was then opened and the position of the esophagus was determined.

The first animal was killed 10 months after the left lung had been removed. The left pleural cavity seemed to be somewhat smaller than the right and the left side of the diaphragm was slightly higher than the right. The filling of the chest appeared to be due in the main to an increase in size of the right lowermost lobe. The lower part of the esophagus occupied a position far to the left of the midline. This is shown in Fig. 1. There was no constriction of the esophagus and no ulceration was noted.

The second animal was killed 5 months following the total removal of the left lung. The findings were similar to those observed in the first experiment.

The third animal appeared to have distemper 5 weeks following the total removal of the right lung. Death was caused painlessly and the usual procedure carried out. The esophagus was found to be markedly deviated to the right. The position is shown in Fig. 2. The 2 sides of the diaphragm were at approximately the same levels.

In the fourth experiment, the left lung was removed and the left phrenic nerve was paralyzed. Fifteen weeks later it was observed that the left diaphragm was very much higher than the right and that the esophagus occupied essentially its normal position.

Summary. Total pneumonectomy in dogs, without an associated paralysis of the diaphragm, was found to be followed by a marked deviation of the esophagus to the side from which the lung had been removed.

8173 C

Reactivation of Ammonia-Inactivated Complement by Leucocytes.

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From the earliest work on the lytic action of serum, the cells of the tissues, particularly the phagocytes, have been considered a source of complement. However, convincing proof is still lacking that these cells either contain or liberate active complement. The possibility that they may contribute substances inactive alone but nevertheless essential to complete complement action has received little consideration.

It seemed of interest to determine whether or not a suspension of leucocytes would contribute complementing substances, either heat-labile or heat-stable, which, like heated serum,[1] might restore the hemolytic activity of complement made inactive by ammonia.

Fresh guinea pig serum was inactivated with half its volume of N/5 ammonium hydroxide at 37°C. for 1½ hours and then neutralized to pH 7.6 with N/5 hydrochloric acid. The final dilution was 1:2.

Leucocytes from guinea pig blood were collected in strata and washed in physiological salt solution by centrifugalization at low speed, according to the technic of Wadsworth and Hoppe.[2] They were washed, however, not once but 5 times. Sufficient salt solution was used in washing so that the dilution of plasma in the final suspension of leucocytes was at least 1:200,000. Also, in order to compensate for the unavoidable mutilation of the cells during the additional washings, the leucocytes from 10 cc. of blood were suspended in 0.5 cc., rather than in 1 cc., of salt solution.

In order to control the reactivating effect of traces of plasma which might be retained in the preparation of the leucocyte suspension, a portion of the supernatant fluid from which these cells had been separated was diluted to the same degree as were the leucocytes in the process of washing, and tested in parallel with them for its reactivating effect. For comparison, the reactivating properties of very small amounts of heated serum were also determined.

Tests for hemolytic action were made as indicated in the protocol. A 5% suspension of sheep cells sensitized with 2 units of amboceptor was used. Readings were made by comparison with color standards.[3]

The leucocyte suspension, unheated or heated for one-half hour at 55°C., completely, or nearly completely, reactivated the ammonia-inactivated complement in 2 hours at 37°C. in the water bath.* The

[1] Gordon, John, Whitehead, H. R., and Wormall, Arthur, *Biochem. J.*, 1926, **20**, 1028.

[2] Wadsworth, A. B., and Hoppe, E. N., *J. Immunol.*, 1921, **6**, 399.

[3] Gilbert, Ruth, Kelley, M. F., and Moore, A. C., *J. Lab. and Clin. Med.*, 1925, **10**, 552.

* The complementary effect of leucocytes may account for the opsonic action which has been observed with ammonia-treated serum.[4-6]

[4] Gordon, John, and Thompson, F. C., *Brit. J. Exp. Path.*, 1935, **16**, 101.

[5] Gordon, John, Whitehead, H. R., and Wormall, Arthur, *J. Path. and Bact.*, 1929, **32**, 57.

[6] Gordon, John, Whitehead, H. R., and Wormall, Arthur, *Biochem. J.*, 1926, **20**, 1044.

TABLE I.
Reactivation of Ammonia-inactivated Complement by Diluted Plasma or Serum and by Washed Leucocytes.

Reagents	Dilution	Amount tested, in cubic centimeters or in drops																	
Ammonia-inactivated complement	1:2	0.2	0.25	0.2	0.25	0.2	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25					
Guinea-pig serum heated ½ hr. 55° C.	1:50							0.1											
	1:400								0.1										
	1:1600									0.1									
	1:6400										0.1								
Plasma from first centrifugalization of cells	1:5000											0.1							
	1:50,000												0.1						
	1:200,000													0.1					
Ammonia-inactivated complement heated ½ hr. 55° C.	1:2														0.25				
Untreated complement	1:2															0.25	0.25		
Leucocyte suspension																			
Unheated				1 gt	1 gt										1 gt		1 gt	4 gtt	
Heated ½ hr. 55° C.						1 gt	1 gt												
		% hemolysis in water bath, 37° C.																	
After 15 min.		0	0	10	25—	25+	50	90+	35	0	0	10	0	0	0	100	100	0	
'' 2 hr.		0	2	75+	90+	100	100	100	90+	30	2	50	0	0	0	100	100	0	

Sensitized cells were used in 0.4 cc. amounts, and the volume in all tubes was equalized with physiological salt solution.

heated suspension was even more active than the unheated. Plasma diluted to the same extent as were the leucocytes in washing—namely, 1:200,000—had no reactivating effect; in an amount 40 times as great, a 1:5000 dilution, it partially restored the activity of ammonia-treated serum; 50% hemolysis was obtained in 2 hours at 37°C. Heated serum in a 1:400 dilution reactivated to about the same degree as the unheated leucocyte suspension; the limit of its activity was reached in about a 1:6400 dilution.

After heating for one-half hour at 55°C., the ammonia-inactivated complement was not reactivated by the leucocyte suspension; the latter, in 4 times the amount used in the other tests, had no hemolytic action alone.

Thus the leucocytes contain or convey complementary substances essential to the hemolytic activity of ammonia-treated serum. Further study may determine more clearly whether the leucocytes are active *per se,* or through the absorption of minute quantities of serum or plasma.

8174 C

Some Analyses of Thyroglobulin.

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In view of the physiological importance of thyroglobulin, it is somewhat surprising that little information has been made available concerning its amino acid composition. Until recently, the investigation of Eckstein[1] represented the only study, by more recent methods of protein analysis, of the distribution of the more important amino acids of thyroglobulin. The basic amino acids yielded on hydrolysis of this protein have been recently determined in this laboratory[2] by isolation procedures, and the results compared with those of Eckstein. Additional data have now been obtained regarding some of the other amino acids of thyroglobulin and are being presented inasmuch as further chemical investigation of this protein is not contemplated at the present time. A portion of these analyses supplement information already available in the literature;

1 Eckstein, H. C., *J. Biol. Chem.*, 1926, **67**, 601.

2 White, A., and Gordon, W. G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 354.