

TABLE II.
Infectivity and Complement-Fixing Potency of Lymphogranuloma Virus Yolk-Sac
Suspensions Before and After Seitz EK Filtration.

Exp.	Strain of virus	Material tested	Infectivity titer	Complement- fixing titer*
A	NH	Unfiltered suspension Seitz EK filtrate	10^{-7} $< 10^{-1}$	1:75 1:10
B	NH	Unfiltered suspension Seitz EK filtrate	2×10^{-7} $< 2 \times 10^{-1}$	1:480† 1:10
C	JH	Unfiltered suspension Seitz EK filtrate	10^{-8} 10^{-1}	1:75 1:10
D	JH	Unfiltered suspension Seitz EK filtrate	5×10^{-9} $< 5 \times 10^{-2}$	1:400 1:20

*The complement fixation tests were performed in Exp. A and B with serum Z diluted 1:50, in Exp. C with serum Z 1:100, in Exp. D with serum W 1:10.

†The period of fixation in Exp. B was longer than that usually employed.

submitted to Dr. A. W. Grace. In the highest dilution tested (1:120) it gave a strongly positive Frei response in known reactors who were at the same time negative to the control filtrate of normal yolk-sac.

The combined series of experiment shows clearly that the ability of Seitz EK filtrates, derived from rich suspensions of lymphogranuloma venereum virus, to fix complement specifically in the presence of lymphogranulomatous serum and to elicit a positive Frei reaction is due not to elementary bodies of the virus but to antigen in a less highly organized, possibly soluble, state. The failure of other investigators to obtain similar results is doubtless due to the low concentrations of virus present in the materials which they employed.

11975

Nature of the Capillary Permeability Factor Present in Extracts of Normal Tissues.

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Menkin¹ has shown that inflammatory exudates of various kinds have the property of increasing capillary permeability as demonstrated by the so-called trypan blue test in rabbits. This consists in

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¹ Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

the localization and concentration of intravenously injected trypan blue at the site of an intradermal injection of the exudate or preparations thereof. He ascribes this property to a specific substance in the exudates which he terms leukotaxine, and which, presumably, is liberated into the exudate from the injured tissues. Bier and Planet,² as well as Rigdon,³ have reported that extracts of normal tissues exhibit this capillary permeability effect. Since Rocha e Silva and Bier⁴ have shown that suitable concentrations of histamine give a positive trypan blue test, and since Bier and Planet found that extracts of rabbit's skin, which is comparatively rich in histamine, gave a positive test, while extracts of other rabbit tissues, which are poor in histamine, gave negative tests, it appeared probable that the capillary permeability factor in normal tissues might be histamine rather than some unknown specific tissue constituent. To test this possibility further, extracts from a variety of tissues have been examined with respect to their ability to give a trypan blue test and with respect to their histamine content.

Method. Simple extracts were prepared by grinding 2 to 4 grams of tissue with sand in saline. The amount of saline was varied in different experiments, so that extracts of varying activity could be studied. The extracts were filtered and assayed for their histamine-equivalent content, using the isolated atropinized intestinal strip of the guinea pig for the assay. A solution of histamine acid phosphate of the same activity as the extract was prepared and both extract and histamine solution were injected into the abdominal skin of previously shaved rabbits. A few minutes after the intradermal injections, trypan blue (approximately 10 cc of 1%) was injected via an ear vein. The intensity of coloration of the respective areas was compared at intervals up to 30 minutes after the trypan blue injection.

The results in 7 experiments with guinea pig tissues are shown in Table I and it will be seen that in general the ability of an extract to produce a trypan blue reaction can be accounted for by the histamine activity of the extract, as determined by its effect on the atropinized guinea pig intestinal strip. Trethewie⁵ has shown that the histamine activity of a saline extract of tissues is frequently increased by heating due to the solution of suspended incompletely fragmented cells. The above extracts were therefore boiled, reassayed as to their histamine activity and retested as to their permeability effect. In

² Bier, O., and Planet, U., *Compt. rend. Soc. Biol.*, 1938, **129**, 65.

³ Rigdon, R. A., *Arch. Surg.*, 1940, **41**, 96.

⁴ Rocha e Silva, M., and Bier, O., *Arq. Inst. Biol.*, 1939, **19**, 219.

⁵ Trethewie, E. R., *Austr. J. Exp. Biol. and Med. Sci.*, 1938, **16**, 225.

TABLE I.
Comparative Trypan Blue Tests Produced by Extracts of Guinea Pig Tissues and
by Solutions of Histamine.

	Histamine*	Trypan equivalent	blue test		Histamine	Trypan Equivalent	blue test
I. Lung	2.5	+++		V. Lung	10.0	++++	
Histamine	2.5	+++		Liver	2.5	+++	
				Histamine	10.0	++++	
II. Lung	3.0	+++		VI. Lung	4.0	++++	
Liver	0.45	0		Liver	0.4	+	
Muscle	0.18	0		Muscle	0.07	0	
Histamine	3.0	+++		Histamine	4.0	+++	
III. Lung	2.5	+++		VII. Lung	15.0	++++	
Liver	—	0		Liver	1.5	+	
Histamine	2.5	+++		Muscle	0.5	0	
IV. Lung	3.3	+++		Histamine	15.0	+++	
Histamine	3.3	+++					

*Micrograms of histamine acid phosphate per cc.

every instance in which the histamine activity increased significantly, the trypan blue reaction likewise increased.

It is well known that, in contradistinction to the guinea pig, the liver of the dog is rich in histamine. Consequently saline extracts of dog's liver, lung, and muscle, were tested as above. The liver extracts gave markedly positive trypan blue reactions, the muscle extracts were negative and the lung extracts were active but less so than the liver. Similarly rabbit's blood contains a large amount of histamine in comparison with the blood of guinea pigs or dogs. Serum, obtained from freshly clotted whole blood of rabbits, guinea pigs, and dogs, was studied. Rabbit's serum gave markedly positive trypan blue reactions, guinea pig serum was usually negative, while dog's serum gave a mild reaction. The latter could not be accounted for by its histamine-equivalent content. Extracts were made by Code's method⁶ from the whole blood of a number of rabbits. These extracts produced trypan blue reactions, indicating that the capillary permeability factor can survive boiling with concentrated hydrochloric acid.

It is clear, therefore, that the ability of tissue extracts to produce a positive trypan blue test in rabbits can, in most instances, be accounted for by their histamine activity. The reason for the occasional slight discrepancy is not apparent. Duthie and Chain,⁷ however, have shown that protein degradation products obtained from a variety of proteins by a variety of proteolytic enzymes, can elicit a

⁶ Code, C. F., *J. Physiol.*, 1937, **89**, 257.

⁷ Duthie, E. S., and Chain, E., *Brit. J. Exp. Path.*, 1939, **20**, 417.

trypan blue reaction. It is not surprising, therefore, that tissue extracts may occasionally manifest an activity of this sort which cannot be accounted for by their histamine content. There seems no necessity, therefore, for postulating a specific factor in tissues which has the property of increasing capillary permeability as evidenced by the trypan blue reaction.

11976

Rapid Response of Guinea Pig Thyroid to a Single Injection of Thyrotropic Hormone.

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Microhistometric studies¹ of the guinea pig thyroid after 3 daily subcutaneous injections of thyrotropic hormone have been published. It had been assumed that 3 or 4 days were required for significant histologic change to occur. Loeb² had noted early hypertrophy one day after an intravenous injection. Aron³ also reported that changes were evident in less than 12 hours. Janssen and Loeser⁴ described structural changes in 2 hours after a single injection of 20 guinea pig units. Kummer⁵ also described changes apparent at 2 hours which were more pronounced at 4 hours. Williams,⁶ by a method of continuous *in vivo* observation, detected a cycle of activity that reached a maximum within 24 hours. Okkels,⁷ in his classical monograph of 1934, states "that 30 minutes after intraperitoneal injection in the guinea pig of Evans' Pituitary Extract structural changes appear in the thyroid cells. The colloid is not yet affected but the thyroid cells develop a tremendous swelling, presumably like a gel-sol transformation of the cytoplasm." A chronological and quantitative representation of the changes occurring in the guinea pig thyroid after a single subcutaneous injection of the thyrotropic hormone is desirable.

¹ Rawson, R. W., and Starr, Paul, *Arch. Int. Med.*, 1938, **61**, 726.

² Loeb, L., and Bassett, R. B., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 490.

³ Aron, M., *Rev. Frac. d'Endocrinol.*, 1930, **8**, 472.

⁴ Janssen, S., and Loeser, A., *Arch. f. Exp. Path. u. Pharmacol.*, 1931, **163**, 517.

⁵ Kummer, H. J., *Endokrinologie*, 1938, **20**, 326.

⁶ Williams, R. G., *Am. J. Anat.*, 1937, **62**, 1.

⁷ Okkels, H., *Skand. Arch. f. Phys.*, 1934, **69**, 97.