body fluid compartments, the spleen and other reservoirs of formed blood elements.[5]

[5] Bonnycastle, D. D., *J. Pharm. and Exp. Therap.*, 1942, **75**, 18.

Conclusion. Preliminary observations seem to substantiate the clinical impression that cyclopropane is a useful anesthetic agent in the presence of circulatory depression due to rapid hemorrhage.

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Yolk Sac Complement Fixation Antigen for Use in Psittacosis-Lymphogranuloma Venereum Group of Diseases.

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The recognition in recent years of the frequency of human infections with several members of the psittacosis-lymphogranuloma venereum group of viruses[1-4] has led to an increased interest in procedures which aid in the laboratory diagnosis of these diseases. The demonstration of complement-fixing antibodies in the sera of patients convalescent from psittacosis and from lymphogranuloma[7,8] has been used for establishing the etiology of these maladies. Considerable confusion has developed recently, however, regarding the interpretation of complement fixation reactions obtained in these diseases because of cross reactions displayed by the patients' sera with antigens prepared from tissues infected with the viruses of classical psittacosis and lymphogranuloma venereum and other members of this group of agents.[2,9-11] Although at present it is impossible to differentiate sharply between human psittacosis and lymphogranuloma by means of serological tests, it is possible, under appropriate conditions, to establish that a given acute illness was caused by a member of this group of viruses.[3]

The observations of several groups of workers suggest that the titers of sera from patients with psittacosis, closely related infections and with lymphogranuloma may differ somewhat when tested against homologous and heterologous antigens prepared from this group of viruses.[2,9,10] Furthermore, pigeons recovered from ornithosis develop antibodies that fix complement with meningopneumonitis antigen but not with lymphogranuloma antigen.[11] The observations reported at this time extend those of others regarding the reactions of human convalescent sera with complement-fixing antigens of psittacosis and lymphogranuloma. In addition, a comparatively simple and safe method for preparing psittacosis antigen from highly infectious material is described.

Materials and Methods. Preparation of Psittacosis Antigen. Antigens containing

[1] Meyer, K. F., *Medicine*, 1942, **21**, 175.

[2] Eaton, M. D., and Corey, M., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 165.

[3] Smadel, J. E., *J. Clin. Inv.*, 1943, **22**, 57.

[4] Shaffer, M. F., Rake, G., Grace, A. W., McKee, C. M., and Jones, H. P., *Am. J. Syph.*, 1941, **25**, 699.

[5] Bedson, S. P., *Lancet*, 1935, **2**, 1277.

[6] Meyer, K. F., and Eddie, B., *J. Infect. Dis.*, 1939, **65**, 225.

[7] Hecht, H., *Wien. Klin. Wschr.*, 1935, **48**, 1389.

[8] McKee, C. M., Rake, G., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 410.

[9] Rake, G., Eaton, M. D., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 528.

[10] Eaton, M. D., Martin, W. P., and Beck, M. D., *J. Exp. Med.*, 1942, **75**, 21.

[11] Eddie, B., and Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 291.

washed inactive elementary bodies of psittacosis prepared from infected agar slant cultures according to the method of Meyer and Yanamura[17] have proved highly satisfactory in our earlier diagnostic studies.[3] However, this method is not well adapted for the production of appreciable quantities of antigen mainly because infectious material is repeatedly handled during the course of differential centrifugation. A safer procedure was therefore adopted, which consisted of a number of steps previously employed by others in the preparation of virus materials. These include the use of yolk sac tissue as starting material,[4,12] inactivation of virus with formalin,[5,12] extractions with ethyl ether[12-14] and washing the virus by differential centrifugation.[3,15] A description of the method now in use follows.

The P-4 strain of psittacosis virus isolated from a dead pigeon in 1942[16] was adapted to growth in the yolk sac of embryonated hen's eggs by 20 serial passages in this organ. The yolk sacs of 30 to 90 six-day-old embryos were injected with 0.25 cc of a 1/30 dilution of sac tissue infected with adapted virus from the 20th to 30th serial passage and incubated at 37.5°C. Yolk sacs were harvested when the embryos became sluggish, usually 56 to 72 hours after injection.

After microscopic examination of an adequate sample of the sacs had shown that impression smears stained by Machievello's method were uniformly rich in elementary bodies, the entire group of yolk sacs was placed in a 500 cc bottle containing glass beads and vigorously shaken on a machine for one-half hour. Seven cc of diluent were then added for each infected sac, the mixture was agitated by hand and then stored at 5°C for 3 days. The diluent consisted of a 1/50 dilution of McIlvaine's citric-phosphate buffer solution, pH 7.6, to which 0.3% formaldehyde, U.S.P. was added. The non-infectious, crude, aqueous suspension was removed by means of a separatory funnel from the floating fat and sedimented material that had separated during storage in the cold. It was then centrifuged in 50 cc tubes in an International horizontal machine at 2,000 rpm for 15 minutes. The aqueous mid-zone was distributed in capped Lusteroid tubes with an internal diameter of 11 mm and spun in the cold in an International angle centrifuge for 2 hours at 4500 rpm. Fat and supernatant fluid were discarded and the sediment was carefully resuspended in sufficient dilute buffered saline to equal the original volume. The buffered saline was made by adding 4 volumes of 1:50 dilution of pH 7.6 buffer to 1 volume of physiological saline solution. The resuspended sediment was next thoroughly shaken in a separatory funnel with an equal volume of ethyl ether and then allowed to stand for one-half hour at room temperature. The heavy opaque emulsion of egg material separated into a moderately opalescent aqueous phase, a mid-zone of emulsion and a clear, faintly yellow layer of ether. If such a separation did not occur, 10 to 20 cc of physiological saline solution were added and the material was again shaken and allowed to stand. The aqueous suspension was removed and extracted twice more with one-half volume of ethyl ether. If the emulsion layer in the first or second extraction was excessive due to incomplete separation, this portion was removed and centrifuged in the horizontal machine at low speed for 5-10 minutes and the aqueous phase then quickly separated.

Elementary bodies were sedimented from the ether-extracted aqueous suspension by centrifugation in Lusteroid tubes in the angle machine at 4500 rpm for 2 hours. The supernatant fluid was removed from the small pellet of grayish white sediment which was resuspended in dilute buffer solution to one-half the original volume. The virus particles were again thrown down by centrifuga-

[12] Nigg, C., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 132.

[13] Ledingham, J. C. G., *Lancet*, 1931, **221**, 525.

[14] Craigie, J., and Tulloch, W. J., *Great Britain Med. Research Council, Special Rep. Series*, No. 156, 1931.

[15] Yanamura, H. Y., and Meyer, K. F., *J. Infect. Dis.*, 1941, **68**, 1.

[16] Smadel, J. E., Wall, M. J., and Gregg, A., in press.

[17] Meyer, K. F., and Yanamura, H. Y., personal communication.

tion in the angle head and finally resuspended in an amount of buffered physiological saline solution equal to 3 cc per yolk sac. Aggregated material was removed by centrifugation in 50 cc tubes in the International horizontal machine at 2,000 rpm for 10 minutes. Microscopic examination of materials was made at various stages in the process of purification of the virus suspensions; for this purpose slides with air dried films of the suspensions were stained with 0.25% basic fuchsin. A progressive decrease in extraneous material was noted and the final antigen was found to consist almost entirely of mono-dispersed elementary body-like structures.

From the results of preliminary experiments it appears that the application of identical methods will yield satisfactory antigens from yolk sacs infected with the virus of lymphogranuloma venereum. A recently isolated strain of virus, LA,[18] partially adapted to growth in yolk sac tissue was used in this portion of the work. Sacs were taken when the embryos became sluggish, *i.e.*, 7 to 9 days after inoculation; only those found rich in elementary bodies on microscopic examination were pooled for antigen, which was tested with convalescent antisera to determine its complement binding power.

Complement Fixation Tests. Complement fixation tests were done by the technic previously described[3] in which two exact units of complement titered in the presence of psittacosis antigen were used. Complement titration was carried out with varying quantities, *i.e.*, 0.08 cc to 0.26 cc at intervals of 0.02 cc, of a 1/30 dilution of commercial lyophilized guinea pig serum. In each test a known immune serum was titered to end point with 2 units of previously standardized antigen. This titration served as a final control on the activity of the hemolytic system and permitted a comparison of the data from one experiment with that of the next.

In addition, Lygranum C. F. *Control Lot 87884 received from Dr. Clara Nigg of the Squibb Institute was used in comparative titrations studies on certain sera. This antigen, in the dilution recommended on the label, was employed with the complement system used with our antigens.

Antisera. Three groups of convalescent sera were studied for their reactions with psittacosis and lymphogranuloma venereum antigens. One group of lyophilized sera was from patients with psittacosis,[3] another group of sera, stored in the frozen state, came from cases of lymphogranuloma among Army personnel, while the final group consisted of samples of lyophilized pigeon sera obtained from birds during an epizoötic of ornithosis.[16]

Results. Antigens consisting of suspensions of washed elementary bodies of psittacosis prepared in the manner described, fixed complement in dilutions 1:4 to 1:8 when tested with appropriate dilutions of sera of patients convalescent from psittacosis. These antigens were not anticomplementary and did not react with sera from normal persons. In addition, these antigens were tested against a series of sera known to give positive Wassermann reactions as well as another series from cases clinically diagnosed as atypical pneumonia. The results of these tests were likewise negative. The physical appearance of the preparations changed little over a period of several months and the suspensions failed to become anticomplementary. However, slight diminution in specific complement binding power occurred during the 2nd and 3rd months of storage in the refrigerator; certain loss of activity has also been noted with antigen prepared from agar slant cultures. Thus, for diagnostic work it was sometimes necessary to increase the concentrations of the stored preparations in order to obtain 2 units of antigen, *e.g.*, from 1:8 to 1:6 or 1:4 to 1:3.

The fact that antigens prepared from psittacosis virus and closely related agents will fix complement with lymphogranuloma antiserum is well established.[2,9,10] We were interested in determining whether differences could be detected in a given antigen when it was titered with psittacosis and lymphogranuloma antisera, each of which was diluted so that it contained 4 units of antibody on the basis of a previous standardization with

[18] Zarafonetis, C. J. D., in press.

* E. R. Squibb & Sons.

psittacosis antigen and Lygranum C.F., respectively. The results summarized in Table I indicate that antigens prepared from eggs infected with the No. 4 pigeon strain of psittacosis virus fix complement to the same titer when tested with comparable amounts of antibody from patients with psittacosis and lymphogranuloma. Similar studies with one lot of elementary bodies of lymphogranuloma suggest that slightly higher titration end points may result when homologous antiserum is used with this type of antigen (Table I).

TABLE I.
Cross Reaction of Psittacosis and Lymphogranuloma Antigens with Convalescent Human Sera.

Antigen*	Sera†	Dilution of antigen 1:1	1:2	1:4	1:8
Psitt No. 5	Psitt	4	4	4	2
	L-V	4	4	4	2
	Normal	0	0	0	0
Psitt No. 6	Psitt	4	4	4	2
	L-V	4	4	4	1
	Normal	0	0	0	0
L-V No. 7	Psitt	4	4	0	0
	L-V	4	4	4	1
	Normal	0	0	0	0

* Antigens were prepared by the method described in the text.
†Four units of antibody from the same samples of convalescent human psittacosis and lymphogranuloma serum were used throughout.
4 indicates complete fixation of complement.
2 indicates 50% fixation of complement.

TABLE II.
Titration of Human and Pigeon Convalescent Sera with Psittacosis Antigen and Lygranum C.F.

Name	Disease	Antigen complement fixation titer* Psittacosis	Lygranum C.F.
E.G.	Psitt	1:40	0
F.S.	"	1:80	1:40
Me.	"	1:160	1:80
F.C.	"	1:320	1:40
W.W.	L-V	1:80	1:40
L.A.	"	1:640	1:160
J.B.	"	1:40	1:40
E.M.	"	1:40	1:40
Do.	"	1:320	1:320
N.G.	"	1:640	1:640
Fr.	"	1:80	1:160
Pigeon 24	Psitt.	1:80	0
" 38	"	1:40	0
" 40	"	1:40	0

* Titers indicate the last tube demonstrating at least a 3-plus fixation.
0 Indicates no fixation with a 1/10 dilution of serum.

Series of serum specimens from patients with psittacosis and with lymphogranuloma venereum and from pigeons with psittacosis were titrated simultaneously with psittacosis antigen and Lygranum C.F. The results of these titrations are summarized in Table II. The data indicate that the end points obtained with sera from patients with psittacosis were regularly slightly higher when tested with homologous than heterologous antigen. The difference was more striking with pigeon serum than with human serum, in that the former failed entirely to react with Lygranum C.F. This agrees with meningopneumonitis studies of Eddie and Francis[11] with pigeon serum. Four of the 7 lymphogranuloma sera showed the same titer when tested with each antigen. Two of the 7 samples gave a slightly higher end-point with psittacosis antigen; while one fixed in a higher dilution with Lygranum C.F. A number of human and pigeon sera listed in Table II were titrated at other times with lymphogranuloma antigen No. 7 (Table I), the end-points for individual sera were comparable to those obtained with Lygranum C.F. antigen.

Discussion. The yolk sac complement fixation antigen is a cleaner and generally more satisfactory antigen than is the tissue agar slant preparation. The method of preparation of this antigen is simpler and a great deal safer. The relative number of elementary bodies in the preparation and its antigenicity compare favorably with the preparations made from tissue agar slants.

The serological data reported at this time indicate that the complement-fixing antigen which is common in the viruses of the psittacosis-lymphogranuloma venereum[1,2,9-11] group is present in about equal amounts in our strains of psittacosis and lymphogranuloma venereum. Not only do antigens prepared from each agent react equally with equivalent amounts of antibody from the two diseases, but also comparable amounts of antibody are generally demonstrable in

patients' sera when tested with either antigen. While slight differences were found in the antibody titers of certain sera when tested with the two antigens, these were neither sufficiently great nor consistent enough to enable one to differentiate between human psittacosis and lymphogranuloma venereum by this method.

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Influence of Lowered Barometric Pressure on the Electroencephalogram.

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Although it is well established that a lowering of the barometric pressure accompanied by a reduction in oxygen tension produces profound changes in the electroencephalogram (EEG), little is known about the effects of reduction in barometric pressure *per se*. In general, it has been found that sensory functions which are very sensitive to anoxia are not altered by reduction in barometric pressure when the oxygen tension is kept at normal levels (*cf.* the review by Gellhorn and Hailman).[1] On the other hand, it has been reported that the cerebro-spinal fluid pressure may increase considerably even at relatively low altitudes. Armstrong[2] thinks that this increase in cerebro-spinal pressure is due to a liberation of nitrogen in gas form. Bergeret and Giordan[3] showed that a rise in cerebro-spinal pressure occurs only at such altitudes in which the oxygen tension is considerably below normal. In view of the fact that alterations in cerebro-spinal fluid pressure may affect the EEG (Forster and Nims)[4] a study of the effect of lowered barometric pressure on the EEG seems to be of interest.

The experiments were performed on 20 unanesthetized male rats which were kept singly in an atmosphere of 100% oxygen. The pressure was reduced in one minute to a level of 130 mm Hg and then maintained for 5 minutes; then the normal atmospheric conditions were restored. In a second control series, the rats were subjected to the same pressure changes in air. The EEG was recorded as in previous investigations (Gellhorn and Kessler).[5]

Results. Uniform results were obtained in 6 animals showing that even this extremely rapid lowering of the barometric pressure to 130 mm Hg did not cause any significant changes in the EEG of rats (*cf.* Fig. 1) nor was there any distinct change in behavior. If, however, the pressure was lowered to the same barometric level in the presence of air (6 experiments), the brain waves were completely abolished (Fig. 2). The experiments indicate that at least in rats the changes in EEG observed under conditions of lowered barometric pressure are solely due to a lowering of the oxygen tension. This conclusion is further supported by the evidence that if the barometric pressure is lowered below 130 mm Hg in an atmosphere of oxygen changes in behavior and EEG occur which are typical of anoxia. Results as illustrated in Fig. 3

* Aided by grant from the Josiah Macy, Jr., Foundation.

1 Gellhorn, E., and Hailman, H., *Federation Proc.*, 1943, **2**, in press.

2 Armstrong, H. G., *Principles and Practice of Aviation Medicine*, Baltimore, 1939, Williams and Wilkins Company.

3 Bergeret, P., and Giordan, P., *J. de Physiol. et de Path. Gen.*, 1938, **36**, 1050.

4 Forster, F. M., and Nims, L. F., *Arch. Neurol. and Psychiat.*, 1942, **47**, 449.

5 Gellhorn, E., and Kessler, M., *Am. J. Physiol.*, 1942, **136**, 1.