

of treatment with B₆ before the hemoglobin had appreciably increased.

Conclusions. Synthetic vitamin B₆ relieves the hypochromic microcytic anemia produced in dogs deficient in this factor. An adequate supply of the non-adsorbable fraction of the vitamin B complex is necessary for the complete disappearance of this anemia.

11222 P

Response of Hypophysectomized Rats to Highly Purified Extracts of Pregnant Mare Serum.

R. I. PENCHARZ, H. H. COLE AND H. GOSS.

From the Endocrine Laboratory, Department of Obstetrics and Gynecology, University of California Medical School, San Francisco, and the Division of Animal Husbandry, University of California, Davis.

Goss and Cole¹ have shown that extracts of mare serum can be prepared testing 4000 to 7000 R.U. per mg total solids (40,000 to 70,000 R.U. per mg nitrogen). The question arises regarding the nature of the hormone present in these extracts as compared to that in untreated mare serum. Evans, *et al.*,² and Hellbaum³ have reported that more than one gonadotropic hormone is present in mare serum though these reports have not been confirmed. In the first mentioned paper the male rats showing only a Leydig tissue response were possibly sacrificed too soon to expect an effect upon the seminiferous epithelium. Further, had the time of autopsy of the females been delayed beyond 72 hours corpora lutea might have been encountered following the treatment with so-called FSH fractions. The extracts prepared by these authors were relatively crude preparations. We were interested, therefore, in determining whether or not highly purified materials would give similar evidence of fractionation of gonadotropic hormones. A fraction giving pure follicular stimulation in the female with little or no effect on the interstitial tissue of the male would give evidence of a purified follicle stimulating hormone whereas a converse relationship would indicate the presence of a luteinizing hormone.

Experimental. Two extracts were used: one, No. 15-19-2, tested

¹ Goss, H., and Cole, H. H., *Endocrinology*, 1940, **26**, 244.

² Evans, H. M., Korpi, K., Simpson, M. E., and Pencharz, R. I., *Univ. of California Publication in Anatomy*, 1936, **1**, 275.

³ Hellbaum, A. A., *Am. J. Physiol.*, 1937, **119**, 331.

TABLE I.
Results on Males Injected with 20 R.U. of No. 15-19-2 Daily for 20 Days.

Rat	Hypophysectomized		Treatment		Autopsy			
	Age in days	B.W. (g)	Age in days	Daily dose, rat units	Age in days	Testes (mg)	Seminal vesicles (mg)	Prostate (mg)
G6953-C	40	112	75	20	96	660	440	418
W6952-C	40	118	75	20	96	681	433	412
BH6941-C	40	114	75	20	96	597	397	351
B6950-C	40	112	None; hyp'd control Uninjected		96	240	9	25
B-6926-C	Normal unoperated	123	0		40	1250	20	90

35,000 and the other, No. 17-76-4, 40,000 R.U. per mg nitrogen.* Four to 6 female rats, hypophysectomized on the 23rd day of age, were used at a given level for each extract. The intraperitoneal administration of a total of 30 R.U. over a 3-day period beginning on the 30th day of age produced ovaries averaging 116 mg for No. 15-19-2 and 127 mg for No. 17-76-4, on the 5th day after the initial injection. Sixty rat units produced ovaries weighing 122 mg and 101 mg, respectively. Estrous smears were encountered in all test animals. The macroscopic observation of many ripe follicles with some corpora lutea was confirmed microscopically.

The extracts were also tested in hypophysectomized male rats. The results on one extract, No. 15-19-2, are shown in Table I. There was a strong interstitial cell response as is indicated by the response of the accessory organs. Two rats treated similarly with No. 17-76-4 gave like results.

Thus it is seen that these extracts containing much less inert material than any previously reported for mare serum give a good follicular response in the hypophysectomized female and a strong interstitial cell response in the hypophysectomized male. In other words, in spite of the high degree of purification attained in the present extracts, no evidence was secured to support the view that mare serum contains 2 distinct hormones, one specifically affecting the interstitial tissue of the ovary and the Leydig tissue of the testis, and a second causing follicular growth in the female and germinal tissue development in the male.

* The potency of the extracts was determined in normal immature rats according to the method of Cole, Guilbert and Goss.⁴

⁴ Cole, H. H., Guilbert, H. R., and Goss, H., *Am. J. Physiol.*, 1932, **102**, 227.