by the intranasal route.[8] From the results included in the table it is apparent that the drugs are active against 100 L_d 50 or 1000 infective doses inoculated by this route.

The demonstration that at least two agents in this group, *viz.*, those of lymphogranuloma venereum and mouse pneumonitis, are susceptible to the action of sulfonamides is of further interest in that it draws attention to the possible relationship of this group to the agents of trachoma and inclusion blennorrhoea which are likewise susceptible to drugs of the same series. Further evidence bearing on this relationship will be published elsewhere.[9] The only other virus-like agent known to be susceptible to chemotherapy is that of heart-water fever,[10] which at present is classed with the rickettsiae.

It should be added that the authors do not believe that these investigations will necessarily have any bearing on chemotherapy of true virus diseases since evidence is accumulating which indicates that the members of the lymphogranuloma venereum-psittacosis group should be separated from the true viruses.

13593

Biochemical Studies of Atheromatous Animals.

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A thesis has been offered[1] suggesting that the fundamental and general causal mechanism of degenerative arterial disease is an impaired nutrition and oxygenation of the vascular wall. This thesis has been based on the production of arteriosclerotic changes by 3 general procedures: first, the use of film and emulsion-forming agents (cholesterol,[2] polyvinyl alcohol,[3] methyl cellulose,[4] etc.):

[8] Shaffer, M. F., Rake, G., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 408.

[9] Rake, G., Shaffer, M. F., and Thygeson, P., to be published.

[10] Neitz, W. O., *J. S. African Vet. Med. Assn.*, 1940, **11**, 15.

[1] Hueper, Wilhelm C., *Arch. Path.*, 1939, **28**, 510; *Medicine*, 1941, **20**, 397.

[2] Anitschkow, N., Experimental Arteriosclerosis in Animals, p. 271, in Cowdry, E. V., *Arteriosclerosis*, New York, The Macmillan Co., 1933.

[3] Hueper, Wilhelm C., *Arch. Path.*, 1941, **31**, 11.

[4] Hueper, Wilhelm C., *Arch. Path.*, 1942, **33**, 1.

second, the use of vasculotonic (hypertonic[5] and hypotonic[6]) agents; and third, the production of abnormal hydrostatic conditions[7] in the vessels. The use of the film-forming agents offered the best possibility for a biochemical approach, since it seemed that altered oxygenation speed of erythrocytes would result if films of cholesterol or other agents of this type formed over the surfaces exposed.

In the polyvinyl alcohol experiments dogs were used exclusively. 125 cc of a 5% polyvinyl alcohol solution in normal saline were injected into the jugular vein, from which the blood was withdrawn for the various tests. Purely relative methods were devised to investigate oxygenation speeds. A blood sample of 5.0 cc was evacuated in the Van Slyke manometric apparatus at 20 mm of mercury for 5 minutes; it was then exposed to the atmosphere for 5 seconds and a manometric oxygen determination[8] was carried out immediately thereafter. All samples were treated in exactly the same manner and results on a given sample of blood could be duplicated to within 0.2 vol. %. The results are recorded in Table I. Cell sizes are included in Table I to show that the potentiality of increased exposed surface is not a factor in this determination. Cell size did

TABLE I.
Oxygenation Speed of Blood of Polyvinylized Dogs.

Dog		Vol. % O	Hb.	Cell size, u	Cell No. × 1000	Vol. % O_2/Hb
	Control	11.91	11.8	6.7	5,440	1.01
415	Immed. following 150 cc 5% PVA	12.61	10.2	6.4	4,880	1.23
	24 hr later	7.4	10.8	6.5	4,550	0.68
	Control	13.11	13.0	6.3	5,660	1.01
417	Immed. following 150 cc 5% PVA	12.87	10.9	6.1	5,040	1.18
	24 hr later	8.35	10.1	6.2	4,270	0.82
	Control	11.42	12.0	6.36	5,850	0.95
419	Immed. following 150 cc 5% PVA	10.97	12.5	6.32	6,580	0.88
	24 hr later	6.25	11.0	6.19	6,020	0.57
	Control	11.4	10.3	6.62	5,140	1.10
442	Immed. following 125 cc 5% PVA	9.8	8.8	6.62	4,330	1.11
	24 hr later	7.9	8.8	6.86	4,470	0.89
	Control	9.6	9.8	6.79	4,500	0.98
443	Immed. following 125 cc 5% PVA	7.6	7.6	6.85	3,550	1.00
	24 hr later	4.9	6.9	6.92	4,200	0.71
	Control	11.2	9.9	6.52	4,950	1.13
464	Immed. following 125 cc 5% PVA	9.6	7.7	6.45	3,920	1.25
	24 hr later	4.6	7.4	6.56	3,890	0.62

[5] Hueper, Wilhelm C., and Ichniowski, C. T., *J. Lab. Clin. Med.*, 1941, **26**, 1565.

[6] Hueper, Wilhelm C., and Landsberg, J. W., *Arch. Path.*, 1940, **29**, 633.

[7] Moon, V. H., *Arch. Path.*, 1927, **3**, 404.

[8] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

TABLE II.
Oxygenation Speed of Erythrocytes of Cholesterolinized Rabbits.

Rabbit	Cholesterol, mg/100 cc	R.B.C. × 1000	Hb.	Vol. % O_2	O_2/Hb.
1	796	3,450	7.6	7.95	1.04
2	770	4,250	9.4	10.3	1.10
3	1020	5,050	9.4	12.0	1.26
4	1020	6,800	12.3	14.6	1.18
5	639	6,110	10.2	9.6	0.95
Control a	64	3,910	10.0	14.9	1.49
" b	42	5,200	12.5	18.0	1.44
" c	60	4,190	11.3	16.3	1.42

not vary significantly or consistently. There was a marked reduction of the O_2 uptake of the blood of the polyvinyl-treated dogs.

Identical methods were applied to the study of oxygenation speed of erythrocytes of rabbits fed cholesterol. Two grams of cholesterol in peanut oil were added to the daily ration of rabbit chow over a period of 9 to 12 weeks. The results recorded in Table II are similar to those observed with polyvinyl alcohol on dogs.

Blood chemical studies were carried out on dogs injected with polyvinyl alcohol. Three dogs received 70 cc of a 5% polyvinyl alcohol solution intraperitoneally on the first, second, sixth, eighth, tenth, thirteenth and fifteenth days of the experiment, while an untreated dog served as a control. Table III illustrates significant blood findings in a typical polyvinyl dog. All 3 of the experimental dogs exhibited an increase of N.P.N. reaching pathological values during the terminal stage. After 3 weeks there was also a uniform drop in total protein values. The markedly lowered fibrinogen values existing in 2 dogs suggest the presence of severe liver damage such as is seen in acute liver atrophy. Histological examination of the liver of these 2 animals confirmed this assumption. There were no significant deviations from the normal range in the other blood constituents determined (sodium, potassium, calcium, phosphorus, cholesterol, cholesterol esters, total lipoids, fatty acids, phospholipids, glucose) aside from the normal terminal decreases in glucose.

Plasma volume was measured in polyvinylized dogs by the method of Gregersen and Stewart[9] with the dye T-1824. 0.05 to 0.1 cc of a

TABLE III.
Protein and Non-Protein Blood Chemistry in PVA Dog.

Weeks	1	2	3	4	5	6
N.P.N., mg	31.6	29.6	27.1	41.2	100.0	120.0
Total protein, %	5.0	5.3	2.6	2.9	1.8	3.0
Fibrinogen, %	0.5	0.6	0.22	0.21	0.25	0.27

[9] Gregersen, M. I., and Stewart, J. D., *Am. J. Physiol.*, 1940, **125**, 142.

1% suspension of this dye in water was injected for each kilogram of body weight. Optical densities were read on a Hilger spectrophotometer and introduced into the formula of Gregersen and Stewart.[8] Following the injection of 50 cc of a 5% solution of polyvinyl alcohol in a series of 7 dogs, plasma volumes increased by approximately 100% during the first 24 hours. The osmotic activity of polyvinyl alcohol causes considerable amounts of tissue fluid to come into the vascular system. The macromolecular aggregates of this colloid are not rapidly and readily removed from the blood, but remain there in considerable quantities over a period of several days following a single injection. The tendency of polyvinyl alcohol to coat the intima with a film may accentuate this osmotic effect by interfering with the escape of water from the liquid component of the blood.

It would seem that cholesterol as well as polyvinyl alcohol is capable of causing an altered physico-chemical state of the blood which results in a decreased speed of oxygenation of the red cells. Christie, Phatak and Olney[10] noted a transitory lowering of oxygen saturation immediately following the injection of a 15% solution of gum acacia. They explained their observations as being due to the coating action of the gum acacia. By similar processes of elimination, it is suggested that the results herein reported may be due to a coating action of the macromolecular compounds employed. The time element was not taken into consideration by Moncke[11] who studied oxygen-combining power, not oxygenation speeds, and therefore did not obtain results comparable to those under consideration here.

Summary and Conclusions. Direct biochemical evidence is presented to show that altered or impaired oxygenation of erythrocytes occurs following the parenteral administration of polyvinyl alcohol or the oral administration of cholesterol, both film-forming agents.

The use of polyvinyl alcohol as a medium for the reëstablishment of normal osmotic pressure in the blood is considered.

[10] Christie, A., Phatak, N. M., and Olney, M. B., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 670.

[11] Moncke, J. V., *Am. J. Physiol.*, 1941, **132**, 529.