illustrated this action of lactogenic hormone by production of deciduomata in hypophysectomized rats, and Astwood[4] has employed mucification of the vagina in immature rats as a means of demonstrating the same effect. When gonadotropic substances[1] are employed in attempts to stimulate function of the corpus luteum, the question of dosage must be regarded as highly important. Dosage of a gonadotropin which can cause follicular as well as luteal development is difficult to regulate. If the dose is low only follicles form; if the dose is high both follicles and corpora lutea develop. Demonstration of luteal function in such instances is difficult since the output of estrogen would tend to override effects of luteal hormone. Until such problems are more thoroughly investigated, therefore, it does not seem wise to reject the view that some gonadotropins which cause formation of corpora lutea may also cause the corpora to function.

Summary. Fourteen rats, hypophysectomized 1 to 5 days after mating, were injected with lactogenic hormone (1 or 3 mg daily). In 4 animals implantation failed to occur. Of the remaining 10 animals 2 carried to term or beyond, while pregnancy was interrupted after 6-17 days in 8 rats. It would seem that lactogenic hormone was capable of stimulating corpus luteum function sufficiently to induce implantation and to maintain pregnancy for periods ranging from 6 days to term or beyond.

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Chemotherapy of Lymphogranuloma Venereum with Sulfonamide Drugs.

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It was recognized early that in lymphogranuloma venereum, unlike most other virus diseases, some success attended treatment of infected mice or guinea pigs with drugs of the sulfonamide group. The earlier work has been summarized by Findlay.[1] In a previous communication from this laboratory[2] it was indicated that,

4 Astwood, E. B., *Endocrinology*, 1941, **28**, 309.

1 Findlay, G. M., *Lancet*, 1940, **2**, 682.

2 McKee, C. M., Rake, G., Greep, R. O., and van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

in preliminary experiments, sulfapyridine and sulfathiazole given orally in the form of the free acid twice daily after infection were equally effective in preventing death of mice infected intracerebrally. In these experiments mouse brain was used as inoculum, and even with a 1 in 10 suspension only 63% of the control mice died. In subsequent papers Findlay[1, 3] repeated his earlier results with sulfanilamide, showing a reduction of mortality from 84% in the controls to 49% in those fed sulfanilamide in gum acacia daily.[3] He further attempted to assess the relative value of sulfanilamide, sulfapyridine, sulfathiazole, sulfamethylthiazole and disodium 4:4 bis-o-carboxybenzoylaminodiphenylsulfone.[1] The mice received 10 mg per 20 g of body weight in 2 equal daily doses orally by stomach tube for the first 4 compounds and subcutaneously for the last. One hundred mice were treated with each compound, together with 100 controls, and the ratio of treated survivors to control survivors was used as a method of comparing one experiment with another. On the basis of his results, Findlay maintained that the order of activity ran: sulfamethylthiazole > sulfapyridine > sulfathiazole > sulfanilamide > lutazol. Smaller experiments with compounds inoculated intramuscularly in olive oil indicated slight activity for ammonium 4-nitrobenzene-sulfonate and 4:4-dinitrodiphenylsulfone.

All of the previous work had been carried out with virus material of low titer derived from infected mouse or monkey brain. The higher titer which could be achieved with infected yolk-sac[4] gave rise to the hope that with such material more conclusive evidence of the relative efficacy of the different sulfonamide drugs could be obtained than was possible with material which killed at best only about 80% of the control mice when very high concentrations of tissue were used.

In the early experiments reported here the inoculum was prepared by grinding heavily infected yolk sacs in a mortar with quartz. This material gave an L_D50 of 1/56 for 10 to 12 g mice when 0.03 cc were given intracerebrally, and titered 10^{-8} to 10^{-9} by yolk-sac inoculation of eggs. In later experiments the heavily infected yolk sacs were shaken, without beads, in a shaking machine in the presence of sufficient broth to make a 10% suspension. Such treatment liberated all virus by rupture of the yolk cells but left the greater part of the chick tissue in a tight ball at the bottom of the suspension. With this material the L_D50 for mice inoculated with 0.03 cc intracere-

[3] Findlay, G. M., *Brit. J. Exp. Path.*, 1940, **21**, 356.

[4] Rake, G., McKee, C. M., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 332.

TABLE I.
Results of Chemotherapy with Sulfonamide Drugs in Lymphogranuloma Venereum of Mice.

Infective dose		Sulfanilamide Deaths to 10 d	Sulfanilamide Deaths to 4 wk	Sulfathiazole Deaths to 10 d	Sulfathiazole Deaths to 4 wk	Sulfapyridine Deaths to 10 d	Sulfapyridine Deaths to 4 wk	Sulfaguanidine Deaths to 10 d	Sulfaguanidine Deaths to 4 wk	Sulfadiazine Deaths to 10 d	Sulfadiazine Deaths to 4 wk	Normal diet Deaths to 10 d	Normal diet Deaths to 4 wk
Exp. 1	1/10											5/5	5/5
Ground	1/50	0/10	*3/10	0/10	0/10	7/10	7/10	2/10	3/10			9/10	9/10
Virus	1/100											4/9	6/9
	1/500					(0.5% diet)						0/10	0/10
	1/1000											0/10	†4/10
Exp. 2	1/10	10/10	10/10	7/10	7/10	10/10	10/10	2/10	2/10			10/10	10/10
Ground	1/50	2/10	2/10	1/10	1/10	0/10	0/10	1/10	1/10			6/10	6/10
Virus	1/100											2/10	3/10
Exp. 3	1/10	16/20	17/20	15/20	15/20	16/20	16/20	10/20	11/20			10/10	10/10
Ground	1/50											5/10	5/10
Virus	1/100											2/10	3/10
Exp. 4	1/50	9/20	10/20	8/20	8/20	10/20	10/20	7/20	7/20			10/10	10/10
Shaken	1/500											14/20	14/20
Virus	1/1000											7/20	7/20
	1/5000											1/20	3/20
Exp. 5	1/50	5/20	5/20	7/20	8/20	9/20	10/20	5/19	5/19	20/20	20/20	8/10	9/10
Shaken	1/500											4/19	4/19
Virus	1/1000											2/19	2/19
	1/5000											1/20	1/20
Exp. 6	1/50			6/20	7/20					1/20	3/20	8/8	8/8
Shaken	1/500											3/10	3/10
Virus	1/1000									(0.1% diet)		0/10	1/10
Exp. 7													
Shaken	1/50	23/40	25/40	17/40	17/40	35/40	35/40	19/39	20/39	20/40	22/40	10/10	10/10
Virus										(0.1% diet)			

Except where indicated, the drug was incorporated as 1% of the diet.
*3/10 indicates that of 10 mice in this group 3 died.
†Probably died of intercurrent infection.

brally was 1/416 and the titer of yolk-sac inoculation was increased approximately tenfold.

Treatment with the sulfonamide drugs intimately mixed as 1% of No. 1 Sherman dry diet[2] was commenced 24 to 48 hours before the infecting dose was given in order that the mice might become accustomed to the food. Treatment was continued for 10 days after infection. The results are shown in Table I.

With inoculum prepared by grinding, a dose of 1:10 killed all the control mice within 10 days (*i. e.*, from acute infection; later deaths up to 4 weeks were usually due to hydrocephalus). Sulfaguanidine gave the best results followed by sulfathiazole, sulfanilamide and sulfapyridine. The 1:50 dose, which killed 75% of the controls, was apparently too low to show conclusive differences between the different drugs.

With inoculum prepared by shaking, sulfadiazine* at 0.1% was the most effective drug, followed by sulfathiazole, sulfaguanidine, sulfanilamide and sulfapyridine. With sulfadiazine at 1% all the mice died (see experiment 5) with an average survival time of 73 hours. That their deaths were due to combined toxicity of drug and infection is suggested by the fact that in our hands, contrary to previous reports,[5] only 4 out of 9 mice on 1% sulfadiazine diet for 30 days died, and the earliest death in this group was on the 10th day. Twelve mice receiving 1% sulfadiazine diet showed a blood level of total sulfadiazine from 21.6 to 43.1 mg % (free 17.5 to 31.0 mg %) which is 7 to 8 times the figures obtained on an equal number of mice fed 1% sulfathiazole. With 0.1% sulfadiazine in the diet the blood levels were: total 5.6 to 6.4 mg %, free 5.3 to 6.2 mg %, and this concentration in the diet was therefore chosen for future experiments.

It is thus clear that drugs of the sulfonamide group, and particularly sulfadiazine, sulfathiazole and sulfaguanidine are capable of preventing death from acute infection with lymphogranuloma venereum when given prophylactically. Preliminary experiments on the therapeutic administration of the drugs in the diet (in which the inoculated mice, directly after inoculation, are placed in cages containing the appropriate drug-diet mixture) show that the drugs are just as effective in preventing death under these conditions. Thus

* We wish to acknowledge the kindness of Mr. F. M. Fargo, Jr., and Dr. M. L. Crossley of the Calco Division of the American Cyanamid Company for supplying us with the sulfadiazine used in these experiments.

5 Feinstone, H. W., Williams, R. D., Wolff, R. T., Huntington, E., and Crossley, M. L., *Bull. Johns Hop. Hosp.*, 1940, **67**, 427.

in experiments with shaken virus at 1:50, 14 out of 15 control mice on normal diet died within 10 days compared to 5 out of 25 mice receiving 1% sulfathiazole diet and 4 out of 24 receiving 0.1% sulfadiazine diet.

While the action of the sulfonamide drugs in preventing death from acute infection is thus incontravertible, it was noticed that in practically no case did any mouse, treated or untreated, remain free from symptoms of disease. These symptoms are completely lost within 2 weeks except in those mice which have obvious chronic hydrocephalus with domed cranium and progressive loss of weight. In view of the fact that most humans infected with lymphogranuloma venereum remain Frei positive and show high complement fixing antibodies despite sulfonamide therapy,[6] and that, in our opinion, such persistence of skin sensitivity and serum antibodies probably indicates persistence of infection in a latent form, it seemed important to study the possibility of a carrier state in recovered mice, whether drug-treated or not. Proofs of the persistence of the virus in sick mice for periods up to 43 days after infection have already appeared in the literature.[7-10] In some cases these were control mice and in others mice which had received sulfonamide drugs. In our own experience, mice have been allowed to live for 3½ months after infection. They developed normally and the females produced and reared healthy families. When sacrificed, a certain number of the animals showed varying, but not crippling, degrees of hydrocephalus which was slightly more frequent in the controls (4 out of 6 animals) than in those which had received drugs (6 out of 12 for sulfaguanidine and 2 out of 8 for sulfathiazole). Virus was isolated from the brains of mice in all 3 groups and demonstrated by yolk-sac inoculation. Small amounts of virus were also demonstrated in the pooled spleens from the sulfaguanidine-treated group in these early experiments. A strain of virus recovered from the brains of the sulfaguanidine-treated mice was studied further. It showed a decreased virulence for eggs by yolk-sac inoculation (10-fold) and for mice by intracerebral inoculation (5-fold), and this decreased virulence persisted for 3 egg passages, which was as long as it was studied. Schlossberger and Bär[9] also comment on the decreased virulence of the strains recovered from convalescent or sick mice.

[6] Harrop, G. A., Rake, G., and Shaffer, M. F., *Trans. Assn. Am. Phys.*, 1941, in press.

[7] MacCallum, F. O., and Findlay, G. M., *Lancet*, 1938, **2**, 136.

[8] Levaditi, C., *Compt. rend. Soc. biol.*, 1938, **129**, 490.

[9] Schlossberger, H., and Bär, F., *Zbl. Bakt.*, 1939, **144**, 228.

[10] Bär, F., *Z. f. Immunitätforsch. u. exp. Therap.*, 1940, **97**, 344.

It would appear, therefore, that while the sulfonamide drugs are able to prevent deaths from acute infection with lymphogranuloma venereum, their action is not always curative. Recovered mice may live healthy lives and raise families over periods of 3½ months, but at the end of this time show signs of chronic hydrocephalus and yield active, although slightly less virulent, strains of virus from the brain and other tissues. The importance of this in human chemotherapy cannot be over-emphasized. The idea that patients, receiving such therapy and becoming clinically "cured", are cured despite persistence of positive Frei and complement fixation tests requires reconsideration. Studies are now under way on the relative sterilizing powers of the different drugs, on the duration of the carrier state, and on the possible transmission of the infection from infected mother to the foetus *in utero*.

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Sulfonamide Inhibition of Bacterial Luminescence.*

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Luminescence intensity is inhibited at once on addition of sulfanilamide either to broth cultures, or washed cell suspensions of luminous bacteria in phosphate buffered NaCl solutions containing glucose as oxidizable substrate. At the respective optimum temperatures of marine species, *Achromobacter Fischeri* (25 to 28°C), *Photobacterium phosphoreum* (15 to 17°C), and a fresh-water species, *Vibrio phosphorescens* (28 to 30°C), a concentration of 100 mg % sulfanilamide greatly reduces the intensity of luminescence, with little or no effect on the rate of respiration (Fig. 1). Except at high concentrations of the drug the inhibition of luminescence is largely or completely reversible (Table I) by centrifuging and resuspending the cells in a drug-free solution. In all these respects the effects of sulfanilamide resemble those of typical narcotics, such as the urethanes[1, 2] and barbital.[3] By analogy, it would be predicted that sulfanilamide will inhibit the luminescent

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1 Taylor, G. W., *J. Cell. Comp. Physiol.*, 1934, **1**, 297.

2 van Schouwenburg, K. L., *On Respiration and Light Emission in Luminous Bacteria*, Thesis, Delft, 1938.

3 Johnson, F. H., and Chambers, E. L., *J. Cell. Comp. Physiol.*, 1939, **13**, 263.