

5-hour, the 72-hour or the 144-hour periods, marked activity persists for a long time. Even at the 2 γ dose, the majority of the animals still showed low clotting times at the end of 3 days while the largest dose was remarkable not only for the very low clotting times observed at the 5-hour and 24-hour periods but also for the fact that they were still very low at the end of 6 days. The results of oral and intramuscular injection are also given in Table I.

Summary. The percutaneous route affords a means of conveniently administering slightly soluble materials possessing vitamin K activity. Doses as small as 1 γ per chick successfully cured approximately 50% of a series of depleted chicks. Larger doses afforded protection for relatively long periods of time.

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Quantitative Effects of Implantation of Cattle Anterior Pituitary Powder on Gonads of Immature Rat.

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The use of subcutaneous or intramuscular "depots" for giving a more or less constant absorption of biological substances is a procedure of recognized value. Such slow, steady absorption should and perhaps does approach closely the same effect as glandular secretion within the body of the animal. This paper presents the results of the administration of cattle anterior pituitary powder* to rats by subcutaneous implantation.

The effect of implanting anterior pituitary powder on the ovaries of the rat was studied by Loeser^{1, 2} and Janssen and Loeser.³

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¹ Loeser, A., *Arch. Exp. Path. Pharm.*, 1930, **147**, 106.

² Loeser, A., *Arch. Exp. Path. Pharm.*, 1930, **148**, 377.

³ Janssen, S., and Loeser, A., *Arch. Exp. Path. Pharm.*, 1930, **151**, 188.

They found newly developed corpora lutea after 96 hours. Attempts at quantitative evaluation were made on the basis of the number of rats showing luteal development, in relation to the dosage employed. Since their data showed that 50 mg total dose of powder gave significant results, this dosage was employed in our experiments. Organ weight was used as the basis for evaluation, since D'Amour and D'Amour⁴ and others have found this to be the most sensitive, and adaptable to quantitative measurements.

Procedure. Acetone-dried cattle anterior pituitary powder had been molded into tablets containing 25 mg of the powder with about 20% milk sugar per tablet. These tablets were ether-sterilized previous to the experiments. The implanting procedure was as follows: Rats used were 22-24 days old at the beginning of each experiment. Each animal was weighed on the first, second, and fourth days of the test. First, the hair was clipped from the dorsal thoracic region. Under light ether anesthesia the clipped area was sterilized with 70% alcohol after which an incision 3-4 mm long was made in such a place as to be out of reach of the hind feet and the teeth. With a pair of blunt scissors a deep subcutaneous pocket was made ventrad to the incision. Into this pocket was placed a pituitary tablet, followed by 2 or 3 drops of sterile physiological saline solution. The incision was then dried with ether and covered with flexible collodion. Twenty-four hours later, another implantation was made in the same animal on the opposite side for the first incision. Controls were subjected to the same operative procedures, but no tablets of any kind were inserted. Ninety-six hours after the first implant the animals were killed; the thymus, testes, or ovaries and uterus were carefully removed, cleared of extraneous tissue, and placed in a modified Bouin's solution. Twenty-four hours later each organ was dried with blotting paper and weighed to the nearest milligram. This method of preserving organs and weighing the next day enabled the operator to use larger numbers of rats in a single experiment than would have been possible if the organs were weighed immediately after excision.

In all animals there was a small amount of sclerosing at the implantation site, but as far as could be determined this was due to the action of the concentrated lactose solution, and not to infection. For the short duration of the experiment, this deterioration of subcutaneous tissue was probably not detrimental to the animal as a whole.

⁴ D'Amour, M. C., and D'Amour, F. E., *J. Pharm. and Exp. Ther.*, 1938, **62**, 263.

Results and Discussion. A total of 58 rats was used, both hybrids and white Wistar-strain animals being tested in an effort to determine whether one strain was more responsive than the other. To make organ weights directly comparable, absolute weights were converted into milligrams per 100 g of animal. Although total body weights are not given in these data, one rather striking point was noticed. Of the 35 experimental animals, 25, or 71.5%, either lost weight or did not gain during the first 24-hour period of the experiment, while 91% (21 out of 23) of the control rats gained weight during the same period. Since the controls were operated on in exactly the same manner as the experimentals, this weight loss must have been due to the pituitary administration. With but two exceptions (both experimentals) all of the animals showed a gain in total weight by the end of 96 hours.

The accompanying table shows comparative results on organ weights. It is obvious that the white rats were more responsive than the hybrids. The critical ratio of the difference of weight

TABLE I.
Weights of Organs in Experimental and Control Rats.

Organ	Experimental Avg.				Control Avg.				Critical ratio
	No. of animals	Wt and	Wt in	Sigma	No. of animals	Wt and	Wt in	Sigma	
		range in mg	mg/100 g			range in mg	mg/100 g		
Hybrids.									
Uterus	12	61 23-112	124	44.6	7	38 19-55	74	21.4	3.08
Ovaries	12	30 10-66	66	26.5	7	28 9-40	58	17.9	0.73
Thymus	11	117 70-215	266	62.3	7	162 98-259	332	50.7	2.60
Whites.									
Uterus	14	56 32-127	188	104.4	9	25 19-33	78	19.7	3.69
Ovaries	14	19 9-32	61	18.8	9	13 9-20	40	7.72	3.58
Thymus (female)	14	60 26-105	189	59.6	9	117 72-170	345	73.8	5.03
Thymus (male)	6	69 53-102	201	38.0	5	110 98-148	297	50.1	3.48
Testes	6	233 196-290	648	47.7	5	178 164-234	484	48.8	6.13
Wts of animals in grams:									
Hybrid		{ Experimental		48.3 (32-58)					
		{ Control		47.7 (30-60)					
White		{ ♂ Experimental		34.2 (29-42)					
		{ Control		37.2 (32-46)					
		{ ♀ Experimental		31.2 (23-44)					
		{ Control		34.0 (27-45)					

increase of testes of albino male experimentals and controls was 6.13, which, according to Fisher's tables,⁵ is very significant. Likewise the differential responses of uteri and ovaries of white rats are very significant. Thus we may suggest that any of these organs would be useful in standardization procedures. One animal alone was responsible for the very large standard deviation of the single determination (sigma) upon the uteri. In this animal the uteri were very large, the cavity being filled with fluid (it was over 500 mg per 100 g).

The thymic response of female white rats gave a high critical ratio, but with a large standard deviation. This positive thymic response, however, may or may not be due to operative damage to the animal, as suggested by Selye⁶ and Selye and Masson.⁷ In any case, the thymic responses are possibly not a direct result of the action of pituitary hormones, but reflect hormonal activity of other endocrine glands.⁸

These preliminary experiments show that it is possible to obtain statistically significant responses to cattle anterior pituitary implants in white rats, using weights of testes, ovaries and/or uteri as the criteria of activity. Further work should be directed at determining the minimum amount of extract which will produce significant changes, and in attempting to make comparisons with other standardization procedures. It is interesting to note that the white rat, either male or female, is more sensitive to cattle anterior pituitary than to similar preparations from the sheep.⁴

Summary. 1. A method is described for administration of pituitary powder by the tablet implantation technic. 2. Statistically significant responses were obtained with 50 mg of powder in case of testes, uteri, and ovaries of 22- to 24-day-old white rats. 3. These responses were measured by organ weight gains rather than by histological changes or vaginal smears.

⁵ Guilford, J. P., *Psychometric Methods*, McGraw, Hill and Co., 1936. (See Table K.)

⁶ Selye, H., *Am. J. Physiol.*, 1938, **123**, 758.

⁷ Selye, H., and Masson, G., *Endocrinology*, 1939, **25**, 211.

⁸ Persike, E. C., Jr., *Am. J. Physiol.*, 1940, **130**, 384.