the chemical structure of corticosterone[3,4] and the sex hormones would indicate the possibility of one or more common physiological properties. The prolonged survival of animals adrenalectomized during pregnancy and heat[5,6] and the favorable influence of the induction of estrus in adrenalectomized dogs[7] make it appear probable that a high concentration of sex hormone is beneficial to adrenalectomized animals.

In the present study the authors have injected crystalline fractions of sex hormones into normal male and female dogs. The dogs were maintained under uniform conditions which included a constant fluid and mineral intake. The effect on the twenty-four-hour renal excretion of sodium has been observed. The care of the dogs and the ability to interpret quantitatively such changes in sodium excretion have been described elsewhere.[8] The "sodium retaining effect" of equivalent amounts of crystalline material has been compared to the effect produced by the injection of a known quantity of standardized adrenal cortical extract. For convenience the result has been expressed as "dog units."

The subcutaneous injection of five milligrams of estradiol resulted in a marked and rather prolonged period of decreased sodium excretion in a normal male dog (Table I). The decreased sodium excretion was

TABLE I

THE EFFECT OF ESTRADIOL ON THE RENAL EXCRETION OF SODIUM IN THE NORMAL DOG

Day	Urine Vol. cc.	Sodium m. eq.	
1	425	55.2	Control period*
2	305	32.5	Subcutaneous injection of 5 mgm. of estradiol at 10 A.M.
3	310	39.5	
4	305	37.1	
5	440	60.6	
6	425	57.6	
7	515	73.0	
8	510	73.2	
9	410	50.3	
10	410	56.1	

* Maximum normal daily deviation of twenty-four sodium excretion does not exceed 3 m. eq.

accompanied by a reduced urine output. As the effect of the hormone diminished an increased excretion of sodium was noted ("Rebound"). Continued injections of estrogenic material (Amniotin, 100,000 International Units) into normal male and female dogs did not prevent an ultimate return of sodium excretion to its previous normal level.

[3] I. Reichstein, *Helv. Chim. Acta*, 19: 29, 1936.

[4] E. C. Kendall, H. L. Mason, W. M. Hoehn and B. F. McKenzie, *Prof. Staff Meeting Mayo Clinic*, 12: 136, 1937.

[5] H. A. Stewart, XVII International Congress of Medicine. London, 1913, 173.

[6] J. M. Rogoff and G. N. Stewart, *Am. Jour. Physiol.*, 79: 508, 1927.

[7] W. W. Swingle, W. M. Parkins, A. R. Taylor and J. A. Morrell, *Proc. Soc. Exp. Biol. Med.*, 34: 94, 1936.

[8] G. A. Harrop and G. W. Thorn, *op. cit.*

A comparison has been made of the "sodium retaining effect" of several of the sex hormones (Table II).

TABLE II

A COMPARISON OF THE "SODIUM RETAINING EFFECT" OF SEX HORMONES

Substance*	Quantity	Assay in dog units
Estradiol (crystalline)	0.010	700
Progesterone (crystalline)	0.010	400
Estrone (crystalline)	0.010	200 +
Pregnandiol (crystalline)	0.010	140
Testosterone (crystalline)	0.010	80
Testosterone proprionic acid ester	0.010	25 +

* Each substance was taken up in corn oil and injected subcutaneously.

All the sex hormones thus far investigated have displayed some degree of "sodium retaining effect." Estradiol and progesterone appear to be the most active substances in this respect. It is interesting to note that pregnandiol, a substance not known to have physiological activity as a sex hormone, also displayed the "sodium retaining effect." The relationship of the chemical structure of these substances to that of the adrenal cortical hormone is under investigation. It would appear that a possible explanation of the beneficial effect of estrus and pregnancy on the survival of the adrenalectomized bitch might be accounted for on the basis of the salt and water retention induced by the presence of an excess of the sex hormones. It is not known whether this action is a direct one or mediated through some other endocrine gland.

The single injection of 0.017 gms of estradiol in a patient with Addison's disease, maintained on a diet constant in fluid and mineral content, resulted in a retention of sodium, chloride and water, associated with a gain in body weight and an increase in blood pressure. The duration of this effect was seventy-two hours.

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THE COUMARIN CONTENT OF MELILOTUS DENTATA[1]

IN 1934 one of us (B.) reported the occurrence of a non-bitter form of Melilotus.[2] The non-bitter race did not conform closely in its morphology to any of the described species of sweet clover, so that there was

[1] Papers from the Departments of Genetics (No. 210) and Agricultural Chemistry, Agricultural Experiment Station, University of Wisconsin. Published with the approval of the director of the station. Experiments conducted in cooperation with Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[2] SCIENCE, 79: 301.

considerable doubt concerning its classification at that time. The authors are indebted to B. L. Robinson, of the Gray Herbarium, and H. Harms and O. E. Schulz, of the Botanisches Museum, Berlin, for having identified this sweet clover since as an annual flowering form of the typically biennial *Melilotus dentata* (W. K.) Pers. Schulz describes the species as occurring sparingly from central Europe eastward to central Asia, usually on salty soils. It does not appear to have become naturalized in America.

The non-bitter condition of this one stock of *M. dentata* led us to assemble an extensive collection of the species for further study. We are indebted mainly to several European correspondents for seed. Examination of 28 different lots (27 of which were biennials) mostly from central Europe, but a few from Russia and one from Mongolia, has shown that all are free of the characteristic bitter taste of the common sweet clovers, *M. alba* and *M. officinalis*. Apparently *M. dentata* as a species is typically non-bitter. Suvorov's[3] report on the Russian species of sweet clover bears out this conclusion.

As the bitterness of the common sweet clovers is due mainly, if not entirely, to coumarin and closely related substances, it was anticipated that *M. dentata* would differ in its content of these compounds. The analytical findings show clearly that this is the case. Roberts and Link have recently developed a micro-method, to be described elsewhere, for estimating coumarin, melilotic acid and coumaric acid, which not only permits the determination of these substances separately but to an accuracy of about 0.001 per cent. on the dry basis. The earlier methods of Obermayer,[4] Kanewskaja and Fedorowa,[5] Duncan and Dustman[6] and Clayton and Larmour[7] severally encounter various analytical difficulties, such as failure to separate the three constituents, inclusion of other phenolic substances in the coumarin fraction and interference by plant pigments. These disadvantages are largely overcome by the procedure used in the present investigation.

Using the highly refined method of Roberts and Link no coumarin, melilotic acid or coumaric acid were detected in the vegetative tissues of *M. dentata* at the flowering stage. Seven different races were tested as follows: F.P.I. 90753, Botanic Garden, Peiping, China; Botanic Garden, Copenhagen, Denmark; Thüringen and Saxony, Germany; Moravia, Czechoslovakia; Saratov and West Siberia, U.S.S.R.; Altai Mountains, Mongolia. All these stocks, except those from the botanical gardens and possibly that from Saratov, represent the forms indigenous to these respective regions, according to the information supplied by the correspondents to whom we are indebted for the seed. It is probable, therefore, that if these substances are present at all in the leaves and stems of *M. dentata*, the amounts are less than 0.001 per cent.

A sample of common yellow sweet clover, *M. officinalis*, analyzed at the same stage of development, was found to contain 0.65 per cent. coumarin, 0.25 per cent. melilotic acid and 0.036 per cent. coumaric acid. The corresponding values for a commercial strain of common white sweet clover, *M. alba*, were found to be 0.36 per cent., 0.27 per cent. and 0.048 per cent. The common sweet clovers vary rather widely in composition from strain to strain and at different stages of development so that the above results are not necessarily representative of the respective species.

A small amount of coumarin is present in the seed of *M. dentata*. Analysis of three of the above-mentioned stocks (Peiping, Copenhagen and Moravia) showed 0.021 per cent., 0.074 per cent. and 0.040 per cent., respectively, on the dry basis. A sample of *M. officinalis* seed run concurrently was found to contain 0.63 per cent. coumarin and an *M. alba* lot, 0.46 per cent. It will be noted that these values are roughly ten times as high as those for *M. dentata*. That the material determined as coumarin in the analysis of the seed is actually that substance was shown by the isolation of 25 mg of pure coumarin from 31 g of *M. dentata* (Copenhagen strain) seed. The melting point of the isolated material and the mixed melting point with pure coumarin were 71.0–71.5° C., the same as that for pure coumarin. The iodine-potassium iodide test and certain coupling tests were positive, and the chemical characteristics exhibited during the isolation were identical with those of coumarin. The regular method of analysis indicates that if any melilotic acid and coumaric acid occur in the seed of *M. dentata* the amounts are extremely small.

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[3] Semenovodstvo, No. 2 (quoted from Herb. Abstr. 5: 153).
[4] *Zeits. Anal. Chem.*, 52: 172–191.
[5] *Ibid.*, 93: 176–180.
[6] *Jour. Ind. Eng. Chem.*, 6: 210–213.
[7] *Can. Jour. Res.* (C) 13: 89–100.

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