As is seen from Table II, in 3 cases (Nos. 3, 4, and 8) the concentration of estrogen in the umbilical vein was the same as in the retro-placental blood of the mother. In the other cases the concentration of estrone in the umbilical vein was lower; the minimum ratio was 27%; the maximum ratio 80%; and the average 68%.

Results. The above experiments show that the concentration of gonadotropin in the blood serum of the umbilical vein is far lower than that of the corresponding retro-placental blood serum. Thus it appears that the placenta allows the passage of gonadotropic hormone only to a very limited extent. Goodman and Wislocki[4] obtained similar results in their experiments on animals. They injected gonadotropin in pregnant rabbits or cats, but were unable to recover the injected substance from the amniotic fluid.

Determination of the estrogen content of the retro-placental and umbilical serum showed the concentration to be more or less equal in either case. The differences were at least not as pronounced as those in gonadotropin content. The placenta is, therefore, to a great extent permeable to estrogenic hormone.

Summary. The free estrogen and gonadotropin content of the serum from the umbilical vein and the corresponding maternal retro-placental serum were studied. The maximum concentration of gonadotropin in the serum of the umbilical vein was 12.5%; the minimum 1.5%; and the average 4.38% of that in the retro-placental blood.

The concentration of estrogen in the umbilical vein was either equal or only slightly different from that of the retro-placental blood. It averaged 68% of that in the retro-placental blood.

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Ornithosis (Psittacosis) in Pigeons and Its Relation to Human Pneumonitis.

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In a previous paper,[1] it was reported that a psittacosis-like virus had been isolated from the lung of a patient who had been exposed

4 Goodman, L., and Wislocki, G. B., *Am. J. Physiol.*, 1933, **106**, 323.

1 Meyer, K. F., *Schweiz. med. Wchnschr.*, 1941, **71**, 1377.

to a flock of racing pigeons *(Columba livia)*. Nineteen, or 63%, of the 30 suspected birds gave specific complement-fixation reactions in dilutions of from 1 :8 to 1 :256 (Table I). A virus, indistinguishable from that of the patient, was isolated from the spleens and livers of 4 pigeons which had given a positive, and from 1 pigeon which had given a negative serum reaction. This observation was the prelude to the recognition of the nation-wide existence of a psittacosis-like infection in pigeons—in the future to be described as *"Ornithosis."* At least 10 human atypical pneumonias with 2 deaths [California 4 (2 deaths), New York 4, Minnesota 1 and Boston 1] have been traced to exposure to the different species of pigeons exclusively.

Survey of Pigeon Lofts and Isolation of Virus. In order to secure comparative data relative to the extent of *ornithosis* in lofts and to have available suitable birds for experimental tests, random samples of pigeons were secured from various places in California and other states of the Union. With the exception of 12 squabs from a loft whose old birds were virus-carriers, a universal existence of the infection was recognized with the aid of complement-fixation tests. The virus responsible for these reactions was isolated from the organs of non-reacting as well as of reacting pigeons. The pertinent data thus far obtained are summarized in Table I.

The extraordinarily high percentage of positive results will doubtless raise questions regarding the specificity of the serum-reactions. There is conclusive evidence that they are the result of a response to a psittacotic antigen. Pigeons whose serum is free from antibodies promptly generate them following the intramuscular injection or feeding of parrakeet-psittacotic or pigeon ornithotic viruses. Moreover, a flock of birds in which 60% of reactors had been demonstrated, were used as test animals for a bacterial biological product. At the end of the experiment they were bled again, and 95% of the sera gave positive reactions in the higher dilutions. This observation may correctly be interpreted as a reactivation of antibodies indicative of a previous contact or experience of the pigeons with the ornithotic antigen. The serum-reactions have been correlated with the carrier-state of the birds. The organs of 6 Visalia and 10 Hanford pigeons, the latter connected with a fatal infection, were individually tested in mice. From the Visalia pigeons 4 isolations were successfully obtained, 1 from a reacting, and 3 from non-reacting birds; from the Hanford pigeons 4 isolations were successfully obtained from the 7 reacting birds, and none from the non-reacting birds. No satisfactory explanation is as yet possible for the discrepancies. However, it is not unlikely that the positive complement-

TABLE 1.
Complement-Fixation Reactions of Pigeon-Sera with Ornithotic Virus Isolation of Virus.

California.

Titer	San Leandro Old	Hayward Young	Hayward Old	Stock Old	Racing pigeons	Visalia	Pasadena I Source of fatal case Old ♂	Pasadena I Source of fatal case Old ♀	Pasadena II Old	Hanford Source of fatal case Old	El Monte Source of case ♂	El Monte Source of case ♀
0	6	12	6	6	1	4	3	8	(9)	3	4	2
1:2												1
1:4	1										1	
1:8	1		1					1				1
1:16	1		1	6							1	
1:32	1		1	1	1		4	2	2	2		
1:64	2			1	2		4		2	3		
1:128			2	2		2	3	4				2
1:256			1					1		2		
Total	12	12	12	16	4	6	30		13	10	12	
% reactors	50%	0	50%	62%	75%	33%	63%		30.7%	70%	42%	
Isolation of virus	Not attempted	Not attempted		1	1 C.F.—	4 1 C.F.+ 3 C.F.—	Incomplete 5 4 C.F.+ 1 C.F.—		Incomplete 1 1 C.F.—	4 4 C.F.+	3 1 C.F.+ 2 C.F.—	

United States.

Titer	Indiana Old	Iowa Farm A	Iowa Farm B	Iowa Farm C	N. Y. City Old	Albany, N.Y.	Brooklyn Source of case	South Carolina Young	
0	10	8	2	6	10	15	1	6	
1:2	2								
1:4		1		1	5				
1:8	4				3		1	3	
1:16	2				3		1	1	
1:32	2	1			4				
1:64	2	3		1	2			1	
1:128	2				3		1	1	
1:256	1						1		
Total	25	13	2	8	30	15	5	12	Total
% reactors	60%	38%	0	25%	66%	0	80%	50%	*237*
Isolation of virus	Not tested		Not tested		15 tested *3* C.F. +	2+	Incomplete	*1* 1 C.F.—	25 virus strains

fixation reaction is merely indicative of a past or latent infection, and not necessarily of an active one. There still exists the other possibility that the virus may reside in organs other than the spleen, liver and kidneys which were used as inocula.

The two lots of pigeons offered certain advantages for successful isolations, since the birds were not simultaneously infected with *Salmonella typhi murium*. Concentrated organ-emulsions were well tolerated by the mice, and the ornothotic virus during its residence for 20 days in the spleens was sufficiently enriched so that the intracerebral transfer in the next passage produced lesions and microscopical findings; it was not necessary to filter the suspensions through a collodion membrane of over 400 mμ pore-width. Moreover, one of the Visalia pigeons, with a complement-fixation titer of 1:128++++, presented at necropsy a residual plastic pericardial exudate of recent origin, which showed suggestive pale intracellular plaques but no definite elementary bodies. A flake of the exudate suspended in a few drops of broth and inoculated intracerebrally in mice produced the typical ornithotic choriomeningitis, and in the next passage by intranasal injection a focal pneumonia.

Parasitism of Ornithotic Virus. The serological reactions and the isolation of 25 viral strains from a relatively small cross-country sample makes it clear that ornithosis of pigeons is probably ubiquitous. The agent possesses a highly adapted latent parasitism for pigeons which is somewhat similar to that of the psittacotic virus encountered in the parrot-species of the Australian regions. How it escapes the body of the birds is unknown, although suspicion is cast on the urine and the fecal droppings.

Infection appears to be acquired in the nests or at least in youth. Recovery from this early contact with the parasite ensures a lifelong relative resistance. But, since it is frequently associated with latency, the balance of the immunity may become disturbed in favor of the virus. That improper feeding may arouse these subclinical infections, in particular, that a thiamin-deficient diet may activate them, is beautifully illustrated by the observations of Pinkerton and Swank.[2] Likewise, the accidental crowding of a dozen pigeons in a poorly lighted, unsanitary cage in the Hooper Foundation induced frank clinical ornithosis and death of 6 birds. The nature of the illnesses and the necropsy findings were at first mistaken for pigeon-paratyphoid, since cultures yielded *Salmonella typhi murium*. The emaciated cadavers presented a fibrinous peritonitis

[2] Pinkerton, H., and Swank, R. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 704.

and pericarditis, enlarged spleens (average 8 x 18 mm) with subcapsular hemorrhages, soft enlarged hyperemic livers, grayish kidneys, and catarrhal enteritis. The pneumonic foci reported for pigeon-paratyphoid were likewise present in 2 birds. Microscopical examinations of the serous exudate revealed, aside from the Gram-negative rods, an enormous number of bodies like those found in psittacosis. It is not unlikely that the relationship of the bacterial carrier-stage to the latent ornithosis is the same as that proven for the South American parrots.[3]

The Virus. Morphologically and tinctorially, the *Microbacterium multiforme* of pigeon origin is indistinguishable from that originally obtained from Australian and South American parrots or American parrakeets, or from human infections traced to such sources. It is present in large numbers, free or in monocytic cells of avian or mammalian tissues, or in Zinsser-Fitzpatrick-Wei cultures. Organ suspensions, or cultures of these organisms produce in mice, ferrets and hamsters on intranasal injections, or in guinea pigs and monkeys on intratracheal injection, focal or lobular pneumonias of the same character as induced with the true psittacotic viruses. Even continuous passage through mice fails to enhance the pathogenicity, and fatal infections on intraäbdominal injections are rarely observed except upon the administration of overwhelming doses. This peculiar low pathogenicity for mice has likewise been noted by Coles[4] in connection with his studies of pigeons found infected with "psittacosis" in Johannesburg, Union of South Africa. However, recent observations indicate that occasionally a virus of greater mammalian pathogenicity may be encountered.

Parrakeets and ricebirds *(Munia oryzivora)* are fatally infected by feeding or by inhalation. Pigeons and an occasional dove *(Streplopelia?)* are clinically not affected by feeding or intramuscular injections, but continue to carry the virus in the organs for many weeks. Since Bedson and Western[5] failed to infect pigeons, it was generally believed that individual birds are refractory to the disease. In this connection, it is important to recall that doves *(Streplopelia decaocto* and *Streplopelia semitorquata)* may become infected spontaneously with the psittacotic virus of parrot or parrakeet origin when exposed in zoological gardens (Tomlinson[6]). It was noted in

[3] Meyer, K. F., and Eddie, B., *J. Infect. Dis.*, 1939, **65**, 234.

[4] Coles, J. D. W. A., *Onderstepoort J. Vet. Science and Animal Industry*, 1940, **15**, 141.

[5] Bedson, S. P., and Western, G. T., *Reports on Public Health and Medical Subjects*, N. 61, London, 1930, 84.

[6] Tomlinson, C. T., *Pub. Health Rep.*, 1942, **56**, 1073.

work done in coöperation with Dr. H. Pinkerton, that the ornithotic viruses quite regularly cause fatal meningitic infections, even in pigeons whose sera are strongly positive in the complement-fixation test; while the parrot- or parrakeet-psittacotic viruses, of either avian or human origin, induce these infections only in a few individual pigeons, and only when the viruses are recently isolated.

Crude antigenic analyses have shown no differences in the composition of the various strains, or differences between the psittacotic or ornithotic agents. These pigeon-viruses are indistinguishable from the "meningo-pneumonitis" virus of Francis and Magill,[7] which in comparative tests proved fatal to doves and pigeons on intracerebral, and to ricebirds and parrakeets on intramuscular injections.

The Relationship of Pigeon-Ornithosis to Human Illness. Epidemiologically, the evidence in its broader aspects grew more convincing as the observations became more complete. Due to unavoidable delays or late reporting, the complete transmission-chain, consisting of finding the virus in the patients and then in the birds, has not been established in every case. For example, a patient in New York with an atypical pneumonia and serum-reaction of 1:128+++ on the 16th day, produced sputum on the 19th day, from which a pigeon virus was isolated. He had a brief exposure to barnyard birds, including pigeons, in up-state New York. It was impossible to secure specimens of sera or cadavers of the birds.

However, the difficulties are in no way different from those experienced in connection with psittacosis due to exposure to parrots and parrakeets. A part of the chain depends on inductive evidence supported by a fairly dependable serological test. The early victims of ornithosis had either handled clinically diseased birds, or had been near or within lofts or barns in which they had been housed; the more recent histories fail to stress these observations. One would suspect by analogy with true psittacosis that pigeons in the active stage of infection discharge more virus and are more dangerous spreaders than the apparently healthy latent carriers. In the rural environment, or on the plazas and parks of towns and cities, large groups of people are constantly exposed to pigeons whose sera react in the complement-fixation tests. With such a vast reservoir of ornithotic virus available everywhere, one wonders why many sporadic atypical pneumonias (colloquially and wrongly diagnosed as "virus" pneumonias) are not in some way connected with these sources. An inquiry into the existence of subclinical or latent infections in certain groups would indeed be of interest. That they

[7] Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1938, **68**, 147.

occur is attested by the observation that the pigeon-fancier, whose father died from ornithosis, had specific antibodies against the virus in his serum. Aerogenic contamination of the respiratory mucosa by the public in a great many civic places can be readily visualized. A patient may not have been cognizant of this exposure. Thus, the fortuitous isolation of a saprophytic ornithotic virus from nose- or throat-washings, in the course of an attack of influenza or a cold, might often happen.

In the future, it appears highly desirable (a) that the sera of patients with atypical pneumonias, influenza and other respiratory infections, in the absence of specific bacterial agents, be tested for specific antibodies against the psittacotic or ornithotic virus, (b) that in the course of epidemiological investigations, the prolonged or even fleeting exposure to pigeons or barnyard birds like fowls and ducks be kept in mind, and (c) that the term psittacosis be limited to the human infections definitely proven to be of association with psittacine birds, while those due to contact with other birds be described as ornithosis of fulmar, pigeon, or chicken origin.

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Toxicity of Washings from *Hemophilus pertussis* for Mice.*

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While studying the toxic substance that can be obtained by growing *Hemophilus pertussis* in a liquid medium, as described by Wood,[1] we have observed that a substance with similar toxic properties can be obtained by simply washing the organisms of freshly isolated strains grown on a solid medium. After 48 hours of growth, the organisms are removed from the surface of Bordet's medium with a cotton swab and suspended in 0.85% NaCl solution. When density of the suspension has been adjusted to approximately 40 billion organisms per ml, the suspension is thoroughly mixed for 5 minutes with a pipet. The organisms are then sedimented in an angle centrifuge at a temperature of 4°C. After several periods of centrifuga-

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[1] Wood, M. L., *J. Immunol.*, 1940, **39**, 25.