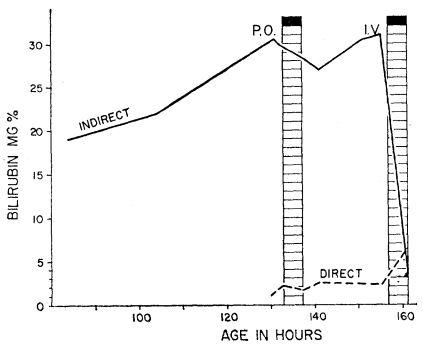

Fig. 2. A hyperbilirubinemic premature infant showed a negligible response to the oral administration of 5 g of glucuronic acid but a large decrease in indirect bilirubin during intravenous administration. Note the slight rise in the direct-reacting pigment during intravenous administration.

details of the reaction are not known at present (*5*). Even though there are no known pathways for the utilization of free glucuronic acid in glucuronide syntheses, there is some experimental evidence that such a pathway may exist. Attempts have been made to promote glucuronide conjugation in man (*6*) and animals (*7*) with glucuronic acid or glucuronolactone, but the results of these investigations are contradictory and inconsistent. Nevertheless, it seemed desirable to make the attempt to control indirect hyperbilirubinemia in the newborn by the administration of glucoronic acid.

This attempt has met with a considerable measure of success. Twenty-eight infants with hyperbilirubinemia have been treated to date by oral administration of glucuronic acid. In 16 of these a striking fall in the concentration of indirect bilirubin was observed, as is illustrated in the accompanying graph (Fig. 1), with an equally striking rebound within a few hours after the discontinuance of the conjugating agent. A simultaneous but less marked rise in direct bilirubin has been observed in some, but not in all instances. Of the 16 patients who responded, 10 were infants with erythroblastosis, the remainder being instances of physiological hyperbilirubinemia in which the concentration of indirect bilirubin exceeded 20 mg/100 ml. Of the patients who failed to respond to the oral administration of glucuronic acid, one, a premature infant, subsequently responded to intravenous administration of the acid (Fig. 2). Administration of glucuronic acid by the oral route produced a mild-to-moderate amount of watery diarrhea and acidosis in most of the infants treated. This prompted us to pursue intravenous glucuronic acid therapy further. We have now treated 14 additional infants by intravenous administration of glucuronic acid (and simultaneous oral administration of sodium bicarbonate); 12 have responded. No untoward symptoms have been noted during the injection or thereafter. The urinary excretion of direct bilirubin has been measured in two patients. In both, a three- to fivefold increase in the conjugated product was noted during and immediately after administration of glucuronic acid as opposed to control periods.

It appears that glucuronic acid per se is successful in lowering indirect bilirubin levels in serum in a significant percentage of hyperbilirubinemic patients. The exact mechanism of this action remains to be determined. These observations have obvious therapeutic implications and suggest that it may be possible to avoid many of the exchange transfusions now given in the neonatal period (*8*). Since glucuronic acid is not without toxicity, caution is required in its clinical use.

STUART DANOFF, CHARLOTTE GRANTZ AUDREY BOYER, L. EMMETT HOLT, JR.
Department of Pediatrics, New York University College of Medicine, New York

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26 November 1957

A Theory of Active State Mechanisms in Isometric Muscular Contraction

This report (*1*) presents in outline a formulation of the interaction of the contractile component and the series elastic component of isometrically contracting muscle. In general our theory is like that of Hill (*2*), but our assumptions and other details are significantly different. Our first assumption is that the basic property of the active state of the contractile component, the capacity to bear a load [given maximally by P_0 (g), the maximal force in isometric tetanus], is dependent on time (Hill's P_0 was independent of time). In simple, first approximation it is assumed that after onset this parameter rises exponentially with time constant α_1 (sec). Thus Hill's classic force-velocity relation,

$$(P + a)(v + b) = b(P_0 + a), \qquad (1)$$

is altered to read

$$(P + a)(v + b) = b[P_0(1 - e^{-t/\alpha_1}) + a]. \qquad (2)$$

In these equations: P(g) = force of the muscle; v(cm/sec) = shortening speed of the contractile component; t(sec) = time; and a(g) and b(cm/sec) are constants. We normalize Eq. 2, and all subsequent ones, so that quantities having dimensions of force are measured relative to $P_0 = 1$. Thus, by defining $p = P/P_0$ and $a_0 = a/P_0$, Eq. 2 becomes

$$(p + a_0)(v + b) = b(1 - e^{-t/\alpha_1} + a_0). \qquad (2a)$$

Our second assumption is based on the now well-known (*3–6*) nonlinear elasticity of the series elastic component [Hill (*2*) assumed a linear elasticity], and we express this by

$$p = f(e^{s/\lambda} - 1), \qquad (3)$$

in which s(cm) = the strain; p = the normalized stress; and f and λ (cm) are constants. By proper choice of f and λ, this equation can be made to fit the data of each of the previously mentioned studies (*3–6*) with remarkable accuracy.

Now, by differentiation of Eq. 3, the velocity of strain of the series elastic structure in an isometric contraction is

$$v = \frac{ds}{dt} = \frac{\lambda}{p + f}\frac{dp}{dt}, \qquad (4)$$

which, on substitution in Eq. 2a, yields

$$\frac{dp}{dt} = \frac{b}{\lambda}\left(\frac{p + f}{p + a_0}\right)(1 - e^{-t/\alpha_1} - p). \qquad (5)$$

This equation cannot be explicitly integrated and must therefore be solved numerically. However, this is required over only a very short initial time interval, for, as will be seen later, α_1 is very small compared with the total contraction period of a tetanus (or even of a twitch), and so for times greater than about $5\alpha_1$, Eq. 5 reduces to

$$\frac{dp}{dt} = \frac{b}{\lambda}\left(\frac{p + f}{p + a_0}\right)(1 - p), \qquad (6)$$

for which the explicit integral is

$$\frac{2bt}{\lambda} = \ln\frac{f}{(f + p)(1 - p)} + \frac{2a_0 + 1 - f}{1 + f}\ln\frac{f + p}{f(1 - p)}. \qquad (7)$$

For computation of Eqs. 5, 6, and 7 we use, for the frog sartorius, here studied at 20°C, the standard average values:

$a_0 = 0.25$, $b = 4.0$ cm/sec, and P_0 as will be involved later for particular muscles. Unfortunately, corresponding appropriate values for f and λ are not available even though, as previously mentioned, they can be calculated from other work: but they vary too greatly, they correspond to muscles of other species than ours (*Rana pipiens*), and, in some cases, certain data (P_0, weight, and length) of the muscles, needed for critical evaluation of the stress-strain data, are not given. We therefore obtain these constants from our theory and experimental tetanus myograms as follows. By using analytical procedures on Eq. 6, we determine the equation corresponding to dp/dt at maximum and put it into the form

$$f = \frac{a_0(1 - 2p_m) - p_m^2}{a_0 + 1}, \qquad (8)$$

in which p_m is the value of p for $(dp/dt)_{max.}$ (Eq. 6 is usable here, and not necessarily Eq. 5, since for p_m, $t > 5\alpha_1$.) Since a_0 is known, and since p_m can be determined from an experimental record as given in Fig. 1 (here, 0.280),

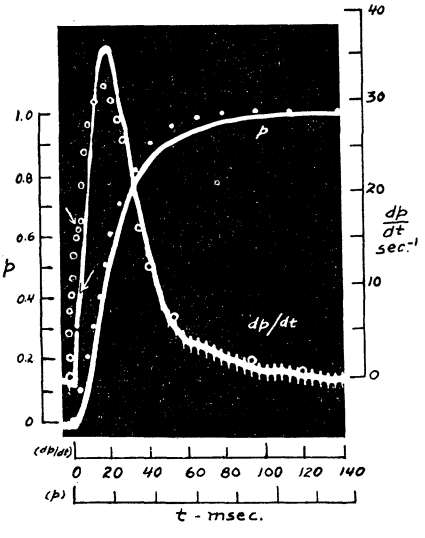

Fig. 1. Dual beam cathode-ray oscillographic records (*8*) of tension development (p) and its derivative (dp/dt) in maximal isometric tetanus contraction of a massively stimulated, curarized frog sartorius muscle (rest length, 3.0 cm; weight, 67 mg; and P_0, 48.3 g) at 20°C. Tension was registered by the RCA Type 5734 mechano-electronic transducer tube. Time is measured from onset of contraction. The time constant of the electronic differentiating circuit is 0.1 msec. (Timing corrections must be made for the direct contraction trace due to imperfect synchrony of its sweep with that of the derivative and because of relative tilt of the vertical axis of that trace.) Corresponding myograms calculated by the theory are represented by the dots for p and the circles for dp/dt. The arrows mark the active state "hump."

f can be computed = 0.0256. Equation 6 is now used to calculate λ, by inserting in it the known constants and matched values of p and dp/dt obtained from the records of Fig. 1. With p varying from 0.1 to 0.74, five such determinations give an average $\lambda = 0.0537 \pm 0.0035$ (S.E.) cm. By way of comparison, our values for f and λ agree fairly well with those, 0.0353 and 0.0476 cm, required, respectively, to make Eq. 3 fit Wilkie's (*6*) data (if it be assumed his $P_0 = 60$ g). Furthermore, using our constants in Eq. 3 and setting $p = 1$, we obtain s_m, (the maximum strain present in the series elastic component at tetanus plateau) = 0.188 cm—that is, 6.3 percent of the rest length (3.0 cm) of our muscle. For the toad sartorius, Hill (*4*) determined that s_m is 3 to 4 percent of muscle length. Our larger value may be due to a species difference.

Since all the constants of Eqs. 5 and 7 are now known, theoretical $p(t)$ curves can be computed. Comparisons will be made with the experimental equivalents (of one frog sartorius muscle) shown in Fig. 1 which have already been used to determine the elasticity constants. Presentation of more extensive results indicating variability is not necessary here since they provide nothing different in principle. The theoretical myogram of Fig. 1 has been computed over the range from 0 to 0.01 sec by an approximate integration of Eq. 5 with $\alpha_1 = 0.001$ sec, and from then on by means of Eq. 7. The theoretical points do not perfectly match the experimental ones, but the general fit is quite good and the theoretical curve especially includes the sigmoid foot [absent in Hill's curves (*2*)] so characteristic of actual myograms.

Figure 1 also presents dp/dt curves, the theoretical determined by means of Eqs. 5 and 6. In general there is good agreement between theory and experiment, as would be expected from the $p(t)$ curves. But of special interest is the little "hump" that appears very early in these myograms (see also Fig. 2). Theoretical considerations prove that the hump, even though it appears too high up on the theoretical curve, marks the termination of development of the active state and that this should occur at about 4.5 msec after onset of contraction, as it does in experimental records. These results are of the greatest interest, for they (i) involve a first observation of a feature of the early phase of contraction which, (ii), according to the theory, marks the instant at which the active state has developed to full intensity, and (iii) they provide verification of our assumption that after onset the active state develops exponentially with time constant α_1.

The preceding deals only with the tetanus contraction period—a relatively

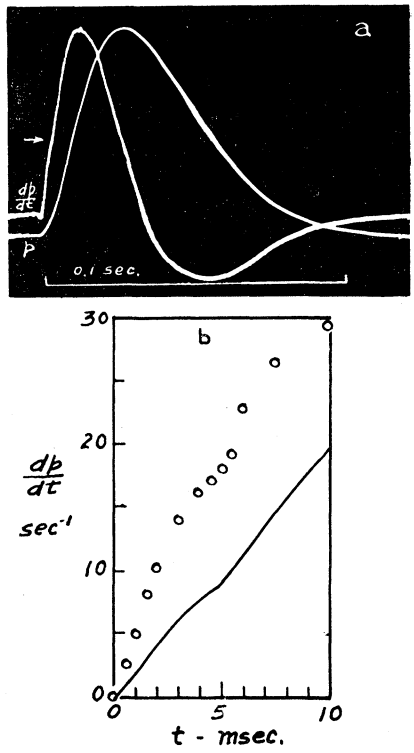

Fig. 2. (*a*) Twitch response of the same muscle as that represented in Fig. 1, showing very clear active state hump on the dp/dt curve. (*b*) Initial part of the dp/dt curves of Fig. 1, showing the active state hump on an extended time scale. Continuous line, experimental; circles, theoretical.

simple response since the active state, once brought to plateau, remains there as long as the tetanus stimulus continues. Systems involving relaxation from this plateau can be included in our theory by means of an appropriate function for the kinetics of active state decay. Making the very simple assumption that this decay is exponential with time constant α_2, a procedure like that leading to Eq. 5 yields the relevant equation,

$$\frac{dp}{dt} = \frac{b}{\lambda}\left(\frac{p+f}{p+a_0}\right)(e^{-t'/\alpha_2} - p), \qquad (9)$$

in which t' measures time from the instant at which the active state plateau ends and decay begins. If $p = 1$ at $t' = 0$, then this equation applies to muscular relaxation from maximal tetanus level. For the twitch, Eqs. 5 and 6 hold up to the moment $t' = 0$ (at which tension is still rising), and from then on Eq. 9 describes the remainder of the twitch, involving the latter part of the rise to peak, when p is always less than 1, and the subsequent relaxation period. Preliminary studies indicate that this theory yields results approximating those of actual twitch responses.

In conclusion, our work leads to the following general results: (i) a confir-

mation of the essentials of Hill's (7) conception of active muscle as a two-component system made up of contractile and series elastic components; (ii) derivation of equations that quite accurately predict the mechanics of the contraction period of an isometric tetanus; (iii) a simple method for determining the stress-strain curve of the series elastic component (though this needs confirmation by direct methods); (iv) a determination of the theoretical and experimental means for studying a newly observed feature of early contraction related to the abruptly rising phase of the active state; and (v) the general mathematical basis for study of active state mechanisms in tetanus relaxation and for the entire course of the twitch. In future research it is planned to complete the analyses regarding systems including active state relaxation, extend the theory to other types of contractions, and apply it, in general, to investigations of muscular responses under a variety of experimental conditions.

ALEXANDER SANDOW
Department of Biology, Washington Square College of Arts and Science, New York University, New York

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25 November 1957

The Two Hemoglobin Components of the Chicken

Recently Saha *et al.* (*1*) reported the presence of two electrophoretically distinct hemoglobin components in the chicken, one of which showed the same mobility as the abnormal human hemoglobin E. In the course of our investigations of animal hemoglobins, we also studied the hemoglobin of the adult chicken, using different techniques. The results of this study will be reported here, for they offer some additional information.

Blood samples of over 50 different chickens (stock for slaughter) were examined first by paper electrophoresis with barbital buffer of *p*H 8.8 and of ionic strength 0.06. These analyses showed the presence of one main component (component I) with a low relative mobility and a second one (component II) with a much higher mobility. Component II, present for about 15 percent of the total amount of hemoglobin, was found in all examined animals. Its relative mobility is about the same as that of the abnormal human hemoglobin E. Our paper electrophoretic investigations confirmed therefore the results reported by Saha *et al.* (*1*).

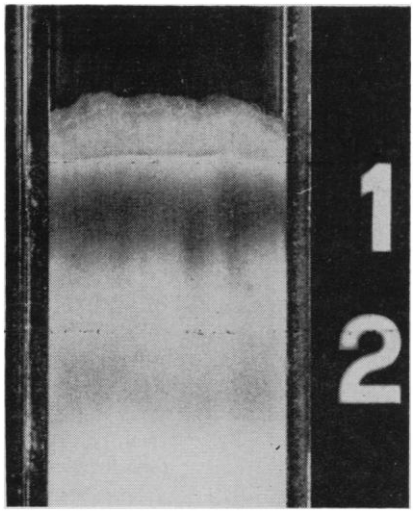

Fig. 1. Separation of two hemoglobin fractions of the chicken on Amberlite IRC-50.

The two hemoglobin components can be separated completely by chromatography on Amberlite IRC-50 with a citrate buffer solution of *p*H 6.0 and a sodium ion concentration of 0.15 (*2*). Figure 1 represents the relative positions of the two fractions. This technique offers therefore a possibility to obtain both components separately and to compare the amino acid compositions of the two hemoglobins. For this purpose about 50 mg of each protein was hydrolyzed with 500 ml of 6*N* hydrochloric acid by boiling under reflux for 48 hours. The amino acid analyses of these hydrolyzates were achieved by the column chromatographic method of Spackman, Moore, and Stein (*3*) with the Amberlite IR 120. The results of duplicate experiments are given in Table 1. Component II contains much more of the acid amino acids (aspartic acid and glutamic acid) and much less of the basic amino acids (lysine, histidine, and arginine). The large difference in the amounts of acid and basic amino acids may explain the great difference in the electrophoretic and chromatographic behavior of the two hemoglobin fractions. Moreover, other marked differences were found. The amounts of serine, valine, and leucine are higher in component II than in the other fraction, and the amounts of alanine, isoleucine, and tyrosine are lower. The results of these amino acid analyses are therefore strongly indicative of the existence of two widely different hemoglobin types in the chicken.

Table 1. Amino acid composition of the 48-hour hydrolyzates of two hemoglobin components of the chicken. The values are given in grams per 100 g of protein.

Amino acid	Component I (g/100 g)	Component II (g/100 g)
Aspartic acid	8.8	11.25
Threonine	4.5	4.1
Serine	3.15	5.15
Glutamic acid	6.8	11.5
Proline	3.2	3.0
Glycine	3.05	2.95
Alanine	8.95	7.55
Valine	8.0	9.45
Methionine	0	0
Isoleucine	5.0	3.75
Leucine	13.2	15.0
Tyrosine	3.1	1.15
Phenylalanine	6.5	6.8
Lysine	14.55	11.8
Histidine	11.8	7.7
Arginine	7.55	4.95
Totals	108.15	106.1

It is remarkable that these two different hemoglobin types were present in all the chickens studied. This situation is different from that found in some other animals—for instance, sheep (*4*) and cattle (*5*), in which the occurrence of two different hemoglobin types seems to be controlled by a pair of allelomorphic genes, both being readily recognizable in heterozygosity. It is obvious from the amino acid analyses that both components are completely different from any human hemoglobin (*6*). Any speculation based on the occurrence of human hemoglobin E (*1*) in birds is therefore without foundation (*7*).

H. J. VAN DER HELM
T. H. J. HUISMAN
Department of Pediatrics, State University of Groningen, Groningen, Netherlands

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21 August 1957