compound in human subjects confirm these observations. In Table III are presented data bearing upon the excretion of sulfathiazole in the urine of human beings. At once, one is struck by the fact that in comparison with sulfapyridine, definitely less of the excreted sulfathiazole is present in the conjugated form. It is also interesting that the excretion of this compound, following a single oral dose, is generally complete within 24 hours, and that following such a dose, from 80% to 90% of the drug was recovered in the urine, thus denoting that the absorption of sulfathiazole under conditions of the test, was much more complete than one would expect had the subject been given sulfapyridine.

Conclusions. It has been shown that the acute toxicity of sulfathiazole (as measured by the parenteral injection of sodium salts) for mice is one-third greater than that of sulfanilamide, and about half that of sulfapyridine, sulfathiazole methyl and sulfathiazole phenyl. Sulfathiazole is absorbed more readily and is excreted more rapidly than is sulfapyridine. Because of its rate of excretion it is probable that doses of sulfathiazole spaced at intervals of 4 hours will maintain adequate concentrations of the drug in the blood of patients.

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Agent of Lymphogranuloma Venereum in the Yolk-Sac of the Developing Chick Embryo.

GEOFFREY RAKE, CLARA M. MCKEE AND MORRIS F. SHAFFER.

From the Squibb Institute for Medical Research, New Brunswick, N. J.

In the course of chemotherapeutic studies, experiments were performed with a strain of the agent of lymphogranuloma venereum* in which passage-mouse brain was used as a source of virus. About one-third of all mice given intracerebrally 0.03 ml of 1:100 dilution died. The L_D50[1] was obtained with a dilution of 1:14. Because of

* Obtained through the courtesy of Dr. Wm. L. Fleming, the School of Hygiene and Public Health, Johns Hopkins University.

[1] Reed, L. H., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

its low mouse-virulence, which is in confirmation of the results of others,[2, 3] an attempt was made to adapt the infective agent to the chorio-allantois of the chick embryo[4] in the hope of increased activity. Lesions appeared on this membrane, albeit irregularly.[5] Thus during 15 passages, approximately two-fifths of all eggs inoculated showed no lesions; one-fifth showed large central foci on the membrane; and in two-fifths the chorio-allantois showed few or many grey foci, 1 mm in diameter. The virus on the chorio-allantois was never lethal for the embryo. When 3rd, 4th and 5th passage membranes were tested in mice, 3 out of 4, 0 out of 4, and 1 out of 4 animals respectively died following intracerebral infection with 1:5 dilution. No mice succumbed when inoculated from the 10 subsequent passages. It appears, therefore, that during propagation in this tissue the particular strain of lymphogranuloma venereum virus either persisted there in very low titer or else suffered a further loss in its virulence for mice.

In view of these conditions, a further effort was made to procure more active infection by recourse to inoculation into the yolk-sac, a method used by Cox[6] to obtain large numbers of rickettsiae. Passages were initiated with mouse-brain material. Five, 6- or 7-day eggs were injected in the yolk-sac with a volume of 0.5 to 1 ml of suspension. In 4 passages in which inoculation was made into the yolk-sac, of the various materials (brain, viscera, chorio-allantois, yolk-sac, yolk and vitelline) which were separately tested after 3 or 4 days' incubation of the egg, virus was demonstrated only in the yolk-sac material and undiluted yolk. From the 5th passage onward only yolk-sac material was used as inoculum. In this passage, eggs receiving 1:15 dilution of yolk-sac were dead by the 4th day and mice died after infection with a 1:100 dilution. Smears made from infected yolk-sacs showed very numerous minute granules which stained with Giemsa stain. These granules, never seen in preparations of normal yolk-sac, resembled the "granulo-corpuscles" seen in the meningeal exudate of mononuclear cells in mice infected with lymphogranuloma venereum[7, 8] and were similar to the elementary

2 Levaditi, C., Ravaut, P., Schoen, R., and Levaditi, J., *C. R. Soc. Biol.*, 1933, **114**, 499.

3 MacCallum, F. O., and Findlay, G. M., *Lancet*, 1938, **2**, 136.

4 Burnet, F. M., *Med. Res. Counc. Spec. Rep.*, Ser. No. 220, London, 1936.

5 Sanders, M., *Arch. Path.*, 1939, **28**, 541.

6 Cox, H. R., *Pub. Health Reports*, 1938, **53**, 2241.

7 Miyagawa, Y., Mitamura, T., Yaoi, H., Ishii, N., Nakajima, H., Okanishi, J., Watanabe, S., and Sato, K., *Jap. J. Exp. Med.*, 1935, **13**, 733.

8 Schoen, R., *Ann. Inst. Pasteur*, 1939, **62**, 260.

bodies of vaccinia. In smears the granules were seen chiefly lying free although they were occasionally observed within cells. In sections the granules were found to be chiefly intracellular, lying in large numbers within the yolk-sac cells. They are probably liberated into the surrounding yolk by disruption of these cells.

Over 25 yolk-sac passages have been completed. The infectious agent is now lethal within 9 days for the developing embryo in dilutions through 10^{-9}. A typical titration at the 20th passage is shown (Table I).

TABLE I.

Dilution of yolk-sac	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
No. of days' infection to death of embryo	7	6,7	7,7	7,8	8,8	9,9

While careful titrations were not carried out in early passages, the virulence of the virus for the embryo does not appear to have increased greatly. At the present time dilutions of 1:10 require 3 or 4 days to kill as they did at first. During the course of the 25 yolk-sac-passages about 1 mouse in 5, of those receiving 1:1000 dilution of material, died and the L_D50 for mice was obtained with a dilution of 1:200.

The granules were purified by differential centrifugation in the cold. The material was spun for 1 hour at 2000 rpm; the sediment was discarded and the supernate recentrifuged for 2 hours at 12,000 rpm. The sediment from this second centrifugation represented a preparation freed from most yolk-sac constituents and contained a rich suspension of the granules which are believed to be the elementary bodies of the infectious agent. Several tests of the sediment and supernate after high speed centrifugation showed nearly all of the mouse-infectious material to be in the sediment. The small amounts found in the supernate were believed to be due to insufficient centrifugation.

Both formalinized and untreated suspensions of such granules evoke specific agglutinins in rabbits (serum titers of 1:400) and, to a lesser degree, in chickens.

We believe that the use of the yolk-sac technic offers a method of great promise in the further experimental study of the agent of lymphogranuloma venereum, as well as in the development of improved reagents for diagnosis, prophylaxis and therapy (*e. g.*, Frei antigen and hyper-immune serum).