sized. Where polymorphism is extensive, on the other hand, the gene pool of the species is essentially broken up into a number of smaller gene pools, each of which corresponds to a chromosome arrangement. There is thus less opportunity for the hereditary variability present to undergo extensive free recombination. One may suggest, therefore, that marginal populations that are essentially homozygous for gene arrangement may be of special interest in evolutionary studies because they have a chromosomal system that allows maximum recombination by crossing over. They would appear to be peculiarly well suited for the attainment of future evolutionary advance.

HAMPTON L. CARSON
Department of Zoology, Washington University, St. Louis, Missouri

References and Notes

1. H. L. Carson and H. D. Stalker, *Evolution* 1, 113 (1947).
2. H. L. Carson, *J. Cellular Comp. Physiol.* 45, 221 (1955).
3. This work was supported by grants from the National Science Foundation and the Office of Naval Research.
4. This collection was made by D. D. Miller of the University of Nebraska, who is responsible for calling my attention to the unique features of the Chadron area.
5. D. D. Williams and D. D. Miller, *Bull. Nebraska State Museum* 3, 1 (1952).
6. A. B. da Cunha and T. Dobzhansky, *Evolution* 8, 119 (1954).
7. H. L. Carson, *Cold Spring Harbor Symposia Quant. Biol.*, in press.

6 October 1955

Influence of White Blood Cells on Lysis of Red Cells by Cobra Venom

It has been known since the early studies of Kyes (*1*) that snake venom hemolysis exhibits a species specificity. The difference in susceptibility of cells has been attributed by some to availability of intracellular lecithin for the action of venom lecithinase. On the other hand, it has not been possible to correlate lecithinase activity of venoms with their hemolytic activity (*2*). A recent discussion of the problem suggests that the facts are best reconciled in the idea that venoms contain, in addition to lecithinase, a lysin that has a direct hemolytic action (*3*). This view was apparently shared by Landsteiner, who compared the action of venoms on red cells with that of the phytoagglutinins and natural antibodies (*4*). The mode of the direct action of a native venom is still unexplained (*2*).

In the course of certain studies (*5*) on splenic cells, it has been found that washed red cells from the spleens of rabbit, swine, and ox are rapidly lyzed by traces of cobra venom although the erythrocytes of the peripheral blood are completely, or almost completely, unaffected by venom in comparatively high concentration. Evidence has been found that this apparently selective action on splenic cells is conditioned by the presence of leucocytes in the red-cell suspensions.

Table 1. Action of venom on splenic cells. Peripheral blood tested in same way showed no hemolysis whatever.

Time (min)	Venom dilution (× 10^3)							Control
	4	8	16	32	64	128	256	
20	++++	++++	++++	0	0	0	0	0
40	++++	++++	++++	++++	++++	+++	0	0
60	++++	++++	++++	++++	++++	++++	0	0

* ++++ = complete hemolysis.

Cobra (*Naja naja*) venom (*6*) stored as a dry powder was serially diluted for tests in 0.85-percent saline containing 0.02*M* phosphate buffer. Unless otherwise stated, *p*H was 7.4. To conserve material, the lowest venom dilution used was generally 1/4000 or 1/8000.

In some experiments, an animal lecithin (Eastman Kodak Co.) emulsion in buffer was added to the system in a volume of 0.1 ml containing 50 μg of lecithin.

Spleens and defibrinated blood of ox and swine were obtained from the slaughterhouse, and cell preparations were made within 1 or 2 hours of death. For studies of rabbit cells, freshly killed animals were used.

Cells of peripheral blood were washed three times in saline and made up to a 1- or 2-percent suspension. Fat and capsule were dissected from spleens, and cell suspensions were made by shaking teased-out pulp in saline. The cells were similarly washed.

Tests for hemolysis were made by adding 0.1 ml of cell suspension to 1 ml of venom dilution. The test tubes were placed at 37°C and read for hemolysis at intervals for 1 to 2 hours, and finally after an overnight interval at 4°C.

The selective action of venom on spleen cells is shown as a typical experiment with ox cells in Table 1. In eight such experiments with the ox, the peripheral blood cells showed hemolysis only once, in the 1/8000 dilution, and not until after 60 minutes of incubation. On the other hand, splenic cell suspensions were invariably hemolyzed rapidly in titers of venom ranging from 1/32,000 to 1/512,000.

Entirely similar findings were obtained with cell preparations from rabbit and swine, but since ox spleens were easier to work with, further studies were made on cell preparations from this animal alone.

It was found that hemolysis of splenic blood was maximum at *p*H 7.4 to 7.9 and that it decreased with lowering of *p*H, disappearing at *p*H 6.0. The lecithinase activity of the venom was tested by adding lecithin to venom and peripheral blood cells, which then hemolyzed rapidly. It was found that the optimum *p*H of this system is also in the range 7.5 to 7.9, and that activity decreases on acidification. Thus, the action of venom on splenic blood and on lecithin could not be distinguished in terms of *p*H optima.

White-cell counts were performed on many suspensions being tested, and random samplings were taken from those showing lysis and from controls. The number of leucocytes in the control tubes of splenic blood usually numbered 20,000 to 40,000/mm^3, while the corresponding preparations of peripheral blood had less than 200. When the content of white cells in spleen blood was reduced by repeated differential centrifugation, it could be shown that lysis of erythrocytes was slow and might fail altogether if the number of white cells fell below about 500/mm^3.

Conversely, it was found that leucocytes concentrated from peripheral whole blood could bring about lysis when they were added to a suspension of red cells that were otherwise insusceptible to the action of venom.

It was of some interest to note that lysis of the white blood cells did not appear to be essential for this effect. Table 2 shows that in the tubes containing the two highest dilutions of venom, the final white-cell counts were comparable to the controls even though hemolysis was ob-

Table 2. Final white counts on venom-splenic cell system.

Expt.	Venom dilution (× 10^3)						Controls	
	8	16	32	64	128	256	C_1	C_2
Lysis	++++	++++	++++	++++	++++	++++		
White count (× 10^3)	2.2	2.5	2.3	2.8	8.4	12.0	8.4	5.1

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.

JOSEPH C. TURNER
Department of Medicine, Columbia University College of Physicians and Surgeons, and *Presbyterian Hospital, New York*

References and Notes

1. P. Kyes, *J. Infectious Diseases* 7, 181 (1910).
2. A. C. Roy, *Nature* 155, 696 (1945).
3. W. E. van Heyningen, in *The Proteins* (Academic Press, New York, 1954), vol. 2, pt. A, chapt. 15.
4. K. Landsteiner, *The Specificity of Serological Reactions* (Thomas, Baltimore, Md., 1936), pp. 5–7.
5. This study was supported by grant C-2473 of the U.S. Public Health Service.
6. Kindly supplied by D. Nachmansohn and Michael Heidelberger.

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*.

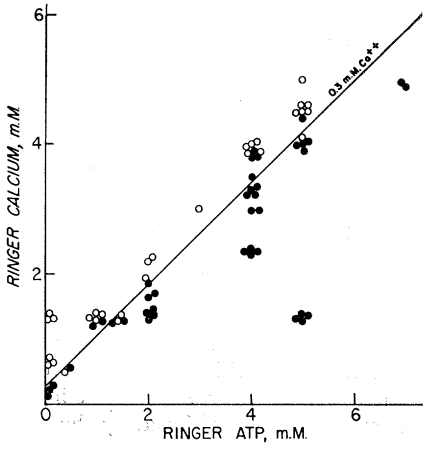

Fig. 1. Relationship of threshold concentration of ATP to Ringer calcium. Based on the mass law, the 0.3mM Ca^{++} line has been drawn. The points indicate the composition of Ringers solution for each muscle tested. Closed circles represent muscles that exhibited spontaneous activity, open circles those 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-

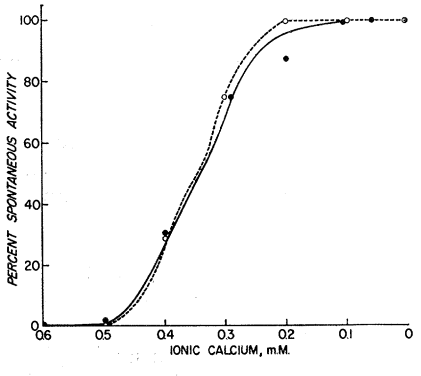

Fig. 2. Relationship of spontaneous activity to ionic calcium concentration. Broken curve: Ringer calcium alone was varied; solid curve: ionic calcium was changed by varying Ringer ATP and calcium.