found after other blocking agents at this hour.[1]

The blocking capacity of Nembutal *per se*, in single dose (30 mg/kg), can endure through most of 4 hours at least, since 2 proestrous rats injected at noon were "blocked." The effect does not last much longer, however, for 2 proestrous rats injected at 10 A.M. ovulated during the following night. In control experiments, 4 proestrous rats were injected intraperitoneally at 2 P.M. with 0.1 ml of 20% propylene glycol in 10% alcohol. All ovulated overnight.

The fact that Nembutal will prevent ovulation when administered at appropriate hours, furnishes additional strong evidence that neurogenic stimulation of the hypophysis is essential for ovulatory discharge of LH. Postponement of hypophyseal stimulation for a full 24 hours following the brief action of Nembutal during the 2-4 P.M. interval, implies a 24-hour periodicity in some neural (presumably hypothalamic) center which constitutes a part of the LH-release apparatus in this species. Evidence of similar nature was obtained recently in a quite different experiment, based on the 24-hour advancement of ovulation time by progesterone in 5-day cyclic rats.[3,4]

It is theoretically possible to block ovulation day after day by treatment with barbiturates at appropriate hours. We have, in fact, accomplished this in several cases (series II), but the question of precise timing on days following proestrus requires further analysis. Whenever we succeeded, the vaginal smears gave evidence of continued estrogen secretion and, therefore, of continued secretion of gonadotrophin at subovulatory level. This recalls the report of Westman[5] that certain rats were in constant estrus during 21 days of treatment with twice-daily subcutaneous 1-methyl-5,5-ethylphenyl barbituric acid.

Summary. Brief action of Nembutal during certain critical hours on the day of proestrus delays the ovulatory discharge of hypophyseal gonadotrophin for 24 hours. This is evidence of a 24-hour periodicity in the neural mechanism which incites such discharge.

[3] Everett, J. W., *Anat. Rec.*, 1949, **103**, 448.

[4] Everett, J. W., and Sawyer, C. H., unpublished.

[5] Westman, A., *Acta Med. Scand.*, 1947, **128**, Suppl. 196, 111.

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17304. Lethal *Brucella* Infections in White Mice Produced with the Aid of the Mucin Technic.

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Although the mucin technic has been employed in order to lower resistance against many microorganisms, no attempts have hitherto been made to produce lethal infections with *Brucellae* by means of this technic. It was found that the experimental infections produced in mice without the use of the mucin technic are mild, following a retrogressive and chronic course, so that the mouse may be used as a reservoir for keeping these pathogenic organisms alive. Lethal infections of mice have so far not been reported.[1,2] The following experiments were undertaken in order to produce more acute or even lethal infections with the aid of the mucin method. We expected that the fatal outcome of such infections would enable us to determine the virulence of *Brucellae*, the immunizing power

[1] Lustig, A., and Vernoni, G., in *Kolle, Kraus, Uhlenhuth, Handbuch der pathogenen Mikroorganismsn*, 1928, **4**, 511.

[2] *Topley and Wilson's Principles of Immunity*, revised by G. S. Wilson and A. A. Miles, 3rd edition, London, E. Arnolds & Co., 1948, **1**, 828.

TABLE I.
Lethal Effect of Different Strains of *B. abortus* Injected Intra-abdominally Into Mice Together with 0.5 cc of a 10% Mucin Suspension.

Strain	Quantity injected (cc)	No. of animals			Cumulative numbers		Mortality, %
		Under experiment	Died	Survived	Died	Survived	
Local strain	0.5	8	5	3	8	3	72.7
	0.2	8	1	7	3	10	23.1
	0.1	8	2	6	2	16	11.1
	0.05	8	0	8	0	24	0
Swiss strain	0.5	8	7	1	13	1	92.9
	0.2	8	4	4	6	5	54.5
	0.1	8	2	6	2	11	15.4
	0.05	5	0	5	0	16	0
Abortus 19	0.5	8	4	4	8	4	66.7
	0.2	8	4	4	4	8	33.3
	0.1	8	0	8	0	16	0

of vaccines as well as the protective and therapeutic action of sera and chemotherapeutic substances.

Test organisms. Two strains, one of *B. melitensis* and another of *B. abortus,* isolated in this country were employed. In addition to these, 3 laboratory strains of *B. abortus* were employed. One was obtained from the National Type Collection in London and 2 others representing the attenuated "Abortus 19" from the Government Veterinary Laboratories in Tel Aviv. One of these strains, designated as "Swiss strain 19" proved to be richer in S-forms than the other.

Mucin. Mucin in scales manufactured by Burroughs Wellcome, London, was employed. A weighed amount was thoroughly ground in a mortar with glass sand, suspended in saline to give the required concentration and autoclaved. Concentrations of 5.0%, 7.5%, and 10.0% were employed.

Experimental infections. White mice from the laboratory stock served as experimental animals. The mice were 5 weeks old at the time of the tests and averaged 20 g in weight. Preliminary experiments with 48-hour broth cultures showed that 0.1-0.5 cc of these cultures given intra-abdominally did not produce lethal infections, though after sacrificing the animals 5 days after injection, pure cultures of *Brucellae* could be obtained from most organs. The spleens of these animals showed marked enlargement. Another group of animals now received varying quantities of 48-hour broth cultures ranging from 0.005 to 0.5 cc, to which 0.5 cc of mucin in varying concentrations was added. These experiments which were carried out with *B. abortus* as well as with *B. melitensis* showed that a mucin concentration of at least 10% must be employed in order to obtain a lethal effect; 0.5 cc of broth culture produced a 100% mortality and 0.2 cc of broth culture a 50% mortality when injected together with 0.5 cc of a 10% mucin suspension. Mucin suspensions of 5.0% and 7.5% given with the same quantities of broth cultures were not able to produce a lethal effect. No differences were observed between *B. melitensis* and *B. abortus.*

We now examined the lethal effect of different strains of *B. abortus* given under the same conditions. The results of these experiments are summarized in Table I. While 2 of the strains, the local strain and the "Abortus" 19 proved to be relatively avirulent, both exhibiting their LD_{50} at 0.35 cc, the Swiss strain proved to be more virulent for mice, if injected along with mucin, having a LD_{50} at 0.19 cc.

The mice which succumbed to the infection yielded pure cultures of the infective strains from their organs. Death generally occurred after 1-2 days, though with small doses such as 0.1 cc it sometimes occurred after 4-8 days. The shorter the period between the injection and death the less was the spleen enlarged.

The surviving animals of the different ex-

periments were killed within a period ranging from the sixth to the 50th day after injection. The organs of the infected animals were cultured on agar plates as well as in broth tubes. In many cases the agar cultures yielded negative results, while after incubation of the broth cultures for 7 days positive results were obtained. The microorganisms isolated from these cultures were identified as *Brucellae* by agglutination with specific antisera. The following organs were examined: heart, lungs, liver, spleen, kidneys and genital glands. Seventy-six mice which were dissected up to the 28th day after injection proved to harbor *Brucellae* in all or in several organs. Fifteen out of 20 mice which were dissected between the 30th and the 50th day after the onset of the infection proved to harbor *Brucellae*, while only 5 yielded sterile cultures. The organs of 145 mice were cultured after death took place or when the mice were sacrificed. The positive results obtained from the different organs were as follows: Heart 131, lungs 126, liver 133, spleen 136, kidneys 136, genital organs 127. These figures prove that significant differences in the infection rate of the different organs do not exist, but only that foci of infection are equally distributed over the different organ systems of the infected animal.

Summary. *B. melitensis* and *B. abortus* did not produce lethal infections in mice even when 0.5 cc of broth cultures were injected intraabdominally. On the other hand lethal infections were produced if the broth cultures were administered together with 0.5 cc of a 10% mucin suspension. The LD_{50} for *B. melitensis* was 0.2 cc, for 2 strains of *B. abortus* 0.35 cc, and for a third strain of *B. abortus* 0.2 cc. In most of the surviving animals chronic infections were noted, if the dissections were performed up to the 50th day after the onset of the infection.

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17305. Absorption of Subtilin in the Rabbit.

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Subtilin,[1,2] an antibiotic active *in vitro* against Gram-positive bacteria[3] and *M. tuberculosis*,[3-6] has also been found active *in vivo* against the more sensitive pathogenic bacteria. For example, Salle and Jann injected subtilin intraperitoneally into mice infected with pneumococcus Type III,[7] *Streptococcus pyogenes*,[8] or *Staphylococcus aureus*,[9] and into guinea pigs subjected to experimental anthrax infections.[10] Prophylactic and therapeutic effects, depending on the relations of infection and dosage times, were striking.

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