Conclusion. The menstrual discharge of normally menstruating women contains a heat-stable factor, insoluble in lipoid solvents, which stimulates increased luteal activity in mature rats. This factor has also been found in venous blood and urine at the time of menstruation. Its progesterone-stimulating effect is not accountable to any free or combined estrogen or to any known gonadotropic hormone.

So far as this work has progressed, the progesterone-stimulating factor of menstrual discharge resembles the substance reported by Astwood and Greep as occurring in the rat placenta.[4] Our material has not yet been tested upon hypophysectomized rats but the fact that alcohol precipitation destroys toxicity without decreasing progesterone-stimulating activity makes it appear that the progesterone stimulation is not a non-specific toxic effect.

11370

Unsuccessful Therapy in Experimental Equine Encephalomyelitis with Salt Solutions of Varied Concentrations and Sulfanilamide Compounds.*

J. EMERSON KEMPF AND MALCOLM H. SOULE.

From the Hygienic Laboratory, University of Michigan, Ann Arbor.

The high mortality of Eastern equine encephalomyelitis in both man and animals has prompted interest in therapy. Specific antisera may have value in the treatment of horses if administered early, but its use after the disease is well established is ineffectual.[1] In horses the disease may be suspected and treated specifically; however, in man the diagnosis must usually await the appearance of neurological signs at which stage the lesions are too far advanced for antiserum therapy. This shortcoming suggested the investigation of other therapeutic procedures employing the highly susceptible rat and mouse.

The beneficial effect of hypertonic solutions, such as 10-25%

[4] Astwood, E. B., and Greep, R. O., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 713.

* This work was aided by a grant from the Clara Ward Seabury Clinic for Infantile Paralysis.

[1] Personal communication, Dr. B. M. Lyon, Assistant Director, Vet. Dept., Lederle Labs., New York.

glucose, in edema of the CNS has long been recognized, particularly in edema of traumatic origin. In contrast, poliomyelitis of man and monkey has apparently responded favorably to injections of hypotonic solutions in the hands of Retan.[2] Therefore, the effects of hypertonic and hypotonic saline solutions were compared in rats with experimental EEE using isotonic saline as a control. Intra-abdominal injections of the solutions were begun 40 hours after intracranial inoculation of the rats, at which time about 10% of the animals showed well advanced signs of the disease. The remaining rats appeared normal. The fluids were administered in 4 cc quantities every 2 or 3 hours, totaling approximately 40 cc daily for a 150 g rat. This is equivalent to 12 liters for a 60 kg man. The saline injections were continued until 83% of the animals were dead. This treatment in no way altered the course of the infection (Table I).

Many workers have reported the use of sulfanilamide and related compounds on virus infections of man and animals. In man sulfanilamides seem to be of value in lymphopathia venereum,[3] and in animals success has followed the treatment of meningo-encephalitis associated with canine distemper,[4] with negative results in poliomyelitis,[5, 6, 7] rabbit myxoma, rabbit fibroma, herpetic encephalitis, chorio-meningitis and St. Louis encephalitis.[8]

The following compounds were selected for evaluation in the treatment of EEE in rats and mice: sulfanilamide, sulfapyridine,

TABLE I.
Effect of Intraäbdominal Injections of Saline Solutions of Varied Concentrations on EEE in Rats Infected with 10 MCLD.*†

Treatment			No. of rats	Mortality
Solution	Dose, cc	Frequency		
				%
None (control)			45	36 (80)
Hypertonic Saline—3%	4	Every 2-3 hr	45	39 (86)
Hypotonic Saline—0.375%	4	" "	45	39 (86)
Isotonic Saline—0.875%	4	" "	45	36 (80)

*Duration of the experiment was 72 hr.
†MCLD = Minimal cerebral lethal doses.

2 Retan, G. M., *J. Ped.*, 1937, **11**, 647.

3 Shaffer, L. W., and Arnold, E., *Arch. Derm. and Syph.*, 1938, **38**, 705.

4 Marcus, P. M., and Necheles, H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 385.

5 Toomey, J. A., and Takacs, W. S., *Arch. Ped.*, 1938, **55**, 307.

6 Kelsen, S. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 718.

7 Toomey, J. A., and Takacs, W. S., *Arch. Ped.*, 1939, **56**, 384.

8 McKinley, E. B., Meek, J. S., and Acree, E. G., *J. Infect. Dis.*, 1939, **64**, 36.

2-sulfanilamidothiazol (sulfathiazol), 2-sulfanilamidomethylthiazol (sulfamethylthiazol), and 2-sulfanilamidophenylthiazol (sulfaphenylthiazol). The animals were inoculated intracerebrally. Treatment with the drugs was started 24 hours later and was continued twice daily by the intraäbdominal route. The rats received 40 mg of sulfanilamide (human dose of sulfanilamide compounds: maximum 6 g daily *per os*); 2 mg of sulfapyridine, and 15 mg of the thiazol compounds. The 5 sulfanilamide compounds were injected into the mice in 2 mg doses following the aforementioned technic. This procedure is open to criticism based on the clinical opinion that in order to maintain a satisfactory blood level of the drug it must be given 4 times daily. However, in the hands of Barlow and Homburger[9] the treatment of staphylococcus infections of mice with 2 daily doses of thiazol compounds administered by stomach tube was successful. In addition, 1,000 times the minimal cerebral lethal dose may be considered a too severe inoculum, but in man the virus would be present in high con-

TABLE II.
Chemotherapy of EEE in Rats Infected with 1,000 MCLD.*

Treatment			
Compound	Dose, mg twice daily	No. of rats	Mortality
			%
None (control)		16	16 (100)
Sulfanilamide	40	14	14 (100)
Sulfapyridine	2	14	14 (100)
Sulfathiazol	15	12	12 (100)
Sulfamethylthiazol	15	9	8 (89)
Sulfaphenylthiazol	15	9	9 (100)

*See footnote Table I.

TABLE III.
Chemotherapy of EEE in Mice Infected with 1,000 MCLD.*

Treatment			
Compound	Dose, mg twice daily	No. of rats	Mortality
			%
None (control)		12	12 (100)
Sulfanilamide	2	12	12 (100)
Sulfapyridine	2	12	11 (92)
Sulfathiazol	2	12	11 (92)
Sulfamethylthiazol	2	12	10 (83)
Sulfaphenylthiazol	2	12	12 (100)

*See footnote Table I.

[9] Barlow, O. W., and Homburger, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 792.

centration before the diagnosis could be made and the drug administered.

The data are presented in Tables II and III. The drugs under the conditions of the experiment had no apparent effect on the course of the infection.

11371 P

Growth Promotion of the Tubercle Bacillus by Serum Albumen.

C. H. BOISSEVAIN.

From the Laboratory of the Colorado Foundation for Research in Tuberculosis at Colorado College, Colorado Springs, Colo.

The author[1] described the growth of single colonies of tubercle bacilli in the depth of coagulated rabbit plasma. It was then observed that rabbit serum + agar was a less favorable medium than coagulated plasma. Only a few colonies developed in "hormone agar", but many more in hormone agar + rabbit serum. The growth-promoting effect of serum was quite variable. Evans and Hanks[2] confirmed the favorable effect of rabbit serum and obtained good growth in the depth of Long's medium after the addition of blood or serum. Kallo and Nathan[3] and Pagel[4] found that some human sera support the growth of tubercle bacilli while others fail to do so. Pagel could not demonstrate the presence of either growth-promoting or specific inhibiting substances. Drea[5] confirmed the inhibiting effect of agar and found that tubercle bacilli grew in the depth of a modified Long's medium when it had been inoculated with varying quantities of a bacillary emulsion.

The addition of human, guinea pig, rabbit, sheep or horse serum all enhance the growth of tubercle bacilli in the depth of synthetic medium The growth appears earlier, is more abundant and takes place after inoculation of smaller quantities. In synthetic medium where the nitrogen is supplied by glycine, growth rarely occurs after inoculation of less than 10^{-1} mg tubercle bacilli, in media with asparagine-ammonium citrate as nitrogen source, growth frequently

1 Boissevain, C. H., *Am. Rev. Tuberculosis*, 1926, **13**, 90.

2 Evans, B., and Hanks, J. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 112.

3 Kallós, P., and Nathan, E., *Z. f. Immunitäts forschung*, 1932, **76**, 393.

4 Pagel, W., *Tubercle*, 1934-35, **16**, 256; *J. Path. and Bact.*, 1940, **50**, 111.

5 Drea, W. F., *J. Bact.*, 1940, **39**, 197.