EXTRACT II.

1 " " " " 636 g bull testes, positive
3 " " " " 424 " " " all positive
1 " " " " 212 " " " negative

EXTRACT III.

2 " " " " 122 g deer testes, both positive
3 " " " " 61 " " " 1 neg., 2 positive
3 " " " " 30 " " " all negative

EXTRACT IV.

4 " " " " 106 g deer testes, 1 neg., 3 positive

The shortage of material prevented a closer determination of the minimal effective dosage, but any correction would indicate a greater concentration of estrogens in the testes than shown herein.

It is to be noted that the second extracts of both bull and deer testes are far less potent than the first extracts. This may be due in part to the fact that in the second extracts a much larger lipoidal content was encountered. Efforts to free the hormones from the lipoid by the Thayer, Jordan and Doisy[10] procedure may not have been fully effective in our hands. We are at a loss to explain the presence of this large lipoid content since all the coverings with attached fat were removed from the testes prior to extraction.

The data confirm the observations of Fellner, Brouha and Simonnet, Zondek, and Dorfman, *et al.*, that estrogens occur in the bull testes, and the quantitative results on Extract I agree remarkably well with those of Zondek.

We have demonstrated for the first time, we believe, that estrogens occur in fresh deer testes and that they have a potency about 3 times as great per gram of tissue as those that occur in frozen bull testes.

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Action of Urea and Ether on Agent and Complement-fixing Antigen of *Lymphogranuloma venereum.*

CLARA NIGG. (Introduced by Geoffrey Rake.)

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That the complement fixation reaction is a valuable aid in the diagnosis of *Lymphogranuloma venereum* has been firmly established.[1-3] Two antigens were found to be satisfactory, viz., (a) the

[10] Thayer, S. A., Jordan, C. N., and Doisy, E. A., *J. Biol. Chem.*, 1928, **79**, 53.

resuspended sediment obtained after high speed centrifugation (12,000 rpm for 2 hrs.) of suspensions of yolk sac membranes from chick embryos infected with the agent of *Lymphogranuloma venereum* and (b) suspensions of lung tissue from mice infected with the same agent. Although Rake, Shaffer, Jones and McKee[4] observed that the supernate from the yolk sac material centrifuged at high speed likewise contained significant amounts of complement-fixing antigen, the high speed sediment was nevertheless used in the earlier work because the retention of the other constituents was found to lead to anticomplementary activity. Table I shows the activity

TABLE I.
Sedimentation of the Complement-fixing Antigen of *Lymphogranuloma venereum.*

Antigens	Antigen dilutions						
	1-50	1-75	1-100	1-150	1-200	1-250	1-300
No. 17, prepared 9/8/41 tested 9/10/41							
Fraction a—Crude 10% suspension	4+	4+	4+	4+	4+	3-4+	
" c—Low speed supernate	4+	4+	4+	3-4+	3+	2+	
" d—High " "	4+	3-4+	3+	2+	1+	0	
" e— " " sediment	4+	3-4+	2+	1+	0	0	
No. 19, prepared 9/17/41 tested 9/19/41							
Fraction a—Crude 10% suspension	4+	4+	4+	4+	4+	4+	3-4+
" b—Low speed supernate	4+	4+	4+	4+	3-4+	2+	—
" c—High " "	4+	4+	3-4+	1+	0	0	
" d— " " sediment	4+	4+	3-4+	1+	0	0	

Results are given in terms of fixation.

of the various fractions obtained in the course of fractional centrifugation of suspensions prepared from yolk sac membranes of infected chick embryos.[2, 5] Centrifugation even at low speed (2000 rpm for 1 hr) resulted in a considerable loss of activity from the supernate, while the activity of the low speed supernate was about equally divided between the high speed sediment and supernate respectively.

All fractions except the high speed sediment became so anti-

[1] McKee, Clara M., Rake, Geoffrey, and Shaffer, Morris F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 410.

[2] Rake, Geoffrey, Shaffer, Morris F., Grace, Arthur W., McKee, Clara M., and Jones, Helen P., *Am. J. Syph., Gon., and Ven. Dis.*, 1941, **25**, 687.

[3] Shaffer, Morris F., Rake, Geoffrey, Grace, Arthur W., McKee, Clara M., and Jones. Helen P., *Am. J. Syph., Gon., and Ven. Dis.*, 1941, **25**, 699.

[4] Rake, Geoffrey, Shaffer, Morris F., Jones, Helen P., and McKee, Clara M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 300.

[5] Rake, Geoffrey, McKee, Clara M., and Shaffer, Morris, F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

complementary shortly after formalin was added to inactivate the virus that all but the latter were unsuitable for use as antigens within a week or two after their preparation. The development of this anticomplementary activity is shown in Table II.

TABLE II.
Action of Formalin on Complement-fixing Activity of Yolk Sac Material.

Antigens Prepared 8/14/41	Tested	Tests with positive serum and antigens diluted: 1-50	1-75	1-100	1-150	1-200	1-250	Anticomplementary controls on antigens diluted: 1-50	1-100	1-200
No. 13-1										
Low speed supernate of saline suspension	8/18	4+	4+	4+	3-4+	3+	2-3+	0	0	0
containing formalin to 0.1%	8/27	4+	4+	4+	4+	4+	3-4+	4+	3+	2+
No. 13-5										
Low speed supernate of saline suspension	8/18	4+	4+	4+	4+	3-4+	3+	2+	0	0
containing M/45 phosphate buffer pH 7.4 and formalin to 0.1%	8/27	4+	4+	4+	4+	4+	4+	4+	4+	4+

Results are given in terms of fixation.

It seemed possible that the anticomplementary property developed as the result of the action of the formalin on nonspecific components of the yolk sac suspensions, since the property developed more rapidly and in greater amounts in the crude suspensions than in the centrifuged supernates from which a considerable amount of inactive material had been removed. The high speed sediment was never observed to become anticomplementary at icebox temperature after the addition of formalin. If, therefore, formalin was used as the inactivating agent, the only fraction suitable for complement fixation antigen appeared to be that sedimented at high speed. Since the other fractions contained considerable activity (Table I), it seemed desirable to seek other means of inactivating the virus which would not lead to the development of anticomplementary activity.

It is well known that urea is a protein denaturant and as such its action on certain viruses has been studied by a number of investigators.[6-10] Most of the reports indicate not only inactivation of the

[6] MacKay, Eaton M., and Schroeder, Charles R., PROC. SOC. EXP. BIOL. AND MED., 1936-37, **35**, 74.

[7] Höring, F. O., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1939, **32**, 597.

[8] Frampton, Vernon L., and Saum, A. M., *Science*, 1939, **89**, 84.

[9] Stanley, W. M., and Lauffer, Max A., *Science*, 1939, **89**, 345.

[10] Hoyt, Anson, and Warner, Douglas, PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 154.

TABLE III.
Comparison of Action of Formalin and Urea on Complement-fixing Activity of Yolk Sac Suspensions.

		Tests with positive serum and antigens diluted:						Anticomplementary controls on antigens diluted:			
	Tested	1-50	1-75	1-100	1-150	1-200	1-250	1-50	1-100	1-200	1-300
Antigen No. 18											
Fraction 1											
Crude suspension (prepared 9/12/41) containing 0.1% formalin (added 9/25/41)	10/7	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Fraction 2											
Crude suspension (same as 1) containing 1% urea (added 9/12/41)	11/18	4+	4+	4+	4+	3-4+	2+	0	0		
Fraction 4											
Supernate (prepared 9/12/41 by centrifuging crude suspension 2000 rpm 1 hr) containing 0.1% formalin (added 9/25/41)	9/26	4+	4+	4+	3+	3+	1+	0	0		
Fraction 5											
Supernate (same as in 4) containing 1% urea (added 9/12/41)	9/26	4+	4+	4+	3+	2+	0	0	0		
Antigen No. 13, low speed supernate of 10% saline suspension prepared 8/14/41 and treated as follows:											
Fraction 1											
0.1% formalin	8/18	4+	4+	4+	3-4+	3+	3+	0	0	0	
added 8/14/41	8/27	4+	4+	4+	4+	4+	3-4+	4+	3+	2+	
Fraction 4											
1% urea added 8/14/41	9/4	4+	4+	4+	3-4+	3+	2-3+	0	0	0	
Fraction 6											
Kept frozen. 0.1% formalin added 9/25/41	9/26	4+	4+	4+	2-3+	1+		1+	0	0	
Fraction 7											
Kept frozen. 1% urea added 9/5/41	9/26	4+	4+	4+	3+	2+		0	0	0	

Results are given in terms of fixation.

virus but destruction so complete as to entail loss of antigenicity. In spite of these reports, the action of urea on the agent of *Lymphogranuloma venereum* in yolk sac material was investigated and it

was found that as little as 1% urea inactivated the virus completely within 1-2 weeks' time at icebox temperature, and as much as 10% urea could be used without apparent loss in the complement-fixing activity of yolk sac suspensions so treated (Table III). Not only did small amounts of urea serve to inactivate the virus in the complement-fixing antigens, but even crude 10% suspensions of yolk sac material, containing all of the non-specific components failed to develop anticomplementary activity within 4 months' time at icebox temperature. The crude suspensions constituted antigens with significantly higher titers than other fractions since, as shown above, some activity was lost at each step of the differential centrifugation.

The inactivation of the virus by ether and the action of the latter on the complement-fixing antigen were similarly studied. One volume of 10% crude yolk sac suspension was shaken for ½ hr at room temperature with 2 volumes of ether. The ether supernate obtained by centrifuging at 1000 rpm for 5 min was discarded. The residual suspension was again extracted with ether in the same way. Ether was more completely removed *in vacuo*. This treatment resulted in the complete inactivation of the virus without impairment of the complement-fixing activity or the development of anticomplementary activity[11] within 4 months' time, the longest period so far tested.

Summary. 1. The agent of *Lymphogranuloma venereum* in yolk sac material can be completely inactivated by the addition of 1 or 2% urea and also by extraction with ether. 2. Crude suspensions of yolk sac material treated with urea or ether constitute antigens (devoid of anticomplementary activity) of considerably higher potency than the high speed sediments obtained from the same suspensions.

The technical assistance of Miss Augusta Hollender is gratefully acknowledged.

[11] Howitt, Beatrice F., *J. Immunol.*, 1937, **33**, 235.