paste containing 0.1 percent kinetin were immediately applied to the tops of the decapitated internodes of ten plants by means of gelatin capsules (1 ml), and a mixture of kinetin and IAA each at 0.1 percent was similarly applied to a further ten plants. After 24 hours, 5-cm internode pieces from the stump were harvested from each plant, and were each divided into 1-cm pieces, the uppermost piece from each plant being discarded. The corresponding remaining pieces (that is, from the same part of the stem) from separate plants were pooled in groups of five and extracted with 80 percent methanol. Prior experiments had indicated that when the extracts were chromatographed in paper with a mixture of *n*-butanol, acetic acid, and water (4 : 1 : 1) as solvent, there were two main peaks of radioactivity, which occurred in the region $R_F$ 0.7 to 1.0 (Fig. 1) corresponding to the zone at which authentic kinetin runs in this solvent system. The extracts from the experimental plants were therefore chromatographed in this way and the zone $R_F$ 0.7 to 1.0 was then cut out and placed in tubes to each of which was added 5 ml of scintillation liquid EN 220 (dioxane based) and 0.5 ml of water at *p*H 5.0. After storage in the dark overnight to reduce chemiluminescence and to allow equilibration, the radioactivity was determined with a scintillation counter (Fig. 2).

The 1-cm sections of stem, immediately below the point of application, contained greater activity with kinetin than with kinetin plus IAA. Nevertheless, at a distance of 3 to 4 cm below the point of application there was much greater activity in the plants treated with kinetin and IAA than with kinetin alone.

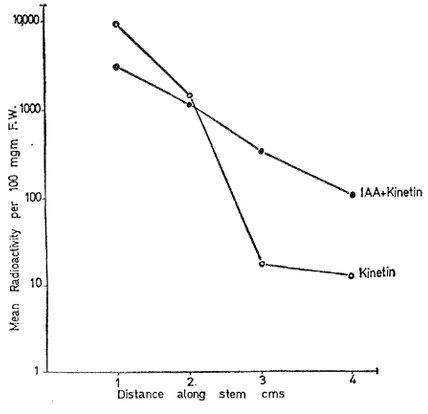


Fig. 2. Effect of IAA on movement of kinetin-$^{14}$C in internodes of decapitated bean plants. Kinetin-$^{14}$C at 0.1 percent in lanolin was applied at the stump of both series of plants and IAA (0.1 percent) was also applied to one series. Abscissa indicates distance from point of decapitation to the top of the stem section in question.

The reduced radioactivity in the top 1-cm sections of the plants to which IAA had been applied may have been due to partial inhibition of kinetin uptake by IAA, or to increased movement away from the site of application. Similar results have been obtained in several experiments in which significantly greater amounts of kinetin moved down the stems of plants to which IAA had been applied. Thus, although kinetin alone appears relatively immobile in the plant, its basipetal movement seems greatly increased in the presence of added IAA. In this respect, the properties of kinetin resemble those of benzyladenine, the basipetal movement of which is enhanced by IAA (*5*), although the benzyladenine is much more mobile in the plant than kinetin when applied alone.

A. K. Seth
C. R. Davies
P. F. Wareing

*Department of Botany,*
*University College of Wales,*
*Aberystwyth, Great Britain*

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6 December 1965

## Swimming Speed of a Pacific Bottlenose Porpoise

Abstract. *Four kinds of speed runs showed a Pacific bottlenose porpoise* (Tursiops gilli) *to have a top speed of 29.9 kilometers per hour (16.1 knots) for 7.5 seconds and a top speed of 21.9 kilometers per hour (11.8 knots) for 50 seconds. These results compare closely with highest predictions based upon rigid body drag calculations, the same power output per unit body weight as for athletes, and a propulsive efficiency of 85 percent.*

Many observations of the speed of porpoises have been made from shipboard (*1*), and analysis indicates unusually high power output or unusually low drag (*2*), or both. Some of these observations indicate various types of assisted locomotion (*3*), where the animal obtains thrust from ship waves. Such field observations are always difficult to interpret in terms of exact speeds, so tests with calibrated instrumentation under controlled conditions are needed.

In 1960, speed tests were conducted on a Pacific striped porpoise (*Lagenorhynchus obliquidens*) in a 97-m tank (*4*). Top speed was only 27.9 km/hr (15.0 knots), which indicates nothing unusual in performance.

On 24 March 1964 a Pacific bottlenose porpoise (*Tursiops gilli*) was captured in Hawaiian waters. This animal (Fig. 1), an approximately 3-year-old male named Keiki, was trained by conditioned-response techniques to swim at high speed in open water and to return upon command (*5*). He weighed 89 kg and was 191 cm long.

Four types of speed runs were conducted from August to December 1964, two in an enclosed lagoon racecourse and two with a speedboat in the ocean. The lagoon runs took place in 3 m of sea water in the 300- by 35-m lagoon at Coconut Island, in Kaneohe Bay, Oahu, Hawaii. The speedboat runs were conducted off Oahu, near Rabbit Island, and in Kaneohe Bay.

Lagoon runs were made by stationing Keiki inside a 9.3- by 9.3- by 3.1-m chain link pen which rested on the bottom of the lagoon. With one side of the pen open, Keiki was stationed under his trainer's hand at the back of the pen. When the hand was lifted, Keiki accelerated toward the entrance. As his snout passed over it, a timer was started and simultaneously an audible "start signal" was projected underwater to the animal. The porpoise raced the entire length of the 61-m course underwater, crossing a submerged finish line. If the run was of acceptable speed, as indicated on a timer at the finish line, a police whistle was blown and a reward of three fish was given. If the run was unusually fast, six fish were given simultaneously. An underwater recall signal returned the animal for another run. If the animal appeared tired, which would usually be indicated by an increased rate

of respiration, a rest period of 5 minutes then ensued. Incorrect behavior was punished by "limited hold or time-out periods" of varying length.

The maximum speed recorded for this test was 29.9 km/hr (16.1 knots) on two runs. Several other runs were in the range from 28.2 to 29.9 km/hr (15.2 to 16.1 knots). During such runs the animal swam partly on its side less than 1 m underwater, seemingly watching the nearby buoy line that marked the course. Though its tail never broke surface, each tail beat was evidenced by a discrete boil on the smooth surface. Spacing of tail beats varied from 0.85 to 1.15 body lengths at a beat rate of about 2.5 per second. On fast runs, swimming was continuous without glides and very straight from start to finish.

The next series of speed runs was conducted offshore of Oahu and consisted of Keiki pacing or overtaking a homing signal transmitted from a moving speedboat (*5*). The tests took place along a 320-m course marked by buoys spaced 15 m apart along an anchored steel cable. The same cage that was used in the lagoon tests was moored nearby and used to house Keiki. Data were recorded from the top of nearby Rabbit Island with a 16-mm Bell and Howell motion-picture camera. The camera was held stationary when Keiki entered one edge of the frame and moved quickly to the next position as he reached the opposite side of the frame.

Frame rate was recorded for each run. Walkie-talkie radios were used for communication between stations. The data were reduced, boat length being used as the length scale, frame rate as the time scale, and the numbered buoys as stationary markers. Keiki was difficult, and generally impossible, to see in the data films when he was underwater. Fortunately, he made several jumps in each test run, so most records were taken during these jumps. Average sustained speed was obtained from elapsed time between jumps. Two outboard boats were used, each capable of high speed. The measured speed of the animal was corrected (increased) for jump height to obtain true speed at water exit. The correction varied with speed and jump height, but was normally around 2.8 to 3.7 km/hr (1.5 to 2.0 knots).

Water currents along the course were measured with a Price current meter at low and high tide. Typically the current ran 0.5 knots from SSE at high tide, and the same from ESE or ENE at low tide, both tidal currents tending to quarter across the course. In view of the small magnitude and the direction, no corrections in ultimate speed were made.

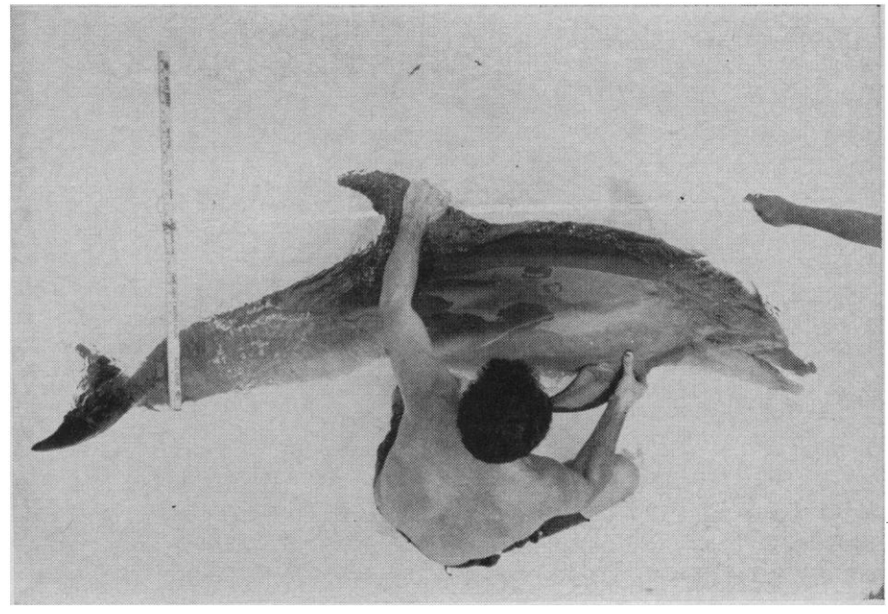

Fig. 1. Side view of Keiki. The measure at the top of the photograph is a yardstick.

Highest recorded speeds were 26.8 km/hr and 26.9 km/hr (14.4 and 14.5 knots), with several other values ranging from 23.8 km/hr to 24.9 km/hr (12.8 to 13.4 knots). Maximum sustained speed between jumps was 19.0 km/hr (10.2 knots) for 6.6 seconds. During all runs Keiki spent some or most of his time swimming in the bow wave, or aft in one of the spilling waves of the wake, doubtless obtaining some thrust from the pressure field of the boat or from the wake.

In view of the many subjective impressions of porpoise swimming speed that have been recorded, it is worthwhile to note that observers of these open-sea runs, including participants, guessed Keiki's speed to be more than 20 knots. The rough sea and the buffeting of the crew as the boat crashed from crest to crest added to the impression of speed. The porpoise was often outpaced by the boat, so recorded speeds may be near maximum for these conditions.

The third test series was run in Coconut Island Lagoon. A homing signal was transmitted from an underwater speaker about 89 m downcourse from Keiki's pen, and a stopwatch was started when he reached a point 4.0 m downcourse. Keiki received a reinforcement that varied from one fish for slow runs to six fish for the fastest runs. The top speed recorded for the 61-m racecourse was 23.2 km/hr (12.5 knots). Moving pictures taken at the finish line from a 3.7-m tower indicated a slowing of about 3.7 km/hr (2 knots) near the end of the run. Further training might have achieved better overall times, but it was decided, instead, to try a fourth and more promising technique.

For the last series of tests, a speedboat was equipped with a calibrated speedometer, and Keiki simply swam with the boat at various preselected speeds until he fell behind. Visual observations of Keiki's position and of boat speed were recorded on a portable tape recorder. The tape speed was accurate within 1 percent and the boat speedometer was calibrated by running each way at various speeds over a measured course. Keiki could remain with the boat for extended periods at a speed of at least 11.1 km/hr (6 knots), for 50 seconds at 21.9 km/hr (11.8 knots), and for 10 seconds at 25.2 km/hr (13.6 knots). In general, he swam under the bow of the boat or in the first stern wave. Obviously he obtained some assistance from the boat until he could no longer keep up. Although there is no way we could be sure that he did or did not exert maximum effort, he appeared to be strongly motivated to stay with the boat, much like a dog chasing an automobile. Keiki blew much more frequently after the faster runs, so he was given a rest period between runs until respiration rate appeared normal.

A detailed analysis of Keiki's body

and appendage drag was conducted with methods applicable to conventional rigid bodies (*6*) in which the boundary layer is predominantly turbulent and the surfaces are smooth. Numerous measurements of Keiki were taken and photographs obtained to aid in the hydrodynamic analysis. Corrections were included for interference drag at the intersection of the body and the appendages. The calculated drag-area coefficient (drag/dynamic pressures) was 0.0644 at 29.9 km/hr (16.1 knots) where the length Reynolds number is $14.2 \times 10^6$. If his power output per unit body weight was the same as that of athletes (*7*) and his propulsive efficiency 85 percent, Keiki could travel 26.8 km/hr (14.4 knots) for 7.5 seconds, 25.8 km/hr (13.9 knots) for 10 seconds, 21.4 km/hr (11.5 knots) for 50 seconds, and 12.8 km/hr (6.9 knots) for a 24-hour day. The experimental results showed a top speed of 29.9 km/hr (16.1 knots) for 7.5 seconds, 25.2 km/hr (13.6 knots) for 10 seconds, 21.9 km/hr (11.8 knots) for 50 seconds, and at least 11.1 km/hr (6 knots) for an indefinite period. Consequently the experimental results compare closely with predicted turbulent values for this animal.

THOMAS G. LANG
KENNETH S. NORRIS
*U.S. Naval Ordnance Test Station, Pasadena, California,* and *University of California, Los Angeles,* and *Oceanic Institute Makapuu Point, Oahu, Hawaii*

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## Sialic Acid Binding Sites: Role in Hemagglutination by Mycoplasma gallisepticum

Abstract. *Hemagglutination of turkey erythrocytes by* Mycoplasma gallisepticum *was inhibited by mucoproteins containing sialic acid, by sialic acid itself, and by treatment of the erythrocytes with neuraminidase. Neuraminidase treatment of the mucoprotein-rich inhibitors reduced or abolished their inhibitory activity. The findings indicate that sialic acid on the erythrocyte surface provides binding sites for* Mycoplasma gallisepticum.

Hemagglutination of turkey red cells by *Mycoplasma gallisepticum* has been described (*1*), and we now report a study of the binding site on erythrocytes for this organism in the hemagglutination reaction.

Fresh cultures of *M. gallisepticum* were grown each day, in PPLO (pleuropneumonia-like organism) broth containing horse serum (20 percent) (*2*). The organisms were harvested by centrifugation at 4°C at 13,800*g*. They were then washed three times in Ringer solution and resuspended to a concentration 100 times that of the original culture. This procedure usually provided $10^{10}$ to $10^{11}$ organisms per milliliter. The organisms were kept at 0°C, and a fresh preparation of mycoplasma was used each day. Turkey blood was drawn from the wing vein to a syringe containing heparin; the erythrocytes were separated and washed three times in Ringer solution and suspended to make a 0.5 percent solution (by volume). In each experiment, the suspension of *M. gallisepticum*, Ringer solution, and the red cells (0.5 percent suspension) were mixed (1 : 10 : 10 by volume). Substances to be tested for inhibitory effects were incorporated in the Ringer solution and incubated with the suspensions of mycoplasmas for 10 minutes at 0°C before the erythrocyte suspension was added. Incubation of the reaction mixture was then continued at 0°C, and sample drops were withdrawn with a Pasteur pipette, placed on a slide, and observed immediately at × 100. The degree of agglutination was graded on a 0 to 4+ scale in which each 1+ represented agglutination of approximately 25 percent of the cells. Readings were made 30 minutes after the erythrocytes were added to the reaction mixtures, and at that time most preparations of mycoplasma gave a 3 to 4+ agglutination. Giemsa stains were made by diluting the reaction mixture tenfold with Ringer solution and then allowing a drop of the diluted suspension to dry on a slide. Such preparations showed many mycoplasmas adhering to erythrocytes, with a lattice of clustered mycoplasmas between clumps of red cells. Heat-killed *M. gallisepticum* did not agglutinate red cells.

Addition of serum to the reaction mixture resulted in inhibition of hemagglutination. This effect was observed with all serums tested, excluding those which themselves agglutinated turkey erythrocytes (*2a*). Thus, human, bovine, fetal bovine, rabbit, and turkey serums inhibited hemagglutination by mycoplasma. When each of these serums constituted 5 percent by volume of the reaction mixture, inhibition was complete. Each serum caused at least partial inhibition in a concentration of 1 percent. Turkey serum was slightly less effective than all others tested. Heating to 56°C for 30 minutes, 65°C for 10 minutes, or 100°C for 5 minutes did not diminish the effectiveness of the serums. Giemsa staining of reaction mixtures inhibited with serum or egg white (see below) showed a striking diminution in the number of mycoplasmas adherent to erythrocytes.

The observations that all of the serums inhibited hemagglutination in low concentrations and that heat did not diminish their effectiveness suggested that the inhibitory activity might be due to the carbohydrate moieties of their glycoprotein or mucoprotein content. When mucoprotein-rich substances such as egg white, or gastric mucin, or ovomucoid were incorporated into the reaction mixture, hemagglutination was completely inhibited. Egg white prevented hemagglutination in a concentration as low as 10 μg/ml, gastric mucin at 500 μg/ml, and ovomucoid at 1.5 mg/ml. Heating these substances at 65°C for 10 minutes or 100°C for 5 minutes did not diminish effectiveness.

Because of the high sialic acid content of the mucoprotein-rich inhibitors and the possibility that this sugar derivative determined inhibitory activity, the effect of neuraminidase was studied.