blood flow; (ii) a transient increase in duodenal motility at the injection site, followed by inhibition for 30 to 90 minutes of spontaneous motor activity in the antrum and throughout the duodenum; (iii) a significant sedating effect characterized by curling up and drowsiness (EEG spindling) if the animal was active, or a transition to persistent slow-wave, high-voltage sleep if the cat was already drowsy; within the hour after intraduodenal injection of milk, the frequency or duration of rapid eye movement (REM) episodes was markedly augmented if the animal was already asleep. Injection of the same amount of milk directly into the stomach had no detectable effect.

Of the components of milk, apparently only fat was able to evoke the response. Equal volumes of water or saline, or solutions of glucose, lactose, or casein had no effect, but 0.5 to 2.0 ml of corn oil elicited the typical response. The duration of the changes (approximately 30 to 90 minutes) was proportional to the amount of oil injected; the latency was shortened by administration of 0.3 to 0.5 ml of cat bile or detergent (approximately 0.5 mg Alconox) with the fat. The response was elicitable from any segment of the duodenum, but most readily from the second portion (Fig. 1).

Intravenous injection of atropine sulfate, at a dose (0.03 mg/kg) that does not seem to modify the EEG pattern of the awake cat, blocked completely the appearance of mesenteric vasodilatation after intraduodenal introduction of fat. Also, the spontaneous intraluminal pressure waves in the duodenum, which persisted after this low dose of atropine, were not inhibited after introduction of the oil. However, a sedating effect apparently remained. Mesenteric vasodilatation after instillation of oil into the duodenum could be elicited in animals under light chloralose anesthesia, but not under pentobarbital. In some cases, duodenal motility was apparently augmented by fat in the anesthetized animal (Fig. 2).

Because fat in the duodenum releases cholecystokinin-pancreozymin (CCK-PZ) (*2*), we tested the effect of intravenous infusion (4 unit kg^{-1} $hour^{-1}$) of a partially purified preparation of this hormone (*3*). This induced an increase in mesenteric blood flow and a variable increase in duodenal motility in anesthetized and awake cats (see Fig. 2). In two trials on an initially awake but drowsy cat, high-voltage sleep ensued during the CCK-PZ infusion.

The extent to which the increase in mesenteric blood flow is in pancreas or intestine remains to be determined; vasodilatation in the pancreas might accompany the increased metabolic activity in that organ induced by CCK-PZ. In any case, the facts that CCK-PZ induces mesenteric vasodilatation, and that atropine, which interferes with release of endogenous CCK-PZ (*4*), prevents the fat-induced vasodilatation, point to a role of CCK-PZ in the response.

The inhibition of duodenal motility by fat, comparable to that described in the dog (*5*), cannot be ascribed to CCK-PZ, because administration of that substance tended to increase motility, in consonance with the observations of Hedner *et al.* (*6*). It is unknown whether the mechanism is humoral, nervous (involving intrinsic or extrinsic innervation of the gut), or a combination of these.

The sedation induced by intraduodenal fat may be mediated by either (i) a direct central action of a released gastrointestinal hormone, perhaps comparable to the effect of gonadotrophins in promoting the appearance of high-voltage and REM sleep in the rabbit (*7*); or (ii) an indirect neurogenic mechanism through the stimulation of duodenal receptors by the released gastrointestinal hormone or by the food (fat), as described for glucose and amino acids (*8*). The observations suggest that the sedating effect of intraduodenal fat may be important in the correlation between the feeding and the sleep-waking cycles, and possibly also in short-term satiation.

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10. After this report was submitted, we saw the article by G. P. Burns and W. G. Shenk [*Arch. Surg.* **98**, 790 (1969)] who observed increased mesenteric blood flow in dogs after a meal or after intravenous injection of crude preparations of gastrin or secretin, the latter containing CCK-PZ.

26 June 1969; revised 4 August 1969

Surface Areas of the Cerebral Cortex of Mammals Determined by Stereological Methods

Abstract. *The surface areas of the cerebral cortex excluding archipallium of 20 human, 11 cetacean, 6 carnivore, and 5 marsupial brains were determined by stereological methods. There exist rather strict relationships between volume, length of superficially exposed gyri, and cortical surface area.*

The volumes of brains of 42 mammals pertaining to widely separated taxonomical groups, fixed in 20 percent formalin, were determined by suspension in water. By a median cut, the hemispheres were separated. One hemisphere of each brain was sliced by frontal sections; the other by horizontal sections of thickness *t*. This thickness was calculated by dividing the measured height or length of each hemisphere by the number of slices. The terms "horizontal" and "frontal" are defined as respectively parallel or per-

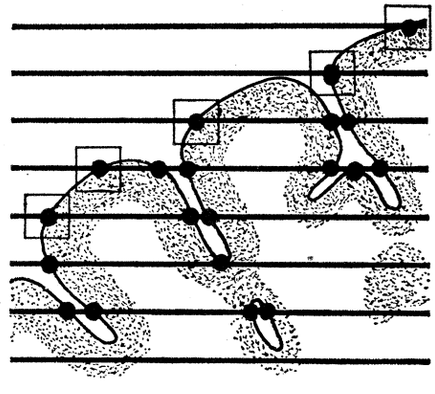

Fig. 1. Method of intersection counts for cerebro-cortical surface determination. The index of folding equals the number of all black dots divided by the number of squares.

Table 1. Brains of mammals (species averages).

Mammal	Body length (cm)	Brain				
		Volume of brain (cm^3)	Total surface of cerebral cortex (cm^2)	Outer surface of cerebrum (cm^2)	Index of folding	Length of exposed gyri (cm)
Marsupialia						
Mouse opossum	10	1.24	4.38	4.24	1.01	5.24
Opossum	44	4.25	13.2	11.8	1.11	10.2
Wallaby	45	25.0	43.3	35.2	1.23	46.0
Kangaroo	47	39.2	74.9	53.0	1.41	75
Carnivora						
Raccoon	79	42.6	122	66	1.85	86
Fox	87	45.3	135	68	2.01	79
Coyote	101	85.3	227	79	1.80	119
Primates						
Man	169	1198	2275	795	2.86	643
Cetacea						
Baird's dolphin	215	722	1717	430	4.0	581
Bottlenose dolphins						
Atlantic	206	1145	2700	567	4.47	935
Pacific	220	1498	3343	693	4.75	898
Risso's dolphin	250	1500	3522	830	4.25	1045
Pilot whales						
Pacific	490	2580	5335	1060	5.03	1260
Atlantic	600	2842	6294	1159	5.55	1468
False killer whale	550	3650	7392	1488	4.97	1544

pendicular to the body of the corpus callosum. In marsupials whose corpus callosum is cylindrical, "horizontal" means parallel to the ground; "frontal" means perpendicular to the long axis of the brain.

On each slice a Lucite plate was laid with parallel equidistant lines engraved into it. The distance between these test lines is designated by the letter h. Points of intersection of these test lines with the pia-cortex boundary were counted as shown in Fig. 1 (large round dots). The number is designated by the letter P. Hennig's (*1*) formula

$$S = 2\ P \cdot h \cdot t$$

gives the absolute surface area. The counts must be made on *all* the slices of each series. In order to compensate for preferred orientation of gyri, the lines must pierce the specimen in three directions perpendicular to each other. On frontal sections, vertical as well as transverse lines can be used. On horizontal slices longitudinal and transverse are the possible directions. The surface of one hemisphere was computed from each of these directional counts. Since transverse lines are used in both hemispheres, the hemispherical surfaces computed from each of the transverse intersection counts were averaged. Finally, the entire cortical surface area was obtained as twice the arithmetic mean of the values for one hemisphere computed from vertical, longitudinal, and transverse intersection counts. Some of these data have been published (*2, 3*); but only surface areas were then considered. Other parameters to be given below had not been considered and correlated with S.

The ratio between the entire cortical surface area and the external surface area (squares in Fig. 1), including places in contact with falx and tentorium, designated by von Bonin (*4*) as the index of folding, was also determined.

The length (L) of superficially exposed gyri, that is, those in contact neither with the falx nor with the tentorium, was determined with a curvimeter before cutting, and by counting external convexities after cutting, by using the formula

$$L = 2\ P \cdot t$$

The cerebro-cortical surface areas of the human brains ranged from 1715 to 3031 cm^2. The average surface area in the sample was 2275 cm^2; it was 2297 cm^2 for 9 males and 2257 cm^2 for 11 females, a negligible difference. The length of exposed gyri in man ranged between 548 and 787 cm, with an average of 643 cm.

The index of folding in these 20 human brains ranged from 2.25 to 3.07, with an average of 2.86.

Considering the members of the order Mammalia we investigated, we find a rather strict relationship between the volume of the whole brain and the cerebro-cortical surface area:

$$\log S \cong 0.93 \log V + 0.5$$

where S is the surface area and V the total volume of the brain. Equally striking is the relationship between the length L of superficially exposed gyri and cerebro-cortical surface area:

$$\log S \cong 1.66 \log L - 0.5$$

These two relationships may permit paleontologists to estimate the cerebro-

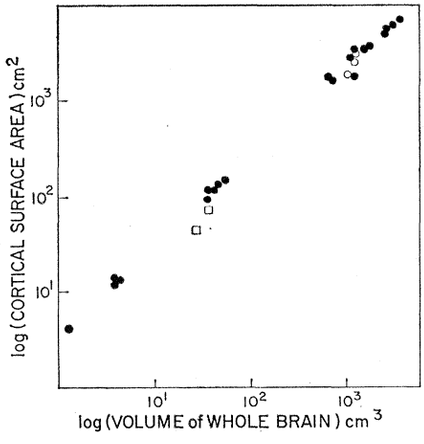

Fig. 2. Relation between volume and cerebro-cortical surface area of mammalian brains. The points plotted represent one to three specimens of each mammal listed in Table 1, except the wallaby and coyote, which are not included in the graph. Upper group of points: whales and dolphins (solid circles) and man (open circles); middle group: carnivores (solid circles) and kangaroos (squares); lower group: opossums; lowest point: mouse opossum.

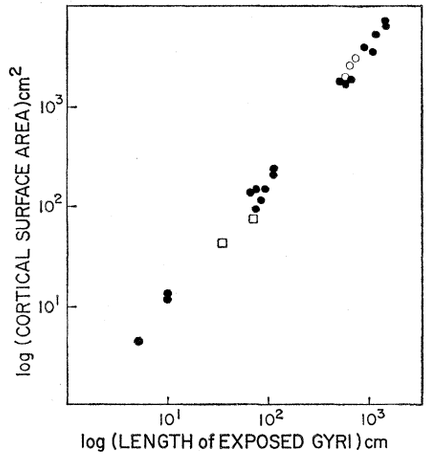

Fig. 3. Relation between length of exposed gyri and cerebro-cortical surface areas of mammalian brains. The points plotted represent one to three specimens of each mammal listed in Table 1, except Risso's dolphin, which is not included. The groups are as in Fig. 2, except that here the squares are one kangaroo and one wallaby.

cortical surface of fossil mammals from endocranial casts.

The results of the entire study are summarized in Figs. 2 and 3 and in Table 1 (*5*). The wallaby and kangaroo deviate most from the straight (undrawn) trend lines in Figs. 2 and 3. A tentative explanation may be found in the fact that these two specimens had been born and raised in captivity.

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5. A more extensive paper on this subject, considering all its aspects in greater detail, with a statistical analysis of the data, will be submitted for publication elsewhere.
6. Supported by PHS grant NB 7104.

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Energy Flux and Membrane Synthesis in Photosynthetic Bacteria

Abstract. *Synthesis of the energy-converting membrane complex of the photosynthetic purple bacterium* Rhodopseudomonas capsulata *during growth under different conditions of energy flux was studied by examining the disorganizing effects of polymyxin B, with or without lysozyme, on integrity of the cell envelope. Cells growing with a limited supply of energy show an elevated bacteriochlorophyll content and increased resistance to breakdown of the "permeability barrier" by these agents. It seems that purple bacteria respond to energy restriction by preferentially synthesizing excess bacteriochlorophyll-membrane which, in effect, toughens the cell integument.*

The purple photosynthetic bacteria may prove to be particularly useful for analysis of mechanisms involved in regulation of energy-converting membrane synthesis. In these organisms the photochemical energy-conversion (photophosphorylation) system is localized in the plasma membrane and its extensions into the cytoplasm (*1, 2*). Bacteriochlorophyll (BChl) is essential for photophosphorylation, and the BChl contents of cells grown under different conditions provides an index of membrane synthesis and, probably, of differentiation. Limitation of the energy supply during anaerobic photosynthetic growth influences formation of BChl and the energy-converting membranes, and this can be achieved without changing the chemical composition of the growth medium by simply altering the light intensity or by using intermittent illumination. When cells growing in continuous bright light are placed in dim light, or intermittent bright light, the growth rate decreases markedly (*3–5*). Such slowly growing cells have an elevated content of BChl [relative to protein content or dry mass (*3, 5*)], and electron micrographs of thin sections indicate a substantially increased quantity of intracytoplasmic membrane (*6, 7*). In other words, it seems that cells of purple bacteria respond to decrease in the average energy flux by producing more light-harvesting membrane or energy-converting membrane, or both. It has been reported (*7, 8*) that the "additional" membrane has the same BChl content as the "original" membrane, but there is contrary evidence (*2, 9*). The ambiguity on this important point may have resulted, in part, from inadequacy of fractionation procedures, and it is also possible that not all the species of photosynthetic bacteria examined show the same pattern (*2*). Phospholipid is generally considered to be uniquely associated with membranes [but see (*10*)], and it is frequently assumed that membrane composition remains constant in this respect. In *Rhodopseudomonas palustris,* phospholipid and BChl contents may indeed change in constant proportion as light intensity is varied (*2*), but this seems not to be true for other photosynthetic bacteria [(*2, 11, 12*); see also relevant observations in (*10*)]. The difficulties in reconciling available analytical chemical data with ultrastructural observations led us at one point to question the physical reality of additional membrane seen by electron microscopy in cells growing slowly because of a restriction on the supply of energy. In this report, independent evidence is presented for the existence of such membrane.

The approach was based on the use of agents that specifically disorganize constituents of the membrane and cell envelope. We reasoned initially that cells with extensive membranous invaginations should show slower leakage of soluble intracellular enzymes, such as inorganic pyrophosphatase, when attacked with a given limited quantity of polymyxin B. This cyclic polypeptide antibiotic reacts with lipid components of bacterial membrane structures (*13*), produces bleblike extrusions on the surface of certain Gram-negative bacteria (*14*), and can induce gross changes in morphology of photosynthetic bacteria (*1, 15*). Addition of polymyxin B to suspensions of *Rhodopseudomonas capsulata* cells leads to appearance of pyrophosphatase activity as illustrated in

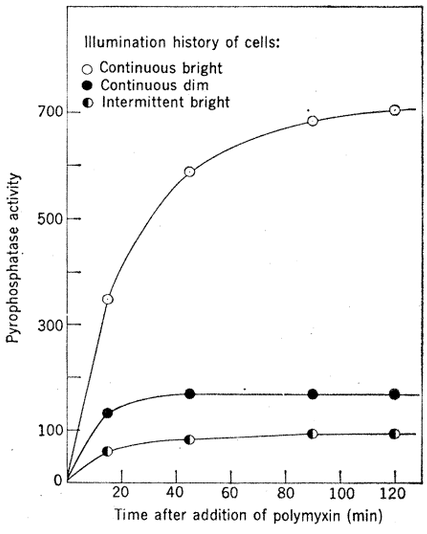

Fig. 1. Evocation of inorganic pyrophosphatase activity in *Rhodopseudomonas capsulata* cell suspensions by polymyxin B. *Rhodopseudomonas capsulata* (American Type Culture Collection No. 23782) was grown with Lumiline lamps as the light source, as described earlier (*5*). Continuous bright light, intensity of 6000 lu/m^2; continuous dim, 440 lu/m^2; intermittent bright, 6000 lu/m^2, on and off at 30-second intervals. In each instance, cells from 10 ml of culture were harvested when the density was approximately 390 μg of dry weight per milliliter (during logarithmic growth). The bacteria were washed with and resuspended in 5 ml of 0.05*M* tris(hydroxymethyl)aminomethane-HCl buffer, *p*H 9.0. At zero time, 5 ml of polymyxin B sulfate (Mann Research Laboratories, Inc., New York; 125 μg/ml in the buffer noted) were added from a syringe, with rapid mixing. Suspensions were incubated at 30°C and 0.1 ml samples removed at intervals (with an automatic pipette) for enzyme activity assay. Each sample was placed in 0.9 ml of solution containing: $Na_4P_2O_7$, 6 μmole; $MgCl_2$, 6 μmole; and tris-HCl buffer (*p*H 9) 22.5 μmole. Following incubation at 30°C for 5 minutes, 1 ml of cold 10 percent trichloroacetic acid was added and inorganic phosphate determined in a suitable portion by a modification of the Fiske-SubbaRow method (*19*). Pyrophosphatase activity is expressed as micrograms of phosphorus liberated (as P_i) per hour per 0.1 ml of suspension.