

Thus, an external application of fat to the skin of the chick caused a lethal dermatitis, whereas the incorporation of the same fat in the diet or its injection (subcutaneously) caused no apparent ill effects.

The histological studies were made under the guidance of Dr. Alexei Koneff of the Department of Anatomy, University of California.

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#### Effect of "Prospermin" on Immature and Mature Hypophysectomized and Normal Male Rats.\*

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The induction or restoration of sperm formation in mature and immature hypophysectomized ground squirrels by Prospermin<sup>†</sup> injections has been reported.<sup>1</sup> However, this material is stated to have only a slight effect on the normal rat testes.<sup>2, 3</sup> The present investigation was undertaken to determine the effect of Prospermin on sperm formation and on the interstitial tissue of hypophysectomized mature and immature male rats.

The majority of the immature male rats were operated at 24 to 28 days of age, a few being 30 to 37 days of age. A postoperative interval of 5 to 10 days was allowed to elapse after hypophysectomy before beginning treatment. The mature rats were 78 to 120 days old at the time of operation; the injections being begun after a postoperative interval of 0 to 60 days. All pituitary capsules were serially sectioned, stained with Masson trichrome and examined microscopically. Data from only completely hypophysectomized animals are presented.

The Prospermin was injected, subcutaneously, in daily doses of 1 and 2 mg. Higher dosages were not given as this extract was poorly

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\* Aided by a grant, administered by Dr. P. E. Smith, from the Rockefeller Foundation, New York.

† Prospermin is stated by the manufacturers, E. R. Squibb & Sons, to be prepared from normal male urine. This material was generously supplied by Dr. J. A. Morrell.

<sup>1</sup> Wells, L. J., and Overholser, M. D., *Anat. Rec.*, 1938, **72**, 231.

<sup>2</sup> Moore, C. R., *Am. J. Anat.*, 1936, **59**, 63.

<sup>3</sup> Morrell, J. A., *Anat. Rec. (Suppl.)*, 1933, **55**, 29.

tolerated, even the 1 and 2 mg doses frequently necessitating the sacrifice of some of the animals before the planned period of treatment had expired.

*Hypophysectomized Immature Males.* Injection of 1 mg of Prospermin daily for 30 days induced sperm formation in the testes of 4 of 6 animals. However, the sperm were not as numerous as observed in the unoperated control animal and there was a marked increase of the interstitial tissue above that of normal males of the same age. Hypophysectomized control animals' testes were devoid of sperm. With the exception of one rat treated for 20 days, all of the 26 rats receiving 1 mg daily for 5 to 23 days failed to exhibit sperm in the testes but invariably had some interstitial tissue increase. Two mg of Prospermin were injected for 18 to 30 days in 7 rats. All had sperm in the testes. Very few sperm were observed after only 18 days of treatment, but they were numerous after treatment for 30 days. None of the 6 animals injected for less than 18 days had sperm although there was a stimulation of the interstitial tissue.

In all cases the testis and seminal vesicle weights were increased. This increase was greater in the longer than in the shorter treatments. After 30 days of treatment, the weights of the testes were approximately 10 times and those of the seminal vesicles 125 times those of hypophysectomized control rats. In no case were sperm present in the epididymis.

*Hypophysectomized Mature Males.* After a 10-day postoperative interval, either 1 or 2 mg daily doses were injected for 30-33 days in 7 hypophysectomized mature rats. Sperm were observed in the testes of all of the rats. However, the testis weights were but half that of the unoperated controls but the seminal vesicle weights were equal to the normal controls. After a 60-day postoperative interval, sperm were present in the testes of one of the 2 rats injected with 1 mg daily for 40 days.

In 9 mature males 0.5 and 1 mg daily was injected for 15 days, the injections being begun on the day of hypophysectomy. Testis weights and spermatogenesis were equal to those of unoperated littermate controls. The seminal vesicles were abnormally large.

*Normal Immature Males.* Normal male rats, 25 days of age, were injected for 15- and for 30-day periods with 1 mg of extract daily. Testis weights were not increased over those of littermate controls, a result which is in accord with that of Moore.<sup>2</sup> However, seminal vesicle weights were increased tenfold in both periods of treatment.

*Summary.* Hypophysectomized immature male rats responded to treatment with Prospermin by exhibiting testis enlargement and

sperm formation. This extract did not increase testis weights in the unoperated immature animal. Restoration and maintenance of spermatogenesis was successfully accomplished in the hypophysectomized mature rat. A marked seminal vesicle weight increase was observed in nearly all of the treated animals.

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### Immunological Distinction Between St. Louis and Japanese B Encephalitic Viruses.

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Experiments by Perdrau published posthumously indicated that St. Louis encephalitic virus, Strain No. 3, and Japanese B encephalitic virus, Strain No. 2, furnished by us, were immunologically identical.<sup>1</sup> These results are contrary to our previous findings<sup>2</sup> and hence have been checked in this laboratory.

Perdrau's technic was followed in preparing immune sera. The standard technic for setting up neutralization-tests used in both sets of experiments was continued. Serum-virus mixtures were tested after 4 hours according to our method, and again after 20 hours according to Perdrau's method.

We found at once that immune sera prepared as above<sup>1</sup> were equal or superior to ours in neutralizing capacity and that 20 hours' standing reduced the activity of the serum-virus mixtures one hundred- to one thousandfold, yet brought out any neutralizing effect more dramatically. Most important, however, was the ready confirmation of Perdrau's results with St. Louis No. 3 and Japanese No. 2 strains (Table I). Further tests in animals showed that the St. Louis strain had retained its distinguishing characteristics, whereas the Japanese virus behaved in every way like the St. Louis virus. Questions arose, therefore, as to whether (a) our previous findings<sup>2</sup> were erroneous, (b) the Japanese virus had become altered through repeated passage, or (c) the Japanese virus had at some time been erroneously labeled.

That an error had occurred in labeling was indicated by the following tests. Seven strains of virus were secured from outside

<sup>1</sup> Perdrau, J. R., *J. Path. and Bact.*, 1940, **50**, 545.

<sup>2</sup> Webster, Leslie T., *J. Exp. Med.*, 1938, **67**, 609.