served. This suggested that the leucocytes might be supplying an enzyme activator that was released from whole cells. This question has been pursued only to the point of establishing that the ash from leucocytes will not substitute for living cells.

It seems clear that the presence of a critical number of leucocytes, about 500/mm^3, will potentiate hemolysis by venom of red cells that are otherwise insusceptible. This finding may be taken into account in studies of the mechanisms of venom lysis. It could perhaps explain some of the curious species specificities hitherto attributed to venoms in their action on erythrocytes.

The precise contribution of white cells to the lytic system is not clear. The white cells may contribute lecithin for the formation of lysolecithin. On the other hand, they might furnish an activator for some other lytic system. It is possible, in either case, that species differences in white cells may at times be more important in determining specificity than differences in red cells.

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11 October 1955

Nonspecificity of ATP-Contraction of Living Muscle

Adenosine triphosphate (ATP) induces contraction both in living muscle and in muscle models (*1–4*). In contrast to muscle models, ATP contraction of the intact muscle cell depends on membrane activation; ATP injected intracellularly is ineffective in altering mechanical state (*2*). Muscle is not unique in being spontaneously excited by ATP; firing of sympathetic ganglia and of anterior horn cells after application of this and related compounds has been reported (*5, 6*).

It can be shown that contraction produced by ATP in intact, isolated striated muscle is nonspecific in nature and depends on the removal of ionic calcium from the bathing medium. It is well known that lowering calcium in the external medium results in spontaneous muscle twitches (*7, 8*). Calcium forms a complex ion with ATP, the dissociation constant of which is considerably lower than that formed with citrate (K for CaATP complex is 8.7×10^{-5} moles/lit; for Cacitrate complex, it is 6.1×10^{-4} moles/lit) (*9, 10*). In order to show that the effect of ATP is due to calcium binding, it is necessary to demonstrate (i) that at the threshold concentration of ATP, the free calcium-ion concentration is sufficiently low to result in spontaneous activity, and (ii) that the results conform to the predictions of the mass-law equation when both initial external calcium and ATP are varied. The experiments were performed on the isolated curarized sartorius of *Rana pipiens*.

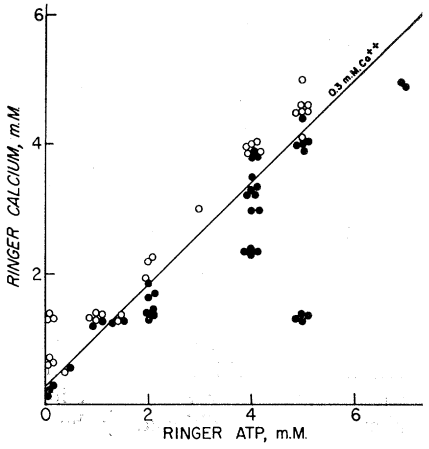

Fig. 1. Relationship of threshold concentration of ATP to Ringer calcium. Based on the mass law, the 0.3mM Ca^{++} line has been drawn. The points indicate the composition of Ringers solution for each muscle tested. Closed circles represent muscles that exhibited spontaneous activity, open circles those that did not.

An equilibration period of ½ hour in Ringers solution was allowed before transfer to the test Ringers solution. Muscle fibers were observed under the microscope for evidence of shortening. When a portion of the calcium in the Ringers solution is omitted, spontaneous twitching occurs in 50 percent of the muscles tested at 0.3mM Ca^{++}. Threshold concentrations for spontaneous activity were 1.3mM ATP or 2.6mM citrate (sodium salts) in normal Ringers solution. From the dissociation constants, ionic calcium was calculated to be 0.3mM. Thus the first criterion appears to be satisfied. The mechanical response to low calcium, ATP, or citrate was indistinguishable. At threshold concentrations, there were repetitive twitches of fibers and occasional tetanic bursts.

The next series of experiments was designed to determine how the threshold concentration of ATP varies with altered initial Ringer calcium. From an equation derived from the mass law,

$$Ca = \frac{[Ca^{++}][ATP]}{[Ca^{++}] + K} + [Ca^{++}]$$

where Ca is the total concentration of calcium, Ca^{++} is the concentration of ionic calcium, ATP is the total concentration of ATP and K is the dissociation constant of the CaATP complex, one can obtain a series of isoionic calcium lines when Ca is plotted against ATP. In Fig. 1, the line for Ca^{++} equal to 0.3mM has been drawn. The points are experimental and indicate the composition of the test Ringers solution for each muscle. The closed circles represent muscles that exhibited spontaneous activity, the open circles those that did not. All points above the line would lie on lines in which ionic calcium was greater than 0.3mM, and conversely for those below. On the whole, the results conform to the predictions based on the mass law.

If the action of ATP is to remove ionic calcium to a critical level, taking into account biological variation, one would expect an S-shaped curve when the percentage of muscles that showed spontaneous activity is plotted against Ca^{++} (Fig. 2). The points on the solid curve were obtained by calculation from the mass-law equation as ATP and Ringer calcium were varied. Points on the broken curve were obtained as Ringer calcium alone was varied. The two curves appear identical. These experiments indicate that ATP-induced contraction is correlated with a critically low calcium-ion concentration. No such correlation is found between the concentration of free (unbound) ATP and contraction (Fig. 3).

The similarity of the stimulating action on ganglia of ATP and low calcium was noted by Feldberg and Hebb (*5*). In harmony with the evidence presented here is the demonstration that inhibition of bone calcification by ATP is due to cal-

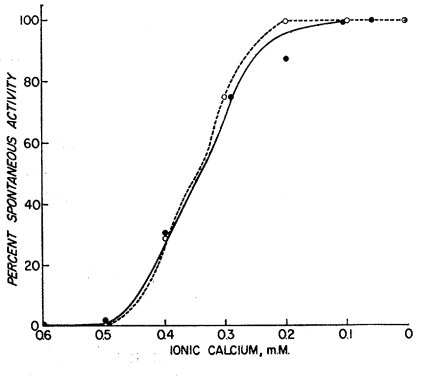

Fig. 2. Relationship of spontaneous activity to ionic calcium concentration. Broken curve: Ringer calcium alone was varied; solid curve: ionic calcium was changed by varying Ringer ATP and calcium.

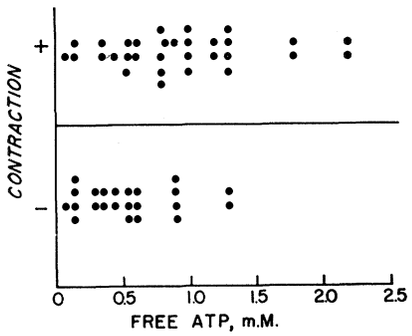

Fig. 3. Absence of correlation between the concentration of free ATP and contraction.

cium binding (*9*). It may be concluded that, in the case of muscle, calcium in the form of its ATP complex is not available to stabilize the muscle membrane. The action potentials so evoked set off the contractile process. Thus the effect of ATP applied to living muscle is unrelated to contraction per se.

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27 September 1955

High Incidence Blood Group Found in Venezuelan Indians

To date no single blood group found exclusively in discrete geographic or ethnical human divisions has been described. It is only through the incidence of the well-known blood groups that the various human stocks can be characterized in a general way (*1*). We present here (*2*) data on the incidence of what appears to be a new blood system with possible anthropological implications.

In 1954, Levine, Koch, McGee, and Hill (*3*) mentioned a new "private" blood factor called "Diego," which was detected in the serum of a Venezuelan woman who had been sensitized through several pregnancies. They found that this serum produced agglutination of the husband's red cells but did not agglutinate the red cells from 200 North American persons. Lately, with no further comments, this finding has been quoted in other publications (*4*). This year, serum was collected during a new pregnancy of the original patient, giving us an opportunity to study the incidence of the Diego factor in her husband's relatives and in various representative sections of the Venezuelan population.

In the study of the family (Ca. family) in which this factor was originally detected, we found eight positive cases out of 29 tested. In the general population from Caracas, we were surprised to find that several unrelated individuals carried this factor. Inquiry about the ancestry and physical features of the various positive cases and of the Ca. family revealed the probability that they all possessed ancestors from Carib Indian stock. The results of 826 tests in groups of people from various Venezuelan regions are given in Fig. 1. They were tested against anti-Diego serum by the indirect Coombs test.

It would seem that the Diego factor is not a "private" blood group, but rather that its incidence is high in Indians, especially in Carib Indians, and in people with mixed Indian ancestry. Since the Indians studied came originally from Brazil, it could be that this factor is prevalent in Brazil and other neighboring countries. The Indian element enters in a high proportion of the general Venezuelan population. This probably explains the positive tests found in the populations of Caracas and Barcelona. The cases found in the Negro population studied may also be explained by mixture with Indians.

From the genetic point of view, the study of the Ca. family and of several Indian families shows that the factor is inherited as a dominant Mendelian character with no sex linkage (examples in Fig. 2). The factor may be followed in some cases through several generations (four in one of the families). Apparently, the antibody is not of the naturally occurring type but is an immune "incomplete" one. Detailed study of the positive cases show that the antibody (anti-Diego

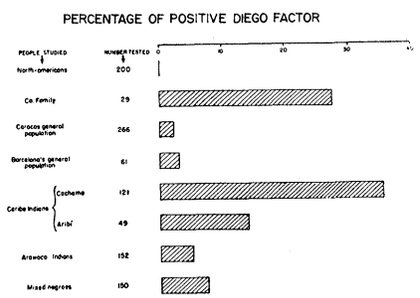

Fig. 1. Results of 826 tests.

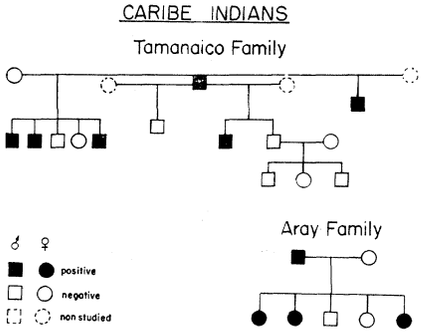

Fig. 2. Examples of genetic studies.

serum), which can be called anti-Di^a, was developed by a homozygous Di^b/Di^b (Mrs. Ca., the original patient). The individuals who reacted against the anti-Diego serum were Di^a/Di^b or Di^a/Di^a, indicating that this blood system is formed by at least two allelic genes. We can postulate that eventually anti-Di^b will be found.

If in the future it can be demonstrated that the Diego factor occurs exclusively or predominantly in Indian populations, it would be wise to change the name of this antigen to a more correct one, such as "Indian factor," related to its anthropological implications.

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26 September 1955

Spiral Male Nuclei in Ragweed Pollen

Stamens from ragweed (*Ambrosia*) growing on the premises of the Southern Illinois University were stained with iron-acetocarmine solution and observed under the microscope at 1000 diameters magnification. At the metaphase of the first meiotic division of the pollen mother cell, 18 round chromosomes were counted. Since the basic number of chromosomes in Compositae is nine, it is not surprising that the chromosome number of the ragweed is $n = 18$.

The chromosomes are regularly arranged on the equatorial plane of the first meiotic division (Figs. 1 and 2). At diakinesis, chiasmata were observed in