scribed with the aid of an antibody produced by transplacental isoimmunization. Evidence is presented to show its genetic relationship to the S factor, which is probably linked to the M and N system.

Received August 23, 1951. P.S.E.B.M., 1951, v78.

Quantitative Biologic Activity of $\Delta^{4,6}$ dehydrocortisone as Compared to Cortisone.* (19026)

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The compound $\Delta^{4,6}$dehydrocortisone acetate was first recognized as a byproduct of the partial synthesis of cortisone acetate(1). It is commonly designated as the "diene" of cortisone. The diene is important from the standpoint of establishing correlations between the chemical structure of steroids and their biologic and therapeutic effects. In a preliminary report, Kendall(1) noted that the diene did not suppress the symptoms of rheumatoid arthritis, although its glycogenic potency seemed to equal that of cortisone. Some apparent differences in the physiologic properties of the two compounds were reported by Higgins, Woods, and Kendall(2).

In the present study we have compared the potency of cortisone acetate and the diene acetate in the muscle-work test of Ingle(3) and in the glycogen deposition test. According to these criteria the quantitative potency of the diene acetate is probably no greater than 50% of the potency of cortisone acetate.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they reached a weight of 200 ± 2 g. The procedures used for the stimulation of muscle were according to Ingle(3). The animals were anesthetized with phenobarbital sodium and cyclopal sodium and were subjected to the work test immediately after removal of the adrenal glands and the kidneys. A Nerve Stimulator, Model B, Upjohn, was used to deliver 5 pulses per second. The duration of each pulse was 20 msec. and the intensity was 20 ma. The distance the weight was lifted was recorded on automatic work adders. Each recorder revolution represented approximately 400 g-cm of work. Stimulation was continued until muscular responsiveness was lost. During the work test, the animals were enclosed in a cabinet with the temperature constant at 28° ± 0.5°C. The steroids were dissolved in peanut oil and were given by intermittent subcutaneous injection at the beginning of stimulation and 6, 24, 30, 48, etc. hours later. Each animal received continuous subcutaneous injections of 0.9% sodium chloride solution at the rate of 20 cc per 24 hours per rat. The liver glycogen deposition tests were carried out in the Endocrinology Department of the Upjohn Research Division by Mr. D. F. Kiel. The procedure has been described by Pabst, Sheppard, and Kuizenga(4).

Experiments and results. The data on dosages, numbers of animals and work performance are summarized in Table I. The work response elicited by cortisone acetate

* We wish to express our appreciation to Dr. E. C. Kendall, The Mayo Foundation, Rochester, Minnesota, who supplied the diene acetate; to Dr. Augustus Gibson, The Medical Division of Merck and Co., who supplied the cortisone acetate; and to Mr. D. F. Kiel, Endocrinology Department, The Upjohn Research Division, who performed the bioassays of these two compounds by the liver glycogen deposition test.

1. Kendall, E. C., *Fed. Proc.*, 1950, v9, 501.
2. Higgins, G. M., Woods, K. A., and Kendall, E. C., *Endocrinol.*, 1951, v48, 175.
3. Ingle, D. J., *Endocrinol.*, 1944, v34, 191.
4. Pabst, M. L., Sheppard, R., and Kuizenga, M. H., *Endocrinol.*, 1947, v41, 55.

TABLE I. Work* Performance of Adrenalectomized-Nephrectomized Rats Given Steroids by Twice-Daily Subcutaneous Injection. Average total recorder revolutions with standard deviations of the averages.

Dose steroid, mg/24 hr/rat	Cortisone acetate		Diene acetate	
	No. rats	Total work	No. rats	Total work
.25	15	17617 ± 877	15	13874 ± 696
.50	14	23697 ± 1703	14	18234 ± 1357
1	19	28205 ± 1433	19	22458 ± 1601

* Each recorder revolution represents approximately 400 g-cm of work.

was significantly greater than the response to the diene acetate at each dosage level. It was necessary to administer amounts of the diene which were twice as great as those of cortisone in order to obtain the same level of work output. Upon this basis it was concluded that the potency of cortisone acetate in the muscle-work test is approximately twice that of the diene acetate.

In the liver glycogen deposition test one unit of activity is defined as the activity equivalent of 0.1 mg of 17-hydroxycorticosterone. The diene acetate assayed 1.86 units per mg and cortisone acetate which was tested in parallel under identical conditions assayed 4.5 units per mg.

Discussion. The results of these experiments show that cortisone acetate is more active than the diene acetate in both the muscle-work test and in the liver glycogen deposition test. The question can be raised as to whether there are any true qualitative differences in the biologic properties of these two compounds or whether the reported differences can be explained on a quantitative basis.

The failure of the diene to suppress the symptoms of rheumatoid arthritis(1) may have been due to insufficient dosage since the only dose tested (100 mg daily) represented the average effective dose of cortisone acetate.

In the studies of Higgins, Woods, and Kendall(2) comparisons of the two compounds were made in normal and in adrenalectomized rats given 2 mg per rat per day. Cortisone acetate suppressed the weight of both non-adrenalectomized and adrenalectomized animals to a much greater extent than did the diene acetate. Cortisone acetate caused a greater regression of the thymus and had a more striking lymphocytolytic effect than did the diene acetate. These differences are explicable by the assumption that cortisone acetate is the more potent of the two compounds and that larger doses of the diene would have caused similar changes. On the other hand, the observations that the diene acetate had a mild lymphocytopoietic effect in normal but not adrenalectomized rats, and that it caused a greater compensatory atrophy of the adrenal cortices than did cortisone acetate may require some other explanation. The question cannot be answered without further comparisons of the biologic properties of the two steroids in which the dose-response relationships are fully explored.

Summary. Cortisone acetate and $\Delta^{1,6}$dehydrocortisone acetate were compared in the muscle-work test and by the liver glycogen deposition test. According to these criteria the quantitative potency of the diene is probably no greater than 50% of the potency of cortisone acetate. It is suggested that the reported differences in the biologic properties of these two compounds may be explicable by the assumption that larger doses of the diene would cause biologic responses which are identical with those caused by cortisone.

Received August 23, 1951. P.S.E.B.M., 1951, v78.