vious paper, such as a gradient of activity from mid-ventral to mid-dorsal positions in the collar, and the concrescence theory do not enter into the present considerations.

At the ventral locus, prior to origin of the after-feather, barb primordia are constantly being initiated on both sides of the formative center located here. This bilateral activity is a fundamental feature of the formative center. When, in the fowl, regenerating feathers of the breast or saddle have reached a length of about 5.5 cm, which occurs after about 33 to 35 days of regeneration in the breast, and after about 40 days in the saddle, barbs begin to arise in the center of the ventral triangle instead of at the sides. These have a strictly vertical arrangement instead of an inclination parallel to the sides of the ventral triangle characteristic of their predecessors. When about six to eight of these have formed it is seen that there are now two ventral triangles, one at each side of the vertical barbs. It is inferred that division of the formative center at the ventral locus has taken place. It is then observed that, as each of the daughter formative centers has the same bilateral type of activity as the parent one, they are gradually forced farther and farther apart by the two series of barbs formed between them. At the meeting point of the two series of barbs between the daughter centers a new feather shaft (the hyporhachis) arises. The barbs formed on the opposite sides of the respective formative centers belong to the main feather. Seriation of barbs is identical in the after-feather and the corresponding part of the main feather.

As developing feathers have their ventral surfaces apposed to the pulp, these surfaces of the main feather and its appurtenant after-feather are also apposed to one another when regeneration is completed. They are thus mirror images of one another. In most breeds of fowl, and very generally in birds, the fluffy part of the feather, to the lower part of which the after-feather corresponds, is without pigment pattern; but in the barred rock fowl the barring is continued to the base of the feather and corresponds in the main and after-feather. In the hybrids between barred rock and brown leghorn, pigment pattern is usually absent in the fluffy part, but is occasionally represented by incomplete bars, or asymmetrical spots of black, on a white background. When this happens the asymmetries in the main and the after-feather are mirror images, occurring in quite exact correspondence on the right side of the one and the left side of the other. This test of twinning also thus holds for the after-feather.

Miss Hosker[3] deserves credit for the first records of stages of development of the after-feather; but the incompleteness of her material did not enable her to reach correct conclusions. Total preparations are indispensable for the study of this subject; these are prepared by splitting the cylinder of epidermal cells surrounding the pulp, as described in our former paper, spreading it out flat and fixing it under pressure between two glass slides. Our preparations show every stage in the formation of the after-feather from the origin of its first barbs to its completion. Sections made in different planes furnish valuable supplementary evidence.

The division of the formative center and the mirrored correspondence of patterns between the main feather and the after-feather demonstrate that this phenomenon belongs in the category of twinning. As a corollary, the formation of two feathers after division of the formative center demonstrates that the latter has properties with reference to the formation of the feather comparable to those of the primitive streak or blastopore with reference to the formation of the axis of the embryo. These conclusions are limited for the present to the material actually investigated.

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THE INFLUENCE OF ELECTROLYTES ON THE OXYGEN DISSOCIATION OF HEMOGLOBIN[1]

Numerous have been the attempts to interpret the equilibrium between oxygen and hemoglobin since Hüffner's first contribution 36 years ago. Adair's intermediate compound hypothesis,[2] a theory which led to an equation with four constants, successfully expressed his own data; Ferry and Green,[3] however, were forced to change the values of these four constants, in order to adapt the equation to their results. In view of this lack of agreement, it was clear that the fundamental assumptions had to be reconsidered.

When blood ferri hemin, dissolved in phosphate and borate buffers of identical pH value, was titrated potentiometrically with a reductant, the E'_0 values in phosphate buffer were 38 millivolts more positive than those of hemin in borate buffer. Hemin, on combining with phosphate and borate, formed complex compounds possessing different free energies (Fig. 1). Hemoglobin, like hemin, combined with a number of anions (chloride, sulfate, citrate, phosphate, bicarbonate) giving complex compounds possessing different dissociation constants for the reaction $\mathrm{Hb} + \text{anion} \rightleftarrows \mathrm{Hb}\ \text{anion}$ and the reaction $\mathrm{Hb}\ \text{anion} + O_2 \rightleftarrows \mathrm{Hb}\ \text{anion}\ O_2$. This is clearly demonstrated in the experiments plotted in Fig. 2, where the per cent. of HbO_2

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[1] From the Lasker Foundation for Medical Research, Department of Medicine, and the George Herbert Jones Chemical Laboratory, the University of Chicago.

[2] G. S. Adair, *Jour. Biol. Chem.*, 63: 529, 1925.

[3] R. M. Ferry and A. A. Green, *Jour. Biol. Chem.*, 81: 175, 1929.

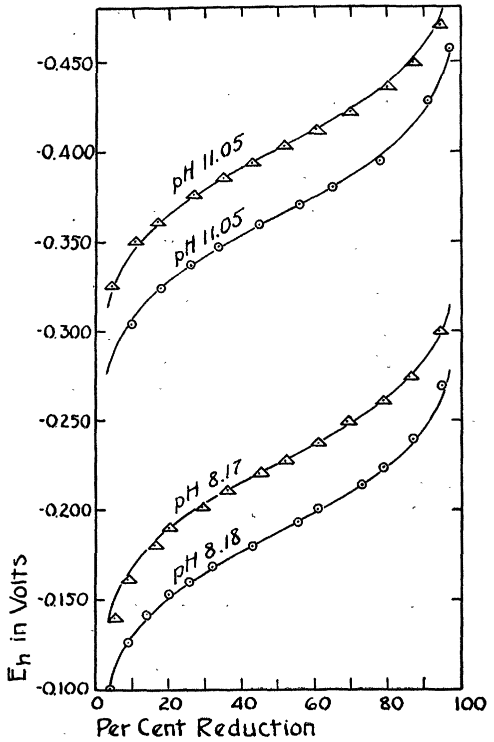

FIG. 1. The effect of anions on the oxidation-reduction potential of blood hemin. Concentration of hemin, 0.0001 M per liter. Titrated with $Na_2S_2O_4$; temp., 30°. ⊙ represents hemin in phosphate buffer; △, hemin in borate buffer.

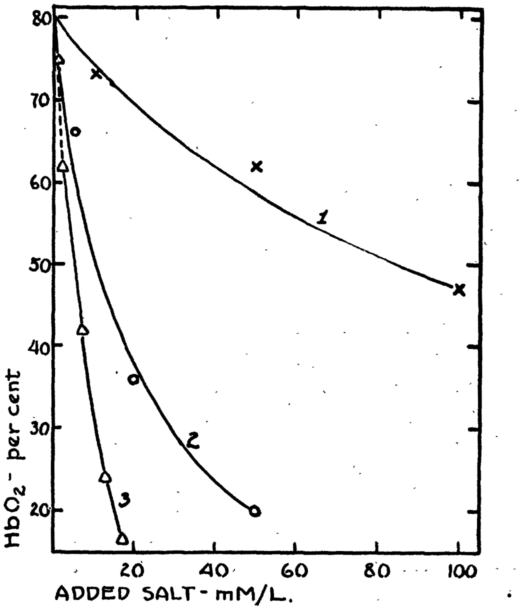

FIG. 2. The effect of anions on the oxygen dissociation of hemoglobin. HbO_2 concentration, 2 mM per liter; pO_2, 5 mm; pH, 6.8; temp., 25°. *1,* NaCl; *2,* Na_2HPO_4: NaH_2PO_4 mixture; *3,* $NaHCO_3$: CO_2 mixture.

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

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[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.