to them that this acid could be synthesized from linoleate. Nunn and Smedley-Maclean[8] also observed that supplementation with linoleic or with linolenic acids resulted in the production of arachidonate.

While this work was in progress, Rieckehoff, Holman and Burr[7] reported on the effect of dietary fat on polyethenoid fatty acids of rat tissues. One of their conclusions was that the deposition of polyunsaturated fatty acids takes place primarily in the phospholipid fraction with very little change in the neutral fat. They also found that the effect of the diet on the occurrence of the unsaturated fatty acids is considerably greater in some organs than in others, and that the polyunsaturated acids are particularly low in the skin and depot fat. Smedley-Maclean and Nunn[9] also found small amounts of arachidonate in the phospholipids of liver and muscle of fat deficient rats, but none in their neutral fat.

On the basis of these observations it becomes evident that the phospholipid fraction from the various organs (especially the heart, liver, brain and kidney) should be isolated and analyzed separately for better evaluation of the changes which take place in the essential fatty acid content of the rat. Otherwise, the small amounts of these acids are diluted in the relatively large quantities of fat extracted from the entire carcass, and the changes become less evident.

Summary. 1. Caloric restriction, causing severe emaciation, followed by *ad libitum* feeding on the same fat-deficient diet, precipitated symptoms of fat-deficiency in mature rats. When the rats, after depletion, were maintained on the fat-free diet *ad libitum* for sufficiently long periods, spontaneous recovery was observed.

2. The tissues of these rats were analyzed for linoleic, linolenic and arachidonic acids by the spectrophotometric method. The concentrations of linoleic and arachidonic acids in the body fat were found to vary with the appearance of the symptoms; it was high at the end of the depletion period, and low at the stage when fat deficiency symptoms were present. The higher levels of linoleic and linolenic acids at the time of recovery, after a long period on the fat-free diet, are considered as further evidence for the synthesis of essential fatty acids in the mature rat.

3. Supplementation of ethyl linoleate increased the level of arachidonate in the body fat.

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[8] Nunn, L. C. A., and Smedley-Maclean, I., *Biochem. J.*, 1938, **32**, 2178.

[9] Smedley-Maclean, I., and Nunn, L. C. A., *Biochem. J.*, 1940, **34**, 884.

17303. The Blocking Effect of Nembutal on the Ovulatory Discharge of Gonadotrophin in the Cyclic Rat.

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In a preceding investigation[1] it was indicated that in 4-day cyclic rats of the Vanderbilt strain, under our colony conditions, neurohumoral stimulation of the hypophysis occurs with great uniformity on the day of proestrus between 2 P.M. and 4 P.M., inciting ovulatory discharge of luteinizing hormone. Administration of intravenous dibenamine (N,N-dibenzyl-β-chloroethyl amine) or subcutaneous atropine at 2 P.M. or earlier characteristically blocks ovulation. Injection of either agent at 4 P.M. or later, however, rarely interferes. We have now found essentially identical time relationships with Nembutal (pentobarbital sodium).

Two dose levels of the barbiturate were

[1] Everett, J. W., Sawyer, C. H., and Markee, J. E., *Endocrinology*, 1949, **44**, 234.

TABLE I.
Blocking of Ovulation by Nembutal When Injected at a Critical Hour (2 P.M.) on the Day of Proestrus.

Exp. series	No. of rats	Nembutal treatment		Autopsy interval post-inj.	Results		
		Hr inj.	Dose, mg/kg		Ovulation prevented	Partial interference	Fully ovulated
I	2	2 P.M.	50	19 hr	2	0	0
	2	"	30	20 hr	2	0	0
II	11	"	30	2-4 days	11*	0	0
III	2	"	50	2-3 "	2†	0	0
	19	"	30	2-3 "	19†	0	0
IV	5	4 P.M.	50	18 hr	1	1	3
	5	"	30	18 hr	1	0	4
Totals	36	2 P.M.	30-50		36	0	0
	10	4 P.M.	30-50		2	1	7

* Ovulation was prevented throughout by certain procedures on the second and third days. (See text).

† Ovulated before termination of experiment, but age of corpora lutea at autopsy indicates clearly that ovulation was retarded one day by the initial treatment.

employed: 30 and 50 mg/kg body weight, in single intraperitoneal injections unless otherwise specified. The concentration was 60 mg/ml in a medium containing 20% propylene glycol and 10% alcohol. The lower dose, the more generally desirable, usually produces moderate anesthesia for an hour or more, with somnolence and other signs persisting in varying degrees for several hours.

The animals were adult virgin females of the Vanderbilt substrain Va, as used in the earlier work.[1,2] In each rat the existence of regular 4-day cycles had been established by the vaginal smear method. In such individuals the expected ovulation time is from 1:00 to 2:30 A.M. during the night following proestrus,[2] 9 to 11 hours after the critical period during which neurohumoral stimulation of the hypophysis presumably occurs (see above).

At the end of each experiment the animal was killed with illuminating gas. Tubal ova were looked for in the excised ampullae compressed in physiological saline between a slide and cover slip.[2] The ovaries were fixed in Zenker's fluid, serially sectioned at 10 μ and stained by a modified Mallory tri-acid technic.

In all rats injected with Nembutal at 2 P.M. during proestrus, ovulation was prevented from occurring at the normal time (Table I). This was proven in series I by the presence of unruptured follicles on the day after treatment. In series II the proof was less direct but no less certain. Although autopsy was delayed 2 to 4 days after the initial treatment, hypophyseal stimulation on intervening days was prevented by either atropine (at 2 P.M. on the second day) or by prolonged Nembutal sedation (repeated injections beginning at 2 P.M. each day). Persistent follicles and complete absence of recent corpora lutea in these 11 rats at the termination of experiment demonstrated that the initial brief action of Nembutal during proestrus in the 2-4 P.M. interval postponed the (potential) activation of the hypophysis for a full 24 hours. In series III various modifications of treatment on the second and third days allowed stimulation of the hypophysis on one or the other of these days, as judged from the histological appearance of the new corpora lutea. Here again, the initial treatment during proestrus had delayed stimulation for a full day.

Quite differently, in series IV, when injections were made at 4 P.M. during proestrus, little interference with gonadotrophin release occurred. The high proportion of animals fully ovulating overnight compares with that

2 Everett, J. W., *Endocrinology*, 1948, **43**, 389.

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.