. Vomiting occasionally succeeded coughing. On the 19th day whoop, previously doubtful, definitely appeared. To this point, though cooperation was bad, we had failed to recover *H. pertussis* on cough plates though subsequently we succeeded in isolating the organism from nose cultures obtained on the 14th day. The disease was so typical clinically that we decided to sacrifice the animal. The white blood cell count on this date was 132,000, lymphocytes: 78.5% (average normal WBC 13-20,000, L. 60-70%).

At autopsy, cultures were taken from upper trachea and its bifurcation, left main bronchus, and small bronchi and bronchioles of both lower lobes. *H. pertussis* was recovered from all places cultured in large numbers. The organism was typical culturally, morphologically, and electrophoretically and agglutinated with our Phase I serum to full titer (1:20,000). A few colonies of *Streptococcus* were present on some plates. In no instance, despite painstaking search, were we able to demonstrate *H. influenzae* although we recovered it in nasal cultures taken during life.

Dr. Harry Goldblatt of the Institute of Pathology kindly performed the post mortem examination. The findings were limited to the chest and were consistent with the usual findings in pertussis. The anatomical diagnosis was acute catarrhal tracheitis, bronchitis,

[11] Toomey, J. A., McClelland, J. E., and Lieder, L. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 403.

and bronchiolitis, both endo- and peri-, and severe acute emphysema. A typical finding was large and small mononuclear cell infiltration of subepithelial stroma and of tissues surrounding some bronchi and many bronchioles. Consolidation of the lungs was not found. No inclusion bodies were found. Although we recovered *H. pertussis* from bronchi and bronchioles, we failed to demonstrate to our complete satisfaction *H. pertussis*-like organisms embedded in the cilia.

7227 P

Inhibition of Cardiac Accelerator Impulses by the Carotid Sinus.*

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It has been shown by Hering,[1] Heymans,[2] and others that stimulation of the afferent endings in the carotid sinus by an increase in the endosinusal pressure causes a slowing of the heart. Some have held that this is entirely due to a reflex stimulation of vagal fibers while others have presented evidence to show that there is a reflex inhibition of the cardiac accelerators as well. The second explanation involving a reciprocal mechanism has generally appeared to be the more probable. In the present investigation we have studied certain aspects of this reflex by observing the sympathetic impulses to the heart while varying the pressure in the carotid sinus.

The experiments were performed on cats under nembutal anesthesia. The carotid sinus on each side was isolated and perfused by way of the common carotid and external carotid arteries. Inasmuch as the nerve supply remained intact an increase of pressure within the sinus caused an increase in the number of afferent impulses going to the medullary centers.[3] The effect of these impulses on the cardiac accelerator discharge was determined by record-

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1 Hering, H. E., *Die Karotissinusreflexe.* Dresden, T. Steinkopff, 1927.

2 Heymans, C., Bouckaert, J. J., and Regniers, P. *Le Sinus Carotidien.* Paris. G. Doin, 1933.

3 Bronk, D. W., and Stella, G., *J. Cell. and Comp. Physiol.*, 1932, **1**, 1.