emphasize the sharks' dependence on visual clues for solving the second problem. For instance, to eliminate any target-specific cues, each target was painted with three black and three white stripes and served as the correct and incorrect target an equal number of times daily for each subject. Consequently, a target remained on the same side during alternate sessions, with correctness being determined by its orientation. Furthermore, when a subject was within one session of reaching criterion on either task, the experimenter randomly varied which target, positive or negative, was lowered first before each trial. Thus, any sound-related cues could not have served as a basis for learning. The possible use of such cues is also ruled out by the fact that the sharks' performances reverted to chance levels at the beginning of training on the second task. During the same precriterion session, both targets were rotated 180° from their standard training positions. A shark was therefore unable to reach criterion by using as a cue a specific stripe always located in the same position relative to the target.

Histological analysis revealed that the optic tectum was totally removed in all subjects except for a small lateral portion (approximately 5 percent) of the right tectum in subject 187. The lesions completely abolished both the upper layers of the tectum which receive the retinal input as well as the lower layers which receive the telencephalic input (*15*); however, there was only minimal damage to the underlying tegmentum. The thalamus remained undamaged except for portions of the pretectal area in subject 184. There was no evidence of any tectal regeneration (Fig. 1).

The present findings demonstrate that the optic tectum is not necessary for some types of visual discriminations. Consequently, we have begun to examine other portions of the shark brain, especially the posterior telencephalon. Bilateral lesions in this region produce severe visual dysfunction (*9, 16*). The observation of short-latency, visually evoked potentials in the same area (*17*) suggests that this deficit can be related to the recently reported similarities between sharks and mammals in some of their afferent and efferent telencephalic connections (*15*). Moreover, these facts suggest that portions of the nonlaminated posterior telencephalon of sharks are remarkably similar to the laminated visual cortex of mammals. Such a view, if correct, would necessitate a revision of our notions on the evolution of the brain, especially the view that the primitive telencephalon is dominated by olfaction (*18*).

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DNA Base Composition of Rickettsiae

Abstract. *There is a small but distinct difference in DNA base composition between the typhus and spotted fever groups of rickettsiae. The molar percentages of guanine plus cytosine for* Rickettsia prowazeki, R. typhi, *and* R. canada *are approximately 30, for* R. rickettsi, R. conori, *and* R. akari *they are about 32.5. The percentage for trench fever rickettsia,* Rochalimaea quintana, *is 38.6.*

Rickettsiae, as other obligate intracellular parasites, have relatively few phenotypic characteristics that can be used for the differentiation of species and strains without undue difficulty. These microorganisms are classified primarily on the basis of arthropod vectors, antigenic differences, and a relatively small number of biological activities (*1*). A need exists, therefore, to establish an objective index of genetic relatedness among rickettsiae. We are describing here an attempt to proceed toward this goal by examining the molar percentage of guanine plus cytosine (percent G + C) in the DNA of a number of strains of the typhus and spotted fever groups, and of a strain of the trench fever rickettsia. Scrub typhus rickettsiae were not included because these organisms are fragile and are separated from host components with considerable difficulty (*2*). Q fever rickettsiae have already been examined (*3–5*).

The species and strains of the organisms studied are listed in Table 1. The rickettsiae were grown in the yolk sac of embryonated chicken and, in some cases, duck eggs. Typhus group rickettsiae, including *Rickettsia canada*, were harvested from moribund embryos, while rickettsiae of the spotted fever group were harvested from embryos that had died 1 or 2 days previously (*6*). The rickettsiae were separated from host cell constituents as described (*7*), except that 0.5 percent trypsin (Difco) was used instead of Pronase. Rickettsiae were grown also in irradiated L cell monolayers and purified as described by Weiss *et al.* (*8*). *Rochali-*

maea quintana was grown in a bacteriological medium (*9*).

DNA was isolated and purified from *Escherichia coli* K12 (ATCC 10798), *Bacillus subtilis* W23S^r (*10*), and *Pasteurella novicida* U112 (*11*) by the procedure of Marmur (*12*). Rickettsial DNA was isolated by the following techniques. Method A: When large numbers of organisms were available, DNA was extracted by the Marmur method, or as modified by Kingsbury (*13*). From small amounts of purified rickettsial material, DNA was isolated and purified either by method B, preparative ultracentrifugation in CsCl (*14*), or by method C, passage of lysates over hydroxylapatite in the presence of 8*M* urea (*15*). Because of suspected nuclease activity of the host cell, some of the rickettsial preparations were heated in triple strength saline–EDTA (0.45*M* NaCl, 0.1*M* sodium ethylenediaminetetraacetate, *p*H 8.0) at 75°C for 20 minutes and subsequently diluted to 0.15*M* NaCl and 0.1*M* EDTA prior to lysis. Purity of the final DNA preparations was determined by ultraviolet absorbance profiles.

Buoyant density determinations in CsCl were done in the Beckman-Spinco (model E analytical) ultracentrifuge (44,000 rev/min, An-F rotor; 25°C for 20 to 24 hours) equipped with electronic speed control and scanner optics (ultraviolet light, 280 nm, passed through a 1.8-mm exit slit). Standard double sector aluminum-filled epoxy centerpieces and quartz windows were used. The volume of the samples was 0.4 ml. Usually, as the rotor reached speed, tracings were made to search for ultraviolet-absorbing artifacts or leaks. DNA of *E. coli* was used as the internal reference in every sample, and the unknown buoyant densities were calculated as described by Szybalski (*16*). Samples were analyzed in triplicate with at least two tracings of each cell, or were assayed in at least three separate runs with multiple tracings of each cell.

The thermal denaturation temperature, T_m, (*4*) of each DNA sample was determined in a Beckman Acta III spectrophotometer equipped with an automatic sampling system. Prior to use the temperature probe of the instrument was calibrated against a thermometer which had been calibrated by the U.S. Bureau of Standards. The T_m of each sample was determined three to six times, with duplicate *E. coli* DNA samples as an internal standard for each experiment.

In addition to *E. coli*, two bacterial DNA preparations were examined as controls: (i) *B. subtilis* yielded density and T_m values in excellent agreement with previous reports (*4, 5, 17*); (ii) *P. novicida*, as expected, yielded results identical to those previously obtained with *P. tularensis* (*4, 5, 18*) and provided an example of a DNA with the G + C content (32.5 percent) similar to that of some of the rickettsiae.

The spectral properties of the purified rickettsial DNA (columns 5 and 6, Table 1) are those of preparations relatively free from contaminating constituents. Possible exceptions, suggested by somewhat lower optical density ratios, were the values for the DNA obtained by method B from rickettsiae grown in L cells. There is no evidence, however, that these impurities or the choice of DNA isolation procedure affected the results. For example: (i) One of the two preparations of *R. quintana* was heated, yet both yielded identical results; (ii) the duplicate preparations of *R. typhi* and *R. akari*, in each case obtained from different host cells and isolation procedures, yielded results that were virtually the same. Two other parameters (not shown) were used to judge the purity and quality of the DNA: (i) In sedimentation experiments peaks other than those attributable to *E. coli* and rickettsial DNA were sought but none were found, suggesting that host cell DNA was reduced to a negligible level; (ii) in thermal denaturation experiments hyperchromicity ranged from 30 to 40 percent, which indicated that the DNA preparations had not undergone appreciable denaturation during isolation procedures.

Table 1 indicates that there is a small but highly significant difference in G + C content between the typhus and spotted fever groups of rickettsiae. The typhus group, represented by two strains of epidemic typhus rickettsia, *R. prowazeki*, and one strain of murine typhus rickettsia, *R. typhi*, have G + C contents indistinguishable from each other,

Table 1. Physical properties of rickettsial DNA. The molar percentages of G + C were derived from density measurements in CsCl by the formula of Schildkraut *et al.* (*5*) and from T_m in SSC (0.15*M* NaCl plus 0.015*M* sodium citrate, *p*H 7.0) by the formula of Marmur and Doty (*4*). Calculations were based on reference DNA of *E. coli* K12, assumed to have a density of 1.7100 g/cm^3 and T_m of 90.5°C. The values shown represent the means of three to six determinations ± standard deviations.

Micro-organism	Strain	Source of purified rickettsia	Method of DNA isolation	Spectral properties of purified DNA		Density (g/cm^3)	G + C (%)	T_m (°C)	G + C from T_m (%)
				260 nm/ 280 nm	260 nm/ 230 nm				
Bacillus subtilis	W23S^r		A	1.9	2.1	1.7032 ± 0.0007	44.1 ± 0.7	87.5 ± 0.1	44.4 ± 0.2
Pasteurella novicida	U112		A	1.8	2.4	1.6917 ± 0.0002	32.3 ± 0.2	82.7 ± 0.2	32.7 ± 0.4
Rickettsia prowazeki	Madrid E	Yolk sac	A	1.8	2.4	1.6897 ± 0.0004	30.3 ± 0.4	81.3 ± 0.4	29.3 ± 0.9
	Breinl	Yolk sac	A	1.8	2.4	1.6891 ± 0.0003	29.7 ± 0.3	81.2 ± 0.3	29.0 ± 0.8
R. typhi	Wilmington	L cells	B	1.5–1.7	1.7–2.1	1.6902 ± 0.0007	30.8 ± 0.7	81.4 ± 0.2	29.5 ± 0.5
	Wilmington	Yolk sac	C	1.8	2.5	1.6892 ± 0.0003	29.8 ± 0.3	81.2 ± 0.1	29.0 ± 0.3
R. canada	McKiel	Yolk sac	A	1.8	2.4	1.6897 ± 0.0001	30.3 ± 0.1	81.3 ± 0.1	29.3 ± 0.2
R. rickettsi	Bitterroot	Yolk sac	C	1.8	2.1	1.6918 ± 0.0001	32.4 ± 0.1	82.9 ± 0.2	33.2 ± 0.5
	R	Yolk sac	C	1.8	2.4	1.6922 ± 0.0007	32.9 ± 0.7	82.4 ± 0.2	32.0 ± 0.5
R. conori	Malish	Yolk sac	B	1.8	2.4	1.6926 ± 0.0003	33.3 ± 0.3	82.8 ± 0.4	32.9 ± 0.9
R. akari	MK	Yolk sac	A	1.8	1.9	1.6918 ± 0.0004	32.4 ± 0.4	82.9 ± 0.3	33.2 ± 0.8
	MK	L cells	B	1.3	1.5	1.6916 ± 0.0001	32.2 ± 0.1	Not done	
Rochalimaea quintana	Fuller		A	1.8	2.0	1.6977 ± 0.0008	38.5 ± 0.8	85.2 ± 0.2	38.8 ± 0.5
	Fuller		A	1.9	2.0	1.6978 ± 0.0006	38.6 ± 0.6	Not done	

ranging, by both procedures, from 29.0 to 30.8 percent. The slight discrepancy with the results of Wyatt and Cohen (*19*), who reported 17.1 percent G and 15.4 percent C in *R. prowazeki*, can be attributed to differences in methods. The spotted fever group, represented by two strains of *R. rickettsi* and one strain each of *R. conori* and *R. akari*, the etiological agents of Rocky Mountain spotted fever, boutonneuse fever, and rickettsialpox, respectively, also have G + C contents indistinguishable from each other with a range of 32.0 to 33.3 percent. The discrepancy between this percentage and that obtained by Price (*20*), who reported G or C contents ranging from 17.3 to 19.4 for *R. rickettsi*, cannot be explained. *R. canada* falls within the range of the typhus group rickettsiae. The trench fever rickettsia, *R. quintana*, has a considerably higher G + C content (38.6 percent).

The following conclusions can be cautiously drawn from the results described above. *R. quintana*, despite its similarity to *R. prowazeki* in ecological association, which involves man and the body louse, has only a remote evolutionary relationship to this organism. It is entirely justified to consider that the biologic characteristics of *R. quintana* which distinguish it from other rickettsiae, that is, extracellular growth in the gut of the louse and in bacteriological media (*21*), are reflections of profound genetic differences. On the other hand, we are justified also in continuing to regard epidemic and murine typhus rickettsiae as closely related organisms. The G + C content of approximately 30 percent for the typhus group and approximately 32.5 percent for the spotted fever group indicates an early evolutionary divergence of these organisms from their common ancestor. In contrast, the three species of spotted fever rickettsiae, despite their wide differences in geographic distribution and arthropod host (*1*), have remarkably similar G + C contents. The Q fever rickettsia, *Coxiella burneti*, not included in this study, has a G + C content of 43 to 45 percent (*3–5*) and it must be considered remotely related to the other rickettsiae.

Of particular interest to the understanding of the evolution of rickettsiae are our findings with *R. canada*. This organism was isolated from a tick in North America, but, surprisingly, several of its phenotypic characteristics link it more closely to the typhus than to the spotted fever group (*22*). Our findings suggest that *R. canada* has evolved from a strain of the typhus group and that it is unlikely that it resulted from a limited number of mutations of a strain of the spotted fever group (*23*).

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Applications of Artificial Intelligence: Relationships between Mass Spectra and Pharmacological Activity of Drugs

Abstract. *The possibility that the mass spectrum and pharmacological activity of a compound may be directly related has been explored with the help of various computer-based pattern-recognition techniques. The relationship appears to hold at least for tranquilizers and sedatives, and compounds with one or the other of these two pharmacological activities can thus be classified from their mass spectra with a high degree of accuracy.*

It has long been a basic assumption of organic mass spectrometry that the mode of fragmentation of an organic compound, which dictates all the details of its mass spectrum, is itself determined by the structure of the molecule (*1*). There is now much evidence in support of this view (*2*), which implies that the mass spectrum of a compound is in fact no more than a representation of its molecular structure, expressed in a complex and incompletely understood code.

Likewise, it has been known empirically for many years, and has always appeared to be entirely acceptable on an empirical basis, that the pharmacological activity of any molecule is dependent upon its structure, and that a change in the structure can lead to a change in the activity (*3*). A vast amount of effort has been expended upon studies of the interplay between these two properties of molecules (*4*).

In view of these two relationships, it appeared to us to be of some considerable interest to examine the one remaining possible correspondence, that be-