

TABLE I.
Average Range of Hearing.

	Stock	Deficient
No. of animals	56	64
No. of measurements	176	373
Avg range	10,630	7,328

freedom). It also shows that animals fed stock diet had significantly wider range of response to audio-signals than those fed deficient diet ($F = 18.34$ with 1 and 118 degrees of freedom).

Summary. Guinea pigs fed a diet deficient

in the anti-stiffness factor may ultimately develop inability to respond with the ear-flick to auditory stimuli. At the conclusion of the experiment, 28% of the deficient animals were deaf according to the ear-flick test, in contrast to 0% among the stock animals. It was possible to trace developing deafness according to the ear-flick test in a considerable number of animals maintained on a deficient diet during the experimental period of 7 months.

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Evidence that Copper Acetate Induces Ovulation in the Rabbit by Direct Stimulation of the Adenohypophysis. (17368)

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Copper salts induce ovulation in the estrous rabbit,^{1,2} a species which normally ovulates only after copulation. The natural mating stimulus, which results in the release of luteinizing hormone from the adenohypophysis, involves the hypothalamus and the pituitary stalk³ and includes both adrenergic and cholinergic components.⁴

Bischoff⁵ suggested that copper activated the hypophysis by toxic stimulatory effects on the nervous system. The idea appeared to be confirmed by the results of Brooks,³ who found that copper failed to induce ovulation in rabbits whose hypophyseal stalks had been severed, and Harris,⁶ who reported that very weak dosages of copper acetate injected directly into the third ventricle stimulated the ovulatory response.

We were led to doubt that copper-induced ovulation is purely a nerve-stimulation phe-

nomenon when dibenamine and atropine, in dosages adequate to block the copulation stimulus from reaching the hypophysis,^{4,7} failed to prevent copper acetate from activating the release of an ovulatory surge of LH.⁸ It therefore seemed desirable to test the hypothesis that copper might exert its stimulatory action directly upon the anterior pituitary.

Sexually mature female rabbits ranging in weight from 2.5 to 4.3 kg were employed in this study. To insure an estrous condition, each animal was treated with 85 μ g estradiol benzoate on 2 successive days prior to copper administration, for ovulation is not induced by copper in anestrus rabbits.⁹ A control series of 10 females revealed that the estrogen alone would not stimulate LH release. The hypophysis was approached parapharyngeally as in earlier studies,^{10,11} and 4 attempts were made at 5 or 10 minute intervals to inject a total of 0.15 ml of 0.1% copper acetate, buf-

¹ Fevold, H. L., Hisaw, F. L., and Greep, R., *Am. J. Physiol.*, 1936, **117**, 68.

² Emmens, C. W., *J. Endocrin.*, 1940, **2**, 63.

³ Brooks, C. Mc., *Res. Publ. Assn. Nerv. Ment. Dis.*, 1940, **20**, 525.

⁴ Sawyer, C. H., Markee, J. E., and Townsend, B. F., *Endocrinology*, 1949, **44**, 18.

⁵ Bischoff, F., *Am. J. Physiol.*, 1938, **121**, 765.

⁶ Harris, G. W., *J. Physiol.*, 1941, **100**, 231.

⁷ Sawyer, C. H., Markee, J. E., and Everett, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71**, 670.

⁸ Sawyer, C. H., and Markee, J. E., unpublished.
⁹ Dury, A., and Bradbury, J. T., *Am. J. Physiol.*, 1943, **139**, 135.

¹⁰ Markee, J. E., Sawyer, C. H., and Hollinshead, W. H., *Endocrinology*, 1946, **38**, 345.

ferred with 0.1% sodium acetate,¹² directly into the pars distalis.

In 12 of the 20 experimental animals which survived this operation for 48 hours the copper did not reach the hypophysis; the hypophyseal capsule remained intact. None of these 12 revealed ruptured or hemorrhagic follicles at autopsy. This group included 5 injected with copper acetate in an India ink suspension, and the ink was found in the retropharyngeal region below the sella and in the cavernous sinuses. Of the 8 whose hypophyses actually received copper, 6 ovulated. Two of the 6 had hypophyses penetrated to the top in such a way that some of the copper could possibly have reached the hypothalamus, but in the other 4 no such condition existed. Three of the 8 injected hypophyses were entered with the copper-ink medium and of these, 2 ovulated; but in all 3 most of the ink was located inferior to the sellar region, none above the diaphragma sellae. The effects of mechanical trauma were controlled by a series of 7 animals into each of whose hypophyses a bipolar electrode was inserted,¹⁰ and a second series of 23 rabbits whose hypophyses were injected with acetylcholine-esterine solution.¹¹ None of the former and only one of the latter ovulated.

At first glance our results, ovulation in

¹¹ Markee, J. E., Sawyer, C. H., and Hollinshead, W. H., *Endocrinology*, 1946, **38**, 345. 1948, **2**, 117.

¹² Dury, A., and Bradbury, J. T., *Am. J. Physiol.*, 1942, **135**, 587.

75% of 8 rabbits, on intrapituitary injection of copper, might seem unconvincing compared with Harris' 80% of 13 animals on injection of copper into the third ventricle, especially since Harris used a dosage only a third as great as ours. However, most of our injected material backed out of the sella, away from the hypophysis and hypothalamus, while Harris' copper could have reached and stimulated the hypophysis directly rather than the hypothalamus as he assumed. His copper, deposited in the third ventricle, may well have been picked up by the proximal capillary plexus of the hypophyseal portal system and carried by the portal veins directly to the adenohypophysis. Brooks' failure to induce ovulation with copper in stalk-sectioned animals is difficult to explain in terms of a direct-action hypothesis, since presumably an adequate arterial blood supply to the adenohypophysis remained intact after his operation. The possibility does exist, however, since he could not test them by the mating response and since he supplied no estrogen, that his copper-treated stalk-sectioned animals were anestrus.

To summarize, the intrahypophyseal injection of 1/100th of the systemic dose of copper acetate, an agent whose effect on stimulating the release of hypophyseal LH is not blocked by anti-adrenergic or anti-cholinergic drugs, has led to ovulation in 6 out of 8 rabbits. It is, therefore, concluded that at least part of the copper effect is a direct stimulation of adenohypophyseal cells.

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Reduction of Potassium Tellurite by Living Tissues.* (17369)

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The property of soluble colorless tetrazolium salts to form insoluble red to purplish formazans as reduction products can be used

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for the demonstration and microscopic localization of reducing activity in living tissues.¹⁻⁴

¹ Mattson, A. M., Jensen, C. O., and Dutcher, R. A., *Science*, 1947, **106**, 294.

² Pratt, R., and Dufrenoy, J., *Stain Technology*, 1948, **23**, 137.