significant. This result has been supported by observation of considerable interspecies cross reaction among denatured DNA fragments and DNA-agar of several mammalian species. In addition, DNA-DNA and also RNA-DNA interactions have been used to evaluate quantitatively, genetic relatedness among the Enterobacteriaceae (*11*).

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Zeolite ZK-5: A New Molecular Sieve

Abstract. *An aluminosilicate of novel crystal structure has been synthesized. It has molecular-sieve properties that permit separation of straight-chain from branched-chain and cyclic hydrocarbons. This zeolite is unusually stable in solutions of low* pH.

A crystalline aluminosilicate with a novel crystal structure has been synthesized. Table 1 contains pertinent data derived from x-ray diffraction measurements of this new substance. These data indicate that the crystal structure of the new zeolite, given the name zeolite ZK-5, is body-centered cubic with a lattice parameter $a = 18.72$ Å. A pseudo cell was also observed which is primitive cubic with $a' = a/(2)^{\frac{1}{2}}$. Meier and Kokotailo have elucidated the main features of the crystal structure (*1*).

Table 1. X-ray diffraction data—zeolite ZK-5.

(*h*, *k*, *l*)	*d*(Å)	I/I_{max}*
110	13.3	0.18
200	9.41	1.00
220	6.62	0.06
310	5.93	.41
222	5.41	.48
321	5.03	.02
400	4.69	.06
330	4.41	.50
420	4.19	.34
332	3.98	.22
422	3.81	.18
510	3.66	.06
521	3.41	.13
530,433	3.21	.35
611	3.02	.28
620	2.94	.21
541	2.88	.02
622	2.81	.26
631	2.75	.09
543,710,550	2.64	.11
640	2.59	.02
721,633,552	2.54	.09
730	2.45	.03
732,651	2.37	.01
811,741,554	2.30	.02
822,660	2.20	.03
831,750,743	2.17	.02
662	2.14	.01
910,833	2.06	.03
842	2.04	.02
921,761,655	2.02	.03
830,851,754	1.97	.005
932,763	1.93	.02
941,853,770	1.89	.02
10,2,0,862	1.83	.05
10,3,1,952,765	1.79	.05

*I/I_{max} is the intensity of each reflection relative to the reflection of maximum intensity.

The mole ratio of SiO_2 to Al_2O_3 in zeolite ZK-5 varies from 4.0 to 5.1. The more silica-rich samples can undergo cation exchange with dilute hydrochloric acid solution (about 0.1*N*) with no significant loss in crystallinity. Stability of this type is not frequently observed in either naturally occurring or synthetic zeolites. Various cation forms of this zeolite, prepared by standard ion-exchange techniques, are capable of adsorbing about 13 percent by weight of straight-chain hydrocarbons while excluding branched-chain or cyclic hydrocarbons. In this respect, zeolite ZK-5 has molecular sieve properties similar to those of zeolite A (*2*) and zeolite ZK-4 (*3*).

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20 May 1963

Blood Group Studies with Turtles

Abstract. *Groups and individuals of diverse species of turtles have been distinguished by selective agglutinations of their washed red blood cells when undiluted normal serums or plant extracts are used as agglutinins. During these studies, production of hemagglutinins and precipitins has been induced in turtles and certain other poikilotherms.*

Very little immunogenetic research has involved reptiles and amphibians, literature in this field having been reviewed by Hildemann (*1*). Sixty years ago Noguchi (*2*) reported hemagglutination and antibody production in turtles. Later, antibodies to mammalian serum were induced in one species of turtle (*3*); normal isohemagglutinins were demonstrated in two species, and irregular heterospecific agglutinins were found (*4*). The present report deals with blood group differences in turtles and shows that a variety of blood grouping reagents may be used for characterizing individuals and populations.

Most of the blood samples were taken without serious trauma by cardiac puncture. So far I have had some limited success in obtaining microliter samples from retro-orbital sinuses of some turtles (*5*) by using techniques recently used with other organisms (*6*). Blood cells were washed three times with 0.9-percent sodium chloride or Alsever's solution, and small drops of approximately 2-percent cell suspensions were mixed in wells of test plates with equal volumes of individual undiluted serum or lectin-containing plant extract. For certain of the reactions, titers were obtained by using serial dilutions of agglutinins in test tubes. Most serums tested were from unimmunized reptiles, but a few amphibian (toad), mammalian (human and rabbit), fish (chondrichthyes and osteichthyes) and arthropod (crab) serums also caused selective agglutinations. Lectins were extracted in 0.85-percent sodium chloride (weight of plant tissue to solvent being about 1:5), and

0.1-percent sodium azide was added as preservative. Of the plant extracts, those from bean seeds (family Leguminosae) were used most frequently and were very effective. Usefulness of any material for selective agglutinations was determined empirically, some proving more effective than others with cell suspensions from a particular species.

Table 1, which is a typical protocol, shows 36 reactions involving red blood cells of common snapping turtles, *Chelydra s. serpentina* (*7*). As can be seen, any of the three cell suspensions may be distinguished from the other two by selection of suitable serums. Table 2 summarizes observations on the reactions of serums and lectins with cells from a variety of domestic and foreign turtles.

Reagents have been used primarily to distinguish individuals from others of the same species. These results therefore do not indicate numbers of blood group systems or which antigenic factors might be suspected of being controlled by allelic genes; however, there is a suggestion of a considerable number of blood group systems in certain species. Thus for the Greek tortoise, *Testudo hermanni*, which has been studied most extensively, each of 24 cell suspensions could be distinguished readily from all others. Some cells of individuals of the Greek tortoise suspended in 22-percent bovine-serum albumin were agglutinated by arthropod (*Limulus polyphemus* and *Cardisoma guanhumi*) serums and certain fish serums as well as by extract from potato, whereas these same reagents failed to agglutinate saline suspensions of cells from the same individuals. In addition to the species shown in Table 2, one cell suspension each from the Florida softshell (*Trionyx ferox*), an Indian turtle (*Geoemyda trijuga*), and the Sonora mud turtle (*Kinosternon sonoriense*) have been agglutinated by certain of their respective normal saline isoagglutinins, thus suggesting that blood groups exist in these species too. Consideration of the above results leaves no doubt that blood group systems readily can be demonstrated among turtles in general.

Suggestion of population differences was seen between a small number of eastern painted turtles (*Chrysemys p. picta*) captured at different times in two well-separated locations in New Jersey. The northerly population (P_1) is about 75 miles from the southerly population (P_2). Serum from each of ten turtles (six positively identified as females) caught at P_1 agglutinated red blood cells from four turtles (two males and two females) from P_2, giving tube titers as high as 1 in 1024. These tests were performed in October 1960. Identical agglutination patterns were obtained in March 1961 with five of the same P_1 and three of the same P_2 animals which had been maintained in active condition during intervening winter months. Serum from one common snapping turtle (*Chelydra s. serpentina*) also selectively agglutinated cells from P_2 animals. These observations suggest that blood typing methods could be used to good advantage in the identification of individual differences within turtle species, especially in studies of genetic relationships, distributions, and migrations.

In addition to use of naturally occurring antibodies, serum from immunized rabbits has been utilized in some further tests, and heteroagglutinins also have been induced in two pond sliders (*Pseudemys scripta*), one giant toad (*Bufo marinus*), and one yellow rat snake (*Elaphe obsoleta quadrivittata*). After immunization with heterologous serum, three turtles (*Chelydra s. serpentina, Pseudemys scripta, Terrapene c. carolina*) and four giant toads (including the one mentioned above) have produced antibodies as demonstrated by agglutination of tannic-acid-treated and then antigen-coated rabbit erythrocytes (*8*). Control serums for all these tests were separated from blood taken just prior to the first injection and were stored at −20°C. Cell suspensions and serums used as antigens were injected into the coelom during an injection program consisting of two to four injections (in several cases only one injection) at 1- to 3-day intervals, at least 1 week elapsing before the next such series was given. There were as many as five series. Antibodies were demonstrable when serum was checked as early as a week after the second series. These data provide additional evidence that poikilotherms synthesize antibodies, and temperature records suggest that high titers of antibodies are most likely to be present when an animal has been maintained at environmental temperatures in the upper part of its temperature range. Use of reptiles and amphibians as antibody sources should assume greater importance in future immunological research, including advancing work on blood group systems among these organisms and among other vertebrates including man.

A thermolabile hemolytic agent

Table 1. Agglutination of common snapping turtle (*Chelydra s. serpentina*) erythrocytes by normal serums.*

Erythrocytes	Serums											
	Css 6	Css 11	Css 12	Bm 1	Bm 2	Bm 3	Bm 4	Ccc 5	Pse 14	Pse 15	Pse 16	Tf 4
Css 6	0	+++	+	0	0	0	0	0	+	0	0	++
Css 11	+	0	0	+	+	+	+	0	+	0	0	0
Css 12	0	0	0	0	0	0	0	0	0	0	0	0

* Abbreviations: Css, *Chelydra s. serpentina;* Bm, *Bufo marinus;* Ccc, *Caretta c. caretta;* Pse, *Pseudemys scripta elegans;* Tf, *Trionyx ferox.* Symbols: 0, no agglutination; +, ++, +++, weak, moderate, and strong agglutination, respectively.

Table 2. Turtles distinguished by iso- and heteroagglutination of their erythrocytes reacting with normal serums and lectins.

Turtles	Number of individuals	Number of isoagglutination reactions	Number of heteroagglutination reactions	Individuals or groups of individuals distinguished
Terrapene c. carolina	27	768*	94*	9
Testudo hermanni	24	330	2165*	24
Chrysemys p. picta	20	339*	222*	11
Chrysemys p. picta	8	64*	16	2
Pseudemys scripta elegans	19	324*	99*	13
Caretta c. caretta	13	182	49*	5
Emys orbicularis	13	169*	27	4
Emys orbicularis	4	0	308*	4
Emys blandingi	10	84	25*	4
Deirochelys reticularia chrysea	6	36*	24	2
Chelodina longicollis	5	25	60*	5
Sternotherus odoratus	5	25	48*	4
Clemmys caspica	4	0	308*	4
Chelydra s. serpentina	3	9*	45*	3
Chelydra s. serpentina	3	9*	27*	3
Geoemyda p. punctularia	2	0	154*	2

* Individual variation observed.

(complement) exists in turtle serum (*2, 4, 9*). In my work, certain of the agglutinating reagents (normal and immune) have been heated when necessary to prevent lysis of the turtle cells being tested. Fresh serums from two Florida snapping turtles (*Chelydra serpentina osceola*) have lysed sensitized sheep cells (*10*).

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Poliomyelitis in Monkeys: Decreased Susceptibility after Avoidance Stress

Abstract. *Eleven monkeys were subjected to avoidance stress for 24 hours followed immediately by intravenous inoculation with type I poliovirus. Twelve control monkeys not so stressed were similarly inoculated. Seven of 11 stressed animals survived the infection while only one of the controls lived and their average incubation period was significantly longer than the average for controls. The number of circulating lymphocytes decreased significantly in experimental animals during and immediately after exposure to stress.*

Changes in resistance to herpes simplex virus and to anaphylactic shock in mice exposed to the stress of confinement or avoidance in a shuttlebox have been reported (*1–3*).

We now summarize effects of stress on resistance to poliovirus infection in cynomolgus monkeys.

In these experiments the Sidman avoidance procedure was used (*4*). Animals learned to press a telegraph key at a steady rate to avoid a shock to the tail which would be delivered once every 10 seconds if the lever was not pressed. Monkeys learn this procedure well and when fully trained receive few shocks during long periods of exposure. Despite this fact, the avoidance procedure is stressful for monkeys. During prolonged periodic exposure increased amounts of 17-hydroxycorticosteroids are found in blood and urine (*5*), and fatal gastrointestinal disorders develop (*6*).

The experiment was repeated five times with a total of 23 adult male monkeys. Throughout each experiment the animals lived in chairs (*7*) in which they were held loosely at the neck and waist. All 11 experimental animals and 7 of the 12 controls were trained to make the avoidance response; five controls were not trained. At the end of training all animals were permitted to rest for at least 9 days during which three blood samples were taken for base line total and differential white blood cell counts. Experimental animals were then exposed to the avoidance situation continuously for 24 hours, with a 10-minute interruption for feeding midway. Control animals remained in adjacent chairs but were not subjected to stress. In two experiments blood samples were taken at 3-hour intervals during the stress period to determine changes in white blood counts. At the conclusion of the 24-hour period of stress blood samples were taken, and experimental and control monkeys were inoculated intravenously with 100 tissue culture ID_{50} (50 percent infectious dose) of Brunhilde strain of type I poliovirus. Thereafter, blood samples were taken daily and the animals were observed for the development of fever and paralytic disease.

Exposure to the stressful avoidance situation results in a marked decrease in susceptibility to poliovirus infection. Whereas 7 of 11 stressed animals survived the infection and recovered, only 1 of the 12 controls lived. This difference in survival rates is significant at the .01 level (Fisher exact test). Three of the stressed animals showed little or no residual paralysis. The other four survivors, while appearing healthy, showed some residual leg, arm, or facial paralysis. Except for a higher incidence of quadriplegia and bulbar involvement in controls, paralytic patterns did not differ in the two groups. Prolonged incubation is also evidence of the protective effect of exposure to avoidance stress (Table 1). On the average, stressed animals developed symptoms 2 days later than controls (6.8 days as against 4.8). This difference tested by *t*-test is significant at the .001 level.

The number of circulating lymphocytes decreased in experimental animals during exposure to stress. Values for stressed animals were significantly lowered at the end of the 24-hour avoidance period, as well as 4 hours after its termination. In those instances where blood samples were taken at 3-hour intervals during exposure to stress, mean lymphocyte values were significantly lower for experimental animals than

Table 1. Effects of avoidance stress on susceptibility to poliomyelitis. Signs of experimental infection were tremor and paralysis with difficulty in breathing and swallowing. Four monkeys, two stressed and two controls, died overnight without developing detectable paralysis; all four showed tremor a day before death. The remaining animals that died all exhibited paralysis before death.

Mortality ratios		Mean incubation (days)	Number of monkeys developing poliomyelitis at times after inoculation					
Total	Died		Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
			Stressed monkeys					
11	4	6.8	0	2	3	2	1	2
			Control monkeys					
12	11	4.8	5	4	1	1	0	0