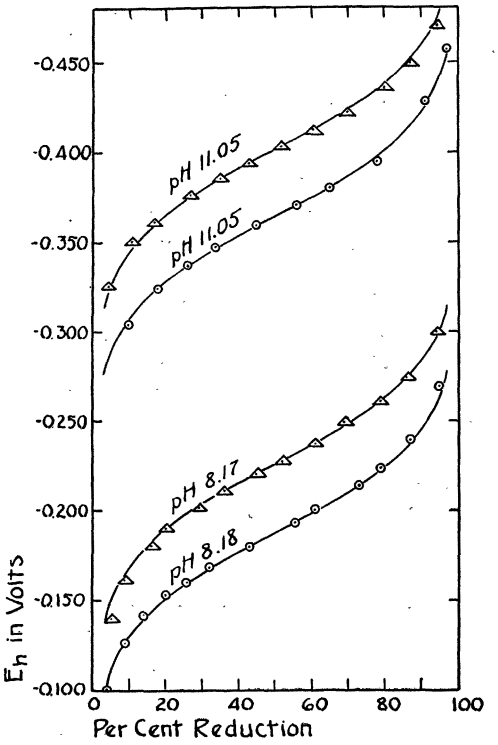

FIG. 1. The effect of anions on the oxidation-reduction potential of blood hemin. Concentration of hemin, 0.0001 M per liter. Titrated with $Na_2S_2O_4$; temp., 30°. ⊙ represents hemin in phosphate buffer; △, hemin in borate buffer.

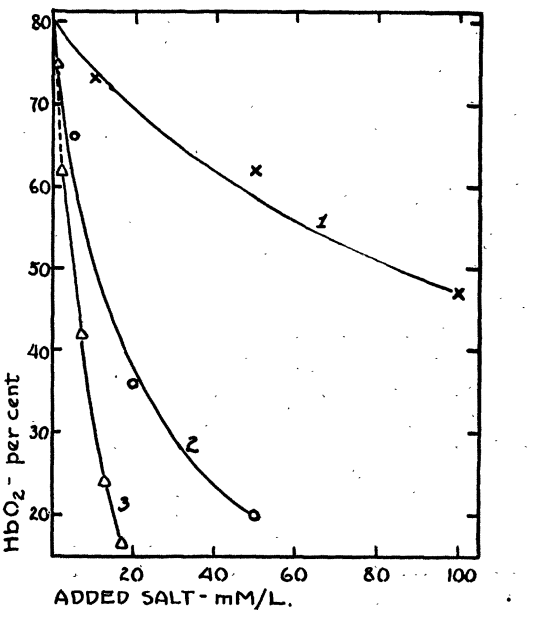

FIG. 2. The effect of anions on the oxygen dissociation of hemoglobin. HbO_2 concentration, 2 mM per liter; pO_2, 5 mm; pH, 6.8; temp., 25°. *1*, NaCl; *2*, Na_2HPO_4: NaH_2PO_4 mixture; *3*, $NaHCO_3$: CO_2 mixture.

has been plotted against the concentration of added salts. The experiments were performed with human dialyzed hemoglobin (2 mM HbO_2 per liter), at 5 mm pO_2, 25°, and pH 6.8. Salt-free hemoglobin possessed the greatest affinity, 50 per cent. HbO_2 being reached at 1.05 mm pO_2; then came, in decreasing order, Hb chloride, Hb sulfate (not in the figure), Hb citrate (not in the figure), Hb phosphate and Hb bicarbonate. Of course in this last case the picture is complicated by the simultaneous formation of carbamino compounds, the hemoglobin having been kept in mixtures of $NaHCO_3$: CO_2. Thus, on increasing the $NaHCO_3$: CO_2 concentration and keeping the hydrogen ion concentration *constant,* the affinity of hemoglobin for oxygen is diminished, a phenomenon of great value for facilitating the diffusion of oxygen from the hemoglobin to the tissues. Curiously enough, this oxygen dissociation curve of hemoglobin bicarbonate is almost a replica of the curve drawn from the data of Henderson, Bock, Field and Stoddard,[4] where the authors attributed this diminished affinity to pH changes. In the experiments reported in Fig. 2 we were dealing in every instance with mixtures of Hb and Hb anion, since the amount of anion used was not enough to prevent dissociation of the Hb anion complex. Similar experiments are being performed on the influence of cations.

These experiments show that all previous attempts to interpret the equilibrium between oxygen and hemoglobin have failed, because the influence of electrolytes on the equilibrium by formation of complex compounds was neglected. In all these experiments the equilibrium measured was that of Hb plus unsaturated mixtures of Hb complexes, each of them possessing different dissociation constants.

The HbO_2 determinations were made with the photoelectric spectrophotometer described by Hogness, Zscheile and Sidwell.[5]

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THE "SODIUM RETAINING EFFECT" OF THE SEX HORMONES

THE positive effect of injections of adrenal cortical hormone on the renal excretion of sodium in normal human subjects[1] and in normal dogs[2] has prompted an investigation of the possible effect of sex hormones on the renal excretion of sodium. The similarity in

[4] L. J. Henderson, A. V. Bock, H. Field, Jr. and J. L. Stoddard, *Jour. Biol. Chem.*, 59: 379, 1924.

[5] T. R. Hogness, F. P. Zscheile, Jr., and A. E. Sidwell, *Jour. Phys. Chem.*, 41: 379, 1937.

[1] G. W. Thorn, H. R. Garbutt, F. A. Hitchcock and F. A. Hartman, *Proc. Soc. Exp. Biol. Med.*, 35: 247, 1936.

[2] G. A. Harrop and G. W. Thorn, *Jour. Exp. Med*, 65: 757, 1937.

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.