

cats were prepared under anesthesia as follows. The stapedius and tensor tympani muscles were cauterized bilaterally. Then recording electrodes were implanted on both cochlear round windows as well as upon the auditory cortex (A_1) of one side. A day or so after the operation, the cats were placed in a cage located in an electrically shielded sound-attenuated experimental room and "habituated" by exposure to clicks of moderate intensity delivered at a rate of one click per 10 seconds day and night for at least 10 days. Complete electroencephalographic recordings, as outlined below, were made on three animals with a Grass model IIIId electroencephalograph located adjacent to the experimental room.

Figure 1 illustrates five successive click-evoked cortical potentials taken from one typical cat in the "habituated" state. Soon after this habituated sample was collected, the animal was conditioned by reinforcing selected clicks with a puff of air directed to its face. Figure 1 clearly shows enhancement of the evoked potentials produced by this reinforcement. The number necessary to produce such marked changes in evoked potentials varied with the animal, but in all three cases, fewer than five puffs produced the "conditioned" changes apparent in the figure.

In three cats amplitude measurements were made on 25 successive responses taken during both the habituated and the conditioned periods, and their means were compared by *t* tests. For each of them, the increase in size of the conditioned evoked potential was significant beyond the .0001 level.

Soon after the conditioned tracings were obtained, the experimenter visually distracted the cat during the delivery of one click by standing at the open door of the laboratory. The last column of Fig. 1 shows the tracings obtained immediately before, during, and after distraction.

Successful removal of the middle ear muscle attachments to the ossicles was verified in two ways. First, the round window response to tone bleeps ranging in frequency from 500 to 5000 cy/sec delivered through earphones is in normal animals reduced in amplitude by middle ear muscle contraction within 15 to 20 msec of its onset; this reduction did not occur in the animals in this series. Second, at autopsy the muscles on both sides were found to have been severed in each cat.

Thus, our data show that in cats without middle ear muscles the variations seen in evoked potentials at the auditory cortex during habituation, conditioning, and distraction closely resemble what is seen in an unoperated

animal subjected to the same procedures. Any participation of the muscles in producing these response amplitude variations must therefore be minimal (6).

GEORGE MOUSHEGIAN, ALLEN RUPERT, JAMES T. MARSH,* ROBERT GALAMBOS
*Department of Neurophysiology,
Walter Reed Army Institute of
Research, Washington, D.C.*

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* Present address: Department of Psychiatry, University of California, Los Angeles.

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Gamma Globulin (Gm group) Heterogeneity in Chimpanzees

Abstract. The serum gamma globulin (Gm) serological system was examined in 24 chimpanzees. Five Gm a, Gm b, and Gm x phenotypes, including Gm (a-b-x-), were observed. Phenotype did not appear to be related to serum gamma globulin concentration. The presence of the Gm system in apes suggests that this polymorphism in man is balanced and ancient.

The various genetic polymorphisms found in human blood are of uncertain antiquity. An estimate of the age of any one polymorphism may be obtained by examining the blood of other animals. Application of this principle to the serum gamma globulin (Gm) polymorphism (1) is the object of the present investigation.

Serum gamma globulin typing is based upon neutralization by normal serum of an indirect Coombs type reaction occurring between selected sera from patients with rheumatoid arthritis (RA) and type O Rh+ erythrocytes sensitized with certain incomplete anti-Rh sera. Allelic specificity is usually conferred by selecting an anti-Rh serum which possesses the allelic product being typed (2, 3) and an RA serum which lacks this product (4). Gm^a, Gm^b, and Gm^x are apparently allelic in man. Gm^a and Gm^b are co-dominant with the result that three phenotypes and corresponding genotypes are distinguish-

Table 1. Ranges of Gm classification scores in chimpanzees.

Allele	RA dilutions employed	Control scores (saline)	Phenotype		
			Gm+	*Gm ⁱ	Gm-
Gm ^a	1/2-1/1024	33-36	0	-	31-36
Gm ^b	1/4-1/64	16-20	0-4	8	12-17
Gm ^x	1/8-1/128	17-18	0	-	12-20

* Intermediate.

able. The phenotype Gm (a-b-) has not been observed except in individuals with agammaglobulinemia. The Gm (a-b-) phenotype has not been observed by us during typing of more than 2000 healthy people.

Sera from 24 chimpanzees (5), 2 gibbons, 25 cynomolgous monkeys, 2 rhesus monkeys, 2 spider monkeys, 1 red monkey, 4 domestic cows, and 5 mongrel dogs were examined. Sera were absorbed overnight in the cold with nonsensitized O Rh+ cells. Without absorption all but chimpanzee sera produced moderately to strongly positive nonspecific reactions. After absorption all sera were diluted 1/8 and tested. The method of examination was that described by Harboe (2). RA serum dilutions employed for typing Gm^a, Gm^b and Gm^x are given in Table 1.

The chimpanzee was the only species whose sera inhibited any of the Gm typing reactions. Agglutination scores (6) were obtained, and these, together with the resulting phenotype distinctions, are shown in Table 1. Scores were repeatedly confirmed for each animal. The observed numbers of animals with various Gm^a and Gm^b phenotypes are given in Table 2. Only one animal of 22 tested was Gm (x+), being also Gm (a+b+). The single instance of an intermediate score occurred in the Gm^b system, and this animal was arbitrarily classified as Gm (b-).

Fourteen of the chimpanzees were affected with extensive pulmonary tuberculosis. The frequencies of Gm phenotypes in this group differed slightly from those observed in healthy animals (Table 2). These differences were not a manifestation of altered amounts of gamma globulin (7), since the concentration of serum gamma globulin, estimated by the product of gamma proportion found on paper

Table 2. Gm classification of 24 chimpanzees.

Presumed genotype	Phenotype	Total	Tuber- culous	Nor- mal
Gm ^a /Gm ^a	Gm (a+b-)	1*	0	1*
Gm ^a /Gm ^b	Gm (a+b+)	11	8	3
Gm ^b /Gm ^b	Gm (a-b+)	10	5	5
- / -	Gm (a-b-)	2	1	1

* Intermediate Gm^b score.

electrophoresis and total protein by the biuret method, did not appear to influence Gm phenotype. In 21 animals (11 tuberculous and 10 healthy) gamma globulin concentrations and Gm^a type were determined on the same serum aliquots. The upper limit of the gamma globulin concentration for Gm (a-) individuals was 4 times the lower limit observed among Gm (a+) individuals. The mean gamma globulin concentration was 1.50 g/100 ml among the Gm (a+) animals and 1.60 g/100 ml among the Gm (a-) animals. Several animals with Gm (a+b+) phenotype had smaller concentrations of gamma globulin than the two chimpanzees with Gm (a-b-x-), that is, "Gm-less" phenotype. The single Gm (a+b+x+) individual had a gamma globulin concentration of 2.01 g/100 ml. This was exceeded by the gamma globulin concentrations of three other animals, two of which were Gm (a-). The individual with an intermediate Gm^b score had a gamma globulin concentration of 0.54 g/100 ml. Lack of correspondence of gamma globulin concentration with Gm phenotype is in accord with observations in man (7). Such findings suggest that the Gm substances are specific proteins rather than a variable feature of all gamma globulins. Further evidence for this view is provided by the failure of large increases in gamma globulin, after immunization of chimpanzees with ovalbumin, to alter either a Gm (a-b-x-) or a Gm (a-b+x-) phenotype.

Our results confirm the earlier observation of Podliachouk (8) who found that of various animals studied the chimpanzee alone possessed the Gm (a+) character. All of 24 chimpanzees examined were Gm (a+). Reagents for typing other allelic products were not then available. The universality of the Gm (a+) character in the earlier study may be the result of inbreeding in small ape isolates.

The appearance of Gm polymorphism in both man and chimpanzee might be due to two independent sets of mutation resulting in at least three similar allelic products in two species. A simpler explanation would be to assume a common origin for the polymorphism in which case the system probably originated at least as early as the Oligocene period. If the latter explanation is correct, then it, in turn, indicates that the polymorphism in both species is balanced rather than transient and subject to rather ubiquitous selective forces.

The observation of heterogeneity of specific gamma globulins which are common to man and chimpanzee suggests a possible means of demonstrating in man the allotype described by Oudin

(9) and others (10) in rabbits. The chimpanzee may be useful as a surrogate man for the purpose of creating isoprecipitins which can then be tested against human sera for reaction with isoantigens. Such an attempt is currently in progress (11).

SAMUEL H. BOYER

WILLIAM J. YOUNG

Departments of Medicine and Anatomy,
Johns Hopkins University School of
Medicine, Baltimore, Maryland

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5. The cooperation of Prof. David Bodian in providing samples of chimpanzee and cynomolgous monkey blood is gratefully acknowledged. Dr. James Wright of the National Zoological Gardens, Washington, D.C., provided blood samples from gibbons.
6. Agglutination with each RA dilution was scored on a scale of 0-4. Complete neutralization by animal serum results in a score of 0 and classification as Gm+.
7. R. Grubb [*Ciba Symposium on Biochemistry of Human Genetics* (Boston, Mass., 1959), p. 264] has observed that the human Gm a substance migrates as a gamma₂ globulin. Grubb also reported that Gm grouping in kindreds with hypergammaglobulinemia gave no evidence of correlation between gamma globulin concentration and Gm (a+) character.
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Fossil and Living Conchostracan Distribution in Kansas-Oklahoma across a 200-Million-Year Time Gap

Abstract. Fifty-nine of 493 ponds sampled in the Wellington fossil conchostracan belt contained *Cyzicus mexicanus* (Claus). Persistent habitat preference and faunal association were also found for four orders of insects (Odonata, Ephemeroptera, Neuroptera, and Homoptera). Comparative limnology is detailed. Greater geographic fractionation of Permian conchostracan gene-pools is attributed to a more arid climate indicated by evaporites.

For the past 3 years, as part of a paleolimnological research project, one of us (P.T.) has been tracing the conchostracan-bearing beds of the Wellington formation (Permian, Leonardian) of Kansas and Oklahoma (1). It occurred to him that a survey of living clam shrimp distribution in the entire area of mapped Wellington conchostracan-bearing beds might provide useful in-

formation for comparison. Accordingly, Zimmerman was assigned to the project as limnologist-entomologist. Well over 550 ponds were sampled during the summers of 1958 to 1960. Collecting during 1958-59 was sporadic, and only during June, July, and August of 1960 was systematic and persistent daily sampling carried out. It is this last sampling of 493 ponds that is reported on here.

Of 493 ponds sampled in and near the Wellington outcrop belt [Fig. 1, Table 1, and (2)], 59 contained clam shrimps. An over-all percentage of 12.1 percent of all ponds sampled contained clam shrimps, the range being from 6.4 percent to 16.4 percent.

Weather records (3) show that, for the belt of investigation, average temperatures and rainfall were similar for the 3-year sampling period.

The conchostracan, *Cyzicus mexicanus* (Claus), was the only species present in all collections. It was found to be presently distributed, though more irregularly, in the same general area as the Wellington fossil-conchostracan beds. A persistence of habitat-preference from Paleozoic time to the present has thus been demonstrated. In fact, several ponds bearing clam shrimp were discovered within the outcrop belt of fossil occurrences (Marion County line, northern Kay County, northwest Noble County; see Fig. 1).

In addition, most other clam shrimp ponds were found to be adjacent or proximate, or both, to such outcrop belts. A few exceptions were noted. Most present-day clam shrimp ponds in southern Dickinson County, for example, occur to the north and east of the fossil belt in this region. The relatively rugged topography in the fossil belt compared to the low-lying terrain to the north and east may account for this distribution (4).

Modern ponds in the sampled area were found to possess very specific characteristics. Alongside of these, fossil occurrences in the Wellington will be cited.

Duration of modern ponds is relatively short. In fossil occurrences, conchostracans found on any given bedding plane are separated from younger and older occurrences by rock intervals ranging from millimeters to meters. Hence, we may conclude that Leonardian ponds, like modern ones, were temporary and sporadic in occurrence.

Currents are generally absent in modern ponds. This is also true of Leonardian ponds. However, there is some evidence of microcross-bedding due to currents in clam shrimp-bearing argillaceous limestone. Similarly, highly fossiliferous cross-bedded siltstones were found.