postulated that plasma copper levels in sheep fall due to depletion of copper stores as a result of prolonged molybdenum and sulfate administration. Sulfate intake was not measured in the trials reported here. It would appear, however, that sulfate intake in these sheep was sufficient, together with the added molybdenum to lead to a copper depletion. The requirement for copper in synthesis of heme is known and Anderson and Tove(9) have recently demonstrated a direct role for copper in this synthesis. Copper deficiency in sheep characteristically exhibits a demyelinating lesion in the central nervous system of lambs while only under extreme conditions will anemia occur in the ewe. Anemia is also a characteristic of molybdenosis in non-ruminant animals and has been attributed to inability of these animals to utilize stored copper(10). Bush, *et al.*(11), found a shortened red cell survival using Fe^{59} in copper deficiency of swine and attributed this to subnormal levels of copper within the red cell. If the phenomenon were similar in these sheep, one might expect to find a shortening of survival time of the entire red cell population rather than 30-40% as found in the present study, unless only a marginal deficiency had occurred.

Summary. Median survival times of the erythrocytes of sheep in experimental molybdenosis were determined using glycine-2-C^{14}. The existence of 2 distinct populations of erythrocytes was observed in each sheep. Median survival times for the first erythrocyte populations in 2 sheep were 20 and 28 days, with 80 and 85 days for the second populations.

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Quantitative and Temporal Studies on Effect of Dexamethasone on Corticosterone Secretion in the Rat.* (26798)

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Depression of plasma or adrenal corticosterone concentration by exogenous steroid administration is well known. Pfeiffer and co-workers suggested the substitution of dexamethasone-blocked animals for hypophysectomized animals in assays of ACTH using peripheral plasma corticosterone concentration as the index of adrenocortical response (1). Peron and Dorfman found that measurement of adrenal corticosterone content provided a useful method of assaying the ACTH-suppressing potency of certain corticoids(2). This report describes the suppressive influence of dexamethasone on adrenal corticosterone secretion rate in the rat.

Methods. Adult male Sprague-Dawley rats weighing 200-250 g were used following a 4 to 10 day acclimatization period in the laboratory. Dexamethasone-21-phosphate†

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(stock concentration 4 mg/ml) was diluted daily with physiological saline to bring final injection volumes to 0.5 ml. Control animals received 0.5 ml of physiological saline. All injections were made subcutaneously in the unanesthetized animal. At selected intervals after injection the adrenal venous effluent was sampled. The animals were anesthetized with ether, heparin (100 units in 0.1 ml) was administered *via* the femoral vein, and laparotomy was quickly performed. A #27 gauge needle, fixed on a tuberculin syringe, was inserted through the left renal vein wall opposite the confluence of the left adrenal vein and threaded into the orifice of the adrenal vein to the point of confluence of small diaphragmatic venules. The adrenal effluent was collected for exactly 3 minutes. To avoid backflow through conjoining veins, steady, gentle withdrawal of the syringe plunger was continued through the collection time. The volume of blood obtained usually ranged from 0.6 to 0.9 ml. To minimize errors due to occasional faulty collection, samples not falling within this volume range were discarded. The entire procedure required 6 to 7 minutes per animal. After stoppering the syringe barrel with a small rubber cap it was used as a centrifuge tube, and the plasma was separated and frozen. Plasma corticosterone was determined by a modification of the fluorometric method of Silber and co-workers(3). Silber's correction for "blank" fluorescence of rat plasma was used in calculation of steroid determinations. In preliminary experiments to determine the fluorescence of dexamethasone-21-phosphate, plasma from hypophysectomized rats obtained one day following hypophysectomy was used.

TABLE I. Fluorescence Readings of Steroids in Hypophysectomized Rat Plasma.

Steroid	μg added/ml	Relative fluorescence
Control	—	6 11
Corticosterone	.2	29
	.4	62
	.8	128
	1.6	225
Dexamethasone	.2	7
	.4	7
	.8	11
	1.6	7
	4000.0	11

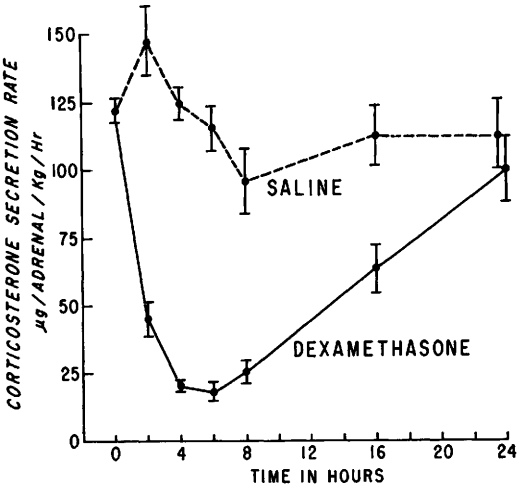

FIG. 1. Comparative effects of a single inj. of dexamethasone-21-phosphate, 100 μg/kg body wt, and saline on the rat corticosterone secretion rate. Each point represents mean secretion rate of 6 or more animals. Stand. errors indicated by vertical bars.

Results. Dexamethasone-21-phosphate itself did not fluoresce under the conditions of the experiment. Table I summarizes fluorescence data obtained after adding dexamethasone-21-phosphate to hypophysectomized rat plasma. The steroid did not alter fluorescence in concentrations up to 4 mg/ml. The steroid was also given intravenously to rats immediately before sampling the adrenal effluent. Corticosterone secretion rate of the dexamethasone-treated animals (120 ± 9.4‡ μg/kg/hr; n = 5) similar to that of untreated controls (123 ± 5.3 μg/kg/hr; n = 17).

Fig. 1 depicts corticosterone secretion rate determinations obtained in dexamethasone-treated (100 μg/kg body weight) and control animals at various intervals following a single subcutaneous injection. Maximal suppression of corticosterone secretion rate occurred 4 to 8 hours following administration of the steroid, and recovery to pretreatment values was nearly complete by 24 hours. Corticosterone secretion rate of saline-injected controls remained at approximately the same values throughout the experiment, suggesting

‡ Values expressed as mean corticosterone secretion rate/kg/adrenal/hr ± standard error.

TABLE II. Dose-Response Effect of Dexamethasone on Rat Corticosterone Secretion Rate 4 Hours after Injection.

Dose, μg/kg body wt	No. of rats	Corticosterone secretion rate*
0	17	123 ± 5.3
10	10	101 ±12.2
40	10	49 ± 6.7
100	10	21 ± 2.6
400	10	23 ± 3.4

* Values expressed as mean μg/kg/adrenal/hr ± stand. error.

that the potential stimulating effect of the injections themselves did not influence secretion rates.

Table II summarizes dose-response data obtained 4 hours after administration of a single dose of dexamethasone-21-phosphate. Maximal suppression of corticosterone secretion rate was produced by 100 μg/kg body weight.

Repeated determinations of corticosterone secretion rate were made in a few animals. Immediately following the initial sampling, 10 mg of hexadimethrine bromide§ was given intravenously to counteract the anticoagulant effect of heparin, and the abdominal incision closed. One week later the adrenal venous effluent was resampled. Minor adhesions were usually encountered at the second operation, but did not prevent the sampling procedure in any animal. Corticosterone secretion rate at the second sampling (131 ± 12 μg/kg/hr; n = 6) was similar to that of untreated controls.

Discussion. Reported values of corticosterone secretion rate from one adrenal gland in the ether-anesthetized rat have been in fairly good agreement despite the variety of methods employed. Collections times have ranged from 15 minutes(4,5) to 1.5 hours(6), but secretion rates have ranged from approximately 100 μg/kg/hr(4) to 150 μg/kg/hr (6). This may reflect near maximal corticotropin release due to ether and/or the stimulus of surgery inasmuch as exogenous corticotropin results in only slight augmentation of the "resting" secretion rate(4).

The degree and duration of suppression of the corticosterone secretion rate following a single dose of dexamethasone agrees closely with the suppression of peripheral plasma corticosterone concentration found in response to exogenous steroids(1). Rate of fall of the secretion rate and onset of maximum suppression also occur at approximately the same time that adrenal steroid secretion rate reaches a minimum in hypophysectomized dogs(7).

The relatively large concentration of corticosterone present in the adrenal effluent of untreated control animals (about 3 μg/ml) compared with peripheral plasma concentration (about 0.2 μg/ml) provides greater amounts of steroid for assay. This technic may be adaptable either to corticotropin assays or to assays of corticotropin-suppressing potency of other non-fluorescing corticosteroids.

Summary. The rat corticosterone secretion rate determined fluorimetrically from 3-minute samples of adrenal effluent (123 ± 5.3 μg/kg/adrenal/hr) agrees well with values reported for more extended collection periods and analyzed by other methods. Dexamethasone-21-phosphate, a non-fluorescing, corticotropin-suppressing, synthetic steroid was found to produce maximal suppression of the corticosterone secretion rate 4 to 8 hours following a single dose. The technic described may be applied to assays of corticotropin itself or assays of corticotropin suppressing activity of other non-fluorescing agents.

§ Polybrene, Abbott.

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