showed that these persons were not ill at the time of our tests. Sanford's method as modified by Holloway was used.

Results. The cells of 72.2% of those tested did not show beginning hemolysis until a solution of 0.4% was reached. In 14.8% of the cases hemolysis did not begin until a solution of 0.36% NaCl was reached. This figure lies within the limits for complete hemolysis as given by several texts. In 22% of the cases complete hemolysis did not occur until a solution of 0.28% was reached.

At present our findings would indicate that the red blood corpuscles of the average Negro are more resistant to hypotonic salt solutions than is indicated for normals in textbooks. It is assumed by the authors that these normal values were probably obtained from the study of average groups of white patients. It is furthermore indicated that the factor of fragility of red blood corpuscles does not account for the high incidence of jaundice observed in lobar pneumonia in the Negro.

9616 P

A Pharmacological Ejaculation Test for Bio-Assay of Male Sex Hormone.

S. LOEWE.

From the Department of Pharmacology, Cornell University Medical College, New York City.

Mammalian tests for the male hormone are preferable to bird tests, a fact which has been stressed by Loewe and Voss since the discovery of the hormone[1, 2, 3] and has gained general recognition through the differentiation of two active principles—testosterone and androsterone (Laqueur,[4] Rucicka[5]). However, mammalian tests in use are unsatisfactory, in that not all of the functions governed by the hormone are observed. This objection does not apply to an ejaculation test since the latter requires the integrity of numerous central and peripheral sex functions. The production of ejacu-

[1] Loewe, S., and Voss, H. E., Letter to Akad. d. Wiss. Wien, Jan., 1927, published in *Akadem. Anzeiger*, Vienna, 1929, No. 20.

[2] Loewe, S., and Voss, H. E., *Klin. Wochenschr.*, 1930, **9**, 481.

[3] Loewe, S., *Dermatol. Z.*, 1932, **64**, 223.

[4] David, K., Dingemanse, E., Freud, J., and Laqueur, E., *Z. Physiol. Chem.*, 1935, **233**, 281.

[5] Rucicka, L., and Wettstein, A., *Helv. Chim. Acta*, 1935, **18**, 1264.

lation by electrical stimulation has been employed in a procedure for detecting the action of the male hormone on the guinea pig,[6] but it has not been developed into a quantitative method, and it is difficult to elicit the response in the smaller rodents. In the mouse the production of ejaculation by the action of drugs[7] provides an excellent method for studying the ejaculatory function. This method not only detects the action of male hormones, but supplies a means for their bio-assay. The experiments reported here deal with its use in a method of bio-assay.

The experiments were performed on adult albino mice. Ejaculation was elicited by the pernostone-yohimbine technic which will be described in detail elsewhere.[7] This consisted essentially of the hypodermic injection of 83 mg./kg. of pernoston in a 0.67% solution followed in 12 minutes by the subcutaneous injection of 10 mg./kg. of yohimbine sulfate in a 0.5% aqueous solution; 5 minutes later the mouse is brought into a lateral position. Ejaculation occurs within 5 to 40 minutes. Only animals which gave a prompt ejaculatory response in the pernostone-yohimbine test before, but not after castration, were used for the hormone injections. After experimenting with various types of hormone administration, preference was given to a slow method, *i. e.*, one daily injection on 5 successive days. A few series with a rapid method—3 injections within 36 hours—will be briefly mentioned. The animals were repeatedly subjected to the pernostone-yohimbine test at intervals of from 2 to 4 days both during and after the period of hormone injection. Testosterone and testosterone propionate, and in further experiments testosterone acetate and androsterone, were used for the hormone treatment. All substances* were injected in a solution of oil, the volume not exceeding 0.5 cc. The experiments are summarized in Table I.

Ejaculation under the conditions described is an all-or-none indicator of sexual regeneration. The individual response was considered positive when a coagulated ejaculate was perceptible inside or outside of the prepuce with or without magnification. In each group the frequency of response (the ratio number of animals responding to the total number of animals in a group) was taken as a measure of the intensity of action. Tentatively, a frequency of 0.5 (50%) was considered the unit of intensity. The persistence of

[6] Moore, C. R., and Gallagher, T. F., *Am. J. Physiol.*, 1929, **89**, 388.

[7] Loewe, S., *J. Pharm. and Exp. Therap.*, in press.

* All four substances were kindly provided by Ciba Pharmaceutical Products, Inc., through the courtesy of Dr. E. Oppenheimer.

TABLE I.
Frequency and Persistence of Ejaculatory Response of Castrated Mice after Administration of Varying Hormone Doses Over a Period of 5 Days.

Substance	Daily gamma	Animals Used	Animals Responding	Frequency of Ejaculation %	Persistence Days
Testosterone	12½	8	1	12	5
	25	11	5	45	10
	37	7	3	43	5
	50	5	4	80	10
	100	4	3	75	5
Testosterone propionate	5	8	3	37	> 7
	10	5	2	40	7
	12½	8	4	50	>10
	25	7	6	86	<13
	50	5	5	100	17
	100	6	6	100	>19
	250	4	4	100	?
	500	8	6	75	?
	1000	2	2	100	>27
Testosterone acetate	12½	8	1	13	3
	20	8	4	50	10
	35	8	4	50	> 7
	50	9	8	89	10
	75	9	7	78	>12
	100	4	2	50	11
Androsterone	500	4	0	00	0
	1000	12	5	42	6
	2000	6	2	33	6
	3000	5	3	60	6

action was also measured, being determined by the time interval between the last injection of the hormone and the last ejaculatory response.

As the table shows, the frequency of ejaculation increased with the dose of the hormone. The unit potency of testosterone was found to be about 40 gamma and of the propionate 12.5 gamma. That of the acetate was close to the propionate and that of androsterone about 2500 gamma. These doses are not essentially larger than those required for the mammalian tests in use;[8, 9, 10] the total amount of hormone required is still lower, since the number of injections is smaller. The relative potency (reciprocal ratio of the unit doses) is androsterone : testosterone : propionate = 1 : 62 : 200, *i. e.*, similar to the ratios of about 1 : 20 : 200 found by other mammalian tests and very different from those of about 1 : 5 : 4 found by the capon test.[8, 9, 10] The propionate showed a greater persist-

[8] Ruzicka, A. L., and Wettstein, A., *Helv. Chim. Acta*, 1936, **19**, 1142.

[9] Tschopp, E., *Arch. Internat. de Pharmacodynamie*, 1936, **52**, 391.

[10] Miescher, K., Wettstein, A., and Tschopp, E., *Biochem. J.*, 1936, **30**, 1977.

ence of action than any of the 3 other substances examined. This property of the propionate was still more striking in the small number of short interval (36 hours) experiments. The unit dose was considerably higher under these conditions but the action persisted more than 20 days.

The absolute values obtained in this study may require revision. Various circumstances indicate that they will be found somewhat lower in a larger series of experiments, when the experimental conditions are better established as, for instance, the volume of the hormone solution injected and the time interval between tests on the same animal. However, if the general results of these experiments are confirmed in a larger series of tests, a new bio-assay method becomes available which offers several advantages, *viz.*, animals need not be sacrificed, autopsy and histological examinations or measurements of organs are not required, and the persistence of action can be registered on the individual animal continually.

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Blood Copper and Iron in Addison's Disease.

ADOLPH SACHS, VICTOR E. LEVINE AND WILLIAM O. GRIFFITH.
From the School of Medicine, Creighton University, Omaha, Neb.

In a series of anemias previously reported,[1,2] we have shown that with few exceptions that low blood iron was accompanied by an increase in blood copper. We report now 3 cases of Addison's disease, a comparatively rare condition accompanied by secondary anemia and characterized in most instances by an increased degree of pigmentation of the skin. The fact that the copper content of the skin has recently been closely associated with increased skin pigmentation[3,4,5] makes these determinations of added interest.

We have made iron and copper analyses on the blood of 3 patients with Addison's disease. Iron estimations were made by a dry ashing method using potassium thiocyanate as the reagent, and

1 Sachs, A., Levine, V. E., and Fabian, A. A., *Arch. Int. Med.*, 1935, **55**, 227.

2 Sachs, A., Levine, V. E., and Griffith, W. O., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 6; 1936, **35**, 332.

3 Cunningham, I. J., *Biochem. J.*, 1931, **25**, 1267.

4 Gorter, F. J., *Nature*, 1935, **136**, 185.

5 Sarata, U., *Jap. J. Med. Sci. II. Biochem.*, 1935, **3**, 79.