of Hepatitis (Natl. Acad. Sci.–Natl. Research Council, Publ. 322, 1954), p. 17.
7. Supplied by R. W. McCollum and J. Paul, Yale University School of Medicine.
8. Supplied by A. M. Wolf, the Michael Reese Research Foundation.
9. J. F. Morgan *et al., Proc. Soc. Exptl. Biol. Med.* 73, 1 (1950).
10. We are grateful to J. Weston and A. R. Taylor for preparation of the photographs.
11. J. Stokes, Jr. *et al., J. Am. Med. Assoc.* 147, 714 (1951).
12. Work is continuing, and a report of progress, as well as further characterization of the group, is in preparation. Recent observations appear to indicate that different lines of Detroit-6 cells vary in their susceptibility to these agents, and certain cultures may be markedly less sensitive than others.

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Metabolism of Glycine by Bovine Spermatozoa

Early attempts to improve bovine semen diluents by the incorporation of glycine were unsuccessful (*1*), in contrast to the favorable response noted with sea urchin and fowl spermatozoa (*2*). Recently, however, glycine solutions have been used in combination with egg yolk or milk to increase the survival time of bovine spermatozoa at 5°C (*3, 4*).

Recent studies (*5*) in this laboratory with glycine-1,2-C^{14} have provided evidence that glycine is metabolized by freshly collected bovine spermatozoa. Previously, a metabolic role for glycine had received little consideration, since it apparently was not utilized (*2, 6*).

In a preliminary trial, 1.7 ml of whole semen containing 2×10^9 spermatozoa was incubated in a diluent of heated skimmilk and 0.5*M* aqueous glycine (1/1) (*4*) containing 4 μc of glycine-C^{14}. Respiratory CO_2, collected in alkali during 3 hours of incubation at 37°C, was converted to barium carbonate and yielded a total of 11,700 count/min.

Subsequent trials involved twice-washed spermatozoa suspended in Ringer-phosphate buffer containing 0.001*M* adenosine triphosphate. In experiment 1, 0.01*M* sodium formate was added as a metabolic trap (*7*); 0.01*M* glyoxylic acid was used similarly in experiment 2. Formate was recovered by steam distillation and was oxidized with mercuric oxide. Respiratory CO_2 and formate were radioassayed as solid barium carbonate. Glyoxylate was recovered as the 2,4-dinitrophenylhydrazone and was radioassayed as such. Results of these two experiments are presented in Table 1. Obviously, spermatozoa are capable of metabolizing glycine. The products recovered indicate that the pathways involved may be similar to those observed in other mammalian tissue (*7*).

Further evidence of the nature of glycine metabolism was sought by determining the glycine and glucose oxidation when one and when both substrates were available. Uniformly labeled glucose or glycine was employed in 0.01*M* concentration with 10^6 count/min, per flask. Osmotic pressure was within normal limits for these cells (*8*). Each flask contained 10^9 spermatozoa in a final volume of 2 ml. The mean results of three trials, presented in Table 2, indicate that each substrate is utilized in the presence of the other under the conditions employed. Recent work has shown that glycine at 0.01*M* concentration is detrimental to bovine spermatozoan survival (*4*) and therefore might be expected to reduce the production of CO_2 from glucose-C^{14}. However, 0.1*M* glycine has been shown to increase spermatozoan survival (*3, 4*).

The action of glycine in reducing CO_2 production from glucose-C^{14} without a reduction in total CO_2 production and the observation that glycine is metabolized lead to the postulation that glycine utilization may have a sparing effect on glycolysis in bovine spermatozoa. Obviously such an effect may not account for all of the benefit derived from glycine.

These studies are being extended to obtain additional information regarding the metabolism of glycine by bovine spermatozoa, particularly with regard to the nature of other possible intermediates and end-products.

Table 1. Formation of labeled products from C^{14}-labeled glycine by bovine spermatozoa. Yields expressed per 10^9 cells for 2 hours.

Expt.	Product			
	CO_2		Formate	Glyoxylate
	μ mole	(count /min)	(count /min)	(count /min)
No. 1 4 μcurie glycine + formate	13	4240	2300	
No. 2 2 μcurie glycine	28	2680		
2 μcurie glycine + glyoxylate	10	1700		1040

Table 2. Oxidation of glucose and glycine in suspensions of bovine spermatozoa in Ringer-phosphate buffer, *p*H 7.1. Results expressed per 10^9 cells for 2 hours.

Substrate	Respiratory CO_2	
	count/min	μmoles
Glycine-C^{14}	5295	32
Glycine-C^{14} + unlabeled glucose (0.01*M*)	4085	31
Glucose-C^{14}	7305	33
Glucose-C^{14} + unlabeled glycine (0.01*M*)	6935	30
Glucose-C^{14} + unlabeled glycine (0.1*M*)	6150	34

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References and Notes

1. A. Tyler and T. Y. Tanabe, *Proc. Soc. Exptl. Biol. Med.* 81, 367 (1952); I. G. White, *J. Biol. Sci.* 7, 379 (1954).
2. A. Tyler and Lord Rothschild, *Proc. Soc. Exptl. Biol. Med.* 76, 52 (1951); F. W. Lorenz and A. Tyler, *ibid.* 78, 57 (1951).
3. A. Roy and W. H. Bishop, *Nature* 174, 746 (1954); J. M. Rakes and O. T. Stallcup, *Science* 123, 224 (1955).
4. R. J. Flipse and J. O. Almquist, *J. Animal Sci.* 14, 1182 (1955).
5. Authorized for publication as a paper No. 2062 in the journal series of the Pennsylvania Agricultural Experiment Station. This work was aided by grants from the U.S. Atomic Energy Commission, contract No. AT(30-1)-1849, and the Pennsylvania State Association of Artificial Breeding Cooperatives.
6. F. X. Gassner and M. L. Hopwood, *Proc. Soc. Exptl. Biol. Med.* 81, 37 (1952); J. Tosic and A. Walton, *Biochem. J.* (*London*) 47, 199 (1950).
7. H. I. Nakada, B. Friedmann, S. Weinhouse, *J. Biol. Chem.* 216, 583 (1955).
8. P. E. Johnson, R. J. Flipse, J. O. Almquist, *J. Dairy Sci.* 39, 180 (1956).

14 May 1956

Maternal and Sexual Behavior Induced by Intracranial Chemical Stimulation

A technique permitting chemical or electric stimulation, or both, of restricted brain areas in unanesthetized rats, and electroencephalographic (EEG) recording from these areas, has been developed and found to be of value (*1*).

Implants are prepared as follows. Two Tygon-insulated copper or silver wires (0.1 mm in diameter) are baked along the outside of No. 22 hypodermic tubing extending from 2 to 9 mm below the base of a plastic holder (*2*). The wires lead from contact points on the holder and terminate at opposite sides of the end of the shaft as a bipolar stimulating-and-recording electrode. The implant shaft is permanently inserted in the brain while the anesthetized rat is held in a stereotaxic instrument. Four holes in the base of the holder permit rigid attachment to the skull with jeweler's screws.

Two or more days later, rats are placed in 3- by 3- by 2.5-ft boxes for stimulation testing. A small clip connects the implant to light overhead leads from a 0- to 12-v, 60 cy/sec stimulator, or to an EEG machine. The clip also contains a

No. 30 metal cannula that penetrates the implant shaft to the depth of the electrode tips or lower. Seven feet of PE 10 polyethylene tubing (0.024 in. in diameter) leads from the cannula to a microsyringe which can release a minimum of 0.0001 cm^3 of solution into the brain. All overhead leads are intertwined and spring-mounted, permitting repeated, controlled stimulation of a freely moving animal. Behavioral tests with a series of external stimulus objects are given before, during, and after chemical stimulation, and placebo solutions and nonhormonal neural excitants are used for control brain injections during initial tests or retests.

The technique is first being used to test whether there are "primary drive centers" under hormonal influence or control (*3*). Thus far, maternal and sexual behavior have been elicited from separate brain loci in a series of males during stimulation with sodium testosterone sulfate in 0.09-percent saline, and, subject to verification, a mixture of a pure salt of estrone and a suspension of progesterone has induced heat behavior in two females (*4*). Maternal behavior elicited during chemical stimulation includes nest building and a persistent retrieving and grooming of litters of young. All aspects of mating behavior have been induced or accentuated. Attendant high-drive states are suggested by an exaggerated speed, compulsiveness, and frequency of all overt responses during positive test periods.

Although individual animals respond positively for up to 4 test days, duplication of effect from animal to animal has imposed difficult problems. Of 130 male operates tested with the testosterone salt, five have shown complete maternal response, 14 have shown nesting behavior only, and six have shown exaggerated sexual response. Histological data suggest that locus of immediate action is a critical factor, with slight variation in placement leading to incomplete, confounded, or diffuse drive states, or negative results. Initial findings tentatively implicate the medial preoptic area in maternal behavior and the lateral preoptic area in sexual behavior.

A variety of effects have been noted during testosterone stimulation at adjacent loci. Those seen in five or more cases include respiratory changes, diffuse hyperactivation, long-lasting exploratory-like behavior, repetitive localized muscular response, digging, leaping, and seizures.

The following list includes other significant points of departure from chemical- or electric-stimulation data reported in the literature. Since positive test responses completely transcend control behavioral data, two single cases of possible theoretical significance are also briefly described.

1) Elicited behavior, whether specific or diffuse, commonly continues without decrement for more than 90 minutes following chemical stimulation.

2) An entire hierarchy of related responses can be brought to the threshold of activation, with adequate stimulus objects insuring integrated behavior.

3) A segment of a response hierarchy may occur alone. Excessive nest-building is often seen, and one aspect of the nesting pattern, "pick up paper and push under body," continued rapidly for more than 1 hour in three cases.

4) Specific behavior has occurred in the absence of appropriate external stimuli. One male continuously "retrieved" his tail when stimulated with testosterone and then repeatedly retrieved a female in heat. When pups and paper were supplied, however, the male built a nest and retrieved and groomed the young, neglecting the objects to which he previously reacted.

5) In one case, maternal and sexual drives were activated simultaneously. The testosterone-treated male reacted to stimuli related to each drive and to a degree never shown in control tests. Double activation was most convincingly illustrated when a female (not in heat) and newborn rat pups were presented. The male attempted copulation twice while a pup he was retrieving to a nest was still in his mouth. Shaft placement was adjacent to both areas previously implicated in sexual and maternal response.

All effects have followed injection of minute amounts of solution, containing from 0.003 to 0.05 mg of the testosterone salt. In this connection, it must be emphasized that the possibility remains that causative factors other than hormonal properties may be operating. Thus far, however, control testing with neural excitants, physiological saline, and electric stimulation has failed to produce or perpetuate these complex behavior patterns.

Initial EEG data are promising. Records from six testosterone-treated "maternal" or "nesting" males have shown single spiking lawfully spaced in a normal record rather than the general spiking seen after picrotoxin or metrazol injection. Selective chemical action seems probable. Also, testosterone-induced spiking occurs before, not during, elicited overt behavior. Correlation of EEG changes with brain stimulation and with elicited response presents technical problems but could become a powerful tool.

The early data suggest other implications and further applications.

1) A neurophysiological definition of drive seems within reach. The role of hormones in eliciting behavior should be clarified as well as the organization of neural circuits that mediate or integrate primary drives. Present data favor a "neural center" theory, but control studies are needed.

2) Responses analogous to symptoms of mental dysfunction often occur during chemical stimulation. These include "obsessive-compulsive acts," tics, diffuse excitation, and states of hypo- and hypersensitivity to sensory stimuli. Further work may establish tie-ins between shifts in chemical balance in the central nervous system and certain forms of mental dysfunction.

3) Males having no adult contact with females, young, or paper have responded to chemical stimulation with integrated maternal behavior on the first trial. The data are pertinent to the problem of whether innate, centrally organized sensory-motor connections exist for complex response systems.

4) Testosterone has ostensibly elicited both sexual and maternal patterns. The findings may reflect multiple properties for the hormone. Limited progesterone-like activity has been proposed for the androgens by Selye, and progesterone has been linked to maternal response.

5) Chemical stimulation has elicited long-lasting, integrated behavior that was free of lapse or interference. The data strongly suggest selective chemical action within the central nervous system. Further work may demonstrate that differential sensitivity to specific physiological change by functionally organized areas of the nervous system is a basic principle of neural function.

In summary, integrated, long-lasting drive states have been induced by direct chemical stimulation of brain loci. Further work with techniques of this type could well lead to breakthroughs in the study of pharmaceutical action, brain organization and function, and the dynamics of behavior.

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References and Notes

1. I initiated the work as a public health postdoctoral fellow at McGill University with D. O. Hebb as sponsor. Work continues at the University of Wisconsin during the second fellowship year with H. F. Harlow as sponsor.
2. The Lucite holder is identical with that described by J. Olds and P. Milner, *J. Comp. Physiol. and Psychol.* 47, 419 (1954).
3. For example, F. A. Beach, *Psychosomat. Med.* 4, 173 (1942).
4. Hormone salts supplied by Ayerst, McKenna, and Harrison, Ltd., Montreal, Canada.

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A practical man is one who practices the errors of his forefathers.—BENJAMIN DISRAELI.