velopment is not confined to calf serum. We have observed the same phenomenon in the serum of young rats and in other cases that will be discussed in a later publication.

Summary. (a) The serum of new-born calves, before the ingestion of colostrum contains no γ globulin and only small amounts of β globulin. (b) During the nursing period the composition of calf serum changes rapidly. The γ globulin appears. Both γ and β increase in concentration and then decrease. The concentration of α and albumin decrease during nursing, with that of the albumin finally increasing. (c) During the period of greatest change in composition of the serum, the protein fractions exhibit an abnormally high mobility.

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Complement-Fixation in Human Pneumonitis with Group-Reactive Virus Antigens.*

MONROE D. EATON AND MARILLA COREY.

From the Research Laboratory, California State Department of Public Health, Berkeley.

Some time ago we reported the isolation of a virus from 4 cases of severe atypical pneumonia by direct intranasal inoculation of mice.[1] This agent was at first thought to be a variant of psittacosis virus, but subsequent investigations have shown that it is related to a group of viruses which includes not only psittacosis, but also the virus of lymphogranuloma venereum,[2] the virus of meningopneumonitis,[3] and a virus of mouse pneumonia recently isolated by Nigg.[4] The members of this group of agents are characterized by the formation of minute coccoid elementary bodies, by the possession of a common complement-fixing antigen,[5, 6] and by similarities in pathogenicity for experimental animals.

These viruses have been isolated not only from man and birds, but also from ferrets and mice by intranasal passage of lung material. A virus isolated from pigeons by Pinkerton and Swank[7] is apparently very similar to the meningopneumonitis virus.[8] However, this latter agent was shown[1] to differ from the virus isolated by us from cases of atypical pneumonia in its greater virulence for Java rice birds and for mice by the intraperitoneal route, and by its ability to induce a carrier state in mice. Furthermore, antigenic differences between the virus of human pneumonitis and the viruses of meningopneumonitis and psittacosis have been demonstrated by active immunity tests in mice.[9] This, however, does not exclude the possibility that pneumonitis or other respiratory diseases in man may also be caused by psittacotic viruses carried by pigeons[10, 11] or even by similar agents which are carried by animals.[3, 4]

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TABLE I.
Cross Reactions by Complement Fixation of Human Sera from Cases of Pneumonitis and Lymphogranuloma Venereum.

Serum	Disease	*Complement fixation titers with antigens of		
		Lymphogranuloma venereum	Meningo-pneumonitis	Mouse pneumonia
St.	Pneumonitis†	4/16	8/32	4/8
Li.	”	8/32	16	4/16
Me.	”	4/32	4/16	8/32
Wk.	”	8/8	4/4	4/8
Ca.	”	4/16	4/4	0/16
Go.	”	4/4	2/4	0/4
Lc.	”	4	32	4
Po.	”	16	0	16
Mc.	”	0	0	16
Kl.	”	16	4	16
Pr.	Pneumonitis‡	16	4/16	32
Bc.	”	64	16/64	—
Cr.	”	64	0/32	64
H9	Lymphogranuloma venereum	64	16	16
H10	” ”	32	16	16
H75	” ”	512	—	32
H76	” ”	512	128	16

*In the three columns to the right, numerator is the titer of serum specimens taken early in the disease; denominator the titer of serum specimen taken late in the disease. Where only one titer is given, this is for the late, or convalescent, specimen.

†Unknown etiology.

‡Virus of meningopneumonitis group isolated from sputum.[1]

In view of the apparent ubiquity of this group of agents, it was considered desirable to determine by serological tests how frequently they are etiologically related to respiratory disease. Sera from cases of atypical pneumonia† and from uncomplicated acute upper respiratory diseases were tested by complement fixation with antigens from the viruses of meningopneumonitis, lymphogranuloma venereum and mouse pneumonia grown in chick embryos and using the methods previously described.[2] All tests were controlled with similar preparations from normal chick embryos.

Complement fixation tests also were carried out with sera of immune animals and antigens prepared from the three viruses. Some of the animal sera, especially those from guinea pigs, gave reactions which were relatively specific for one strain. Others gave group reactions, the titers being approximately equal with all three antigens. These sera gave no fixation of complement with antigens prepared from the viruses of influenza or lymphocytic choriomeningitis.

Table I shows some of the significant reactions of human sera with the 3 antigens. Sera from cases of pneumonitis of unknown etiology gave group specific reactions with the viruses of lymphogranuloma venereum, meningopneumonitis, and mouse pneumonitis. Sera from cases definitely diagnosed as virus pneumonia by isolation of a specific virus, and also sera from cases of lymphogranuloma venereum gave similar reactions. Although in some cases there was evidence of specificity for one of the 3 antigens, in general the group specific character of the reaction was quite definite and this has been observed with many other samples of human serum.

Despite the fact that the complement fixation test with this group of viruses was not specific for any one member of the group when applied to human serum, it was considered of some interest to determine what proportion of specimens gave positive reactions. The results with sera from cases of atypical pneumonia and of uncomplicated upper respiratory disease are shown in Table II. In 6 of 70 cases of pneumonitis tested with

† The term atypical pneumonia is used to denote all forms of acute primary bronchopneumonia or pneumonitis without demonstrable bacterial etiology.

TABLE II.

Complement Fixation Tests with Acute and Convalescent Serums.

Disease	Antigen	Number tested	Change in titer associated with illness: No increase, number	Increase 2-fold, number	Increase 4-fold or over, number
Pneumonitis	Meningopn.	70	60	4	6
Upper Resp.	"	80	76	3	1
Pneumonitis	Lymphogran. ven.	61*	43	12	6
Upper Resp.	" "	40	37	2	1
Pneumonitis	Mouse pneumonia	27	19	5	3

* 4 cases showing increase in complement fixation titer with normal yolk sac not included.

TABLE III.

Complement Fixation Tests with Serum Collected During Convalescence Only.

Disease	Antigen	Number tested	Number with titer of: 0	4	8	16 or over
Pneumonitis	Meningopneumonitis	64	30	14	10	10
Upper Resp.	"	76	60	14	1	1
Pneumonitis	Lymphogranuloma venereum	69	30	13	9	17
Upper Resp.	" "	40	29	5	5	1
Pneumonitis	Mouse pneumonia	63	36	10	9	8

the virus of meningopneumonitis the serum collected during convalescence showed a definite increase in titer of 4-fold or greater over the serum taken during the acute phase of illness. Similar definite increases were observed in 6 out of 61 cases tested with the virus of lymphogranuloma venereum and in 3 of 27 cases tested with the mouse pneumonia virus. In other cases increases in titer of lesser degree were observed. One case of upper respiratory disease having the symptoms of influenza showed a definite increase in titer with both antigens. This case gave negative serological tests for influenza A and B. In a few other cases of upper respiratory disease slight increases were observed. Four cases of atypical pneumonia showed an increase in complement fixation titer with normal yolk sac antigen. This emphasizes the importance of normal antigen controls in all tests of this sort.

In Table III are presented the results of tests on cases of pneumonia from which only sera collected during convalescence were available. The titers of sera of patients convalescing from upper respiratory disease are included for comparison. It will be seen that the proportion of serum specimens having titers of 8 or above with the 3 antigens was relatively higher in the cases of pneumonitis than in cases of upper respiratory disease. Titers below 8 are considered normal. In about 15% of the cases of pneumonitis the titers were over 16 or well above normal limits. In many cases the results represent triplicate tests on the same serums with the 3 antigens.

During the past 2 years sputum specimens from 110 cases and lung specimens from 12 cases of atypical pneumonia, and over 100 throat washings from cases of influenza-like illness have been inoculated intranasally into mice with at least 2 subsequent intranasal passages. From only 3 of these specimens‡ (excluding those previously described[1]) was a virus which forms elementary bodies isolated by the method used. The results obtained in these tests, as well as the failure of other investigators to infect mice with sputum from atypical pneumonia, suggest that this pathological syndrome, particularly in its milder form, is rarely caused by a psittacosis-like agent directly transmissible to mice. The possibility that the 15% of cases of pneumonitis

‡ These cases will be described in a later communication.

which gave positive reactions were caused by any one of a group of several related viruses should be considered. Some of these agents may not be directly transmissible to mice.

Summary. Complement fixation tests were done with sera from cases of pneumonitis and acute upper respiratory disease and antigens from the viruses of lymphogranuloma venereum, meningopneumonitis, and the mouse pneumonia virus of Nigg.[4] Positive reactions of a group-specific character were obtained in 10 to 15% of cases of pneumonitis and in about 2% of cases of upper respiratory disease.

Most of the serum specimens tested in this study were obtained through the courtesy of Drs. W. G. Donald, R. T. Sutherland, and Henry Borson, Cowell Memorial Hospital, University of California; Drs. W. J. Kerr and J. W. Brown, University of California Medical School; and Drs. J. Thomas Hardesty, J. E. Walker, and G. B. Hanson, Seaside Memorial Hospital, Long Beach, California.

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Detection of Tubercle Bacillus Polysaccharides in Infected Material from Animals and Patients.*

JOHN T. RIORDAN. (Introduced by Francis G. Blake.)

From the Section of Preventive Medicine, Yale University School of Medicine.

In a previous communication[1] a series of precipitin and collodion-particle-agglutination tests has been reported with antigens derived *in vitro* from tubercle bacilli. In view of the extreme sensitiveness of some of these tests, in which positive collodion-particle-agglutination tests were obtained with the tubercle bacillus polysaccharides, in a dilution of one part in ten million, it would seem that, if these polysaccharides exist free within tuberculous lesions, they should be demonstrable there by these methods. It has been our purpose, therefore, to see whether or not this is true; so in the present communication a series of these tests which have been performed on pathological material is recorded.

Materials. A single lot of anti-H37 tubercle-bacillus serum (designated No. 5807A and B) was used as in the former series.[1] † It was prepared by the Mulford Biological Laboratories of Sharp and Dohme in 1925, by the injection of a horse with defatted tubercle bacilli. The immunological properties of this serum have been studied by a number of investigators,[2] including Heidelberger,[3] who found its antibody to be almost entirely of antipolysaccharide specificity. In our own tests,[1] this was confirmed, in so far as positive immunological reactions were obtained with polysaccharide fractions of acid-fast bacilli and with Old Tuberculin, but not with purified protein derivatives of tubercle bacilli.

In the present series of tests, the following material was used as a possible source of antigens which might react with this serum:

I. *Infected embryonic tissue.* Twenty, 9-day chick embryos were implanted with tubercle bacilli (human type), and 9 days later the gross lesions noted on the chorio-allantoic membrane were harvested in 3 eggs.[4] This material was washed in saline, minced,

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