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Bachra and Trautz state: "On theoretical grounds it is unlikely that the catalytic action of the enzyme carbonic anhydrase would be required for the precipitation of apatite in vitro." Our report (*1*) states quite clearly (in connection with the experiments in which we used synthetic calcifying solutions): "First it was discovered that when the solutions were saturated with carbon dioxide by bubbling the gas through them, the *enzyme was not required* in order for a deposit to form" (italics added).

They say, "It is unlikely that carbonate (or bicarbonate) ions are essential for the precipitation of apatite in vitro or in vivo." Inasmuch as the carbonate hydroxyapatite (bone mineral) which forms in vivo *universally* contains (*2*) a small percentage of carbon dioxide (as carbonate), it makes no sense whatever to assume that it can form in the absence of carbonate (or bicarbonate) ions. This statement is true regardless of whether or not a precursor of bone mineral is involved.

Since our original results appeared (*1*), we have reduced the period of time required to obtain the precipitations from several days to several hours. The fact that Bachra and Trautz obtain different results does not appear to be justification for their publicly questioning our results, particularly in view of the fact that they did not investigate the same systems. Our systems were open either to laboratory air or to carbon dioxide at atmospheric pressure (as clearly indicated), whereas theirs were in erlenmeyer flasks which were "closed with paraffinized cork stoppers."

If, as they imply, our depositions were caused by bacterial or fungal growth, we found no visible evidence of it. Application of their speculation to our later experiments (those requiring merely 6 to 8 hours) would certainly

require modification of existing theories concerning the proliferation of bacteria (or fungi) in environments unfavorable to their growth. Under any circumstances the production of carbonic anhydrase by microorganisms cannot be eliminated as a principal cause of the precipitations which Bachra and Trautz obtained.

Furthermore, Bachra and Trautz choose to ignore the fact that sulfanilamide was found to function as a specific inhibitor of carbonic anhydrase in our experiments, both in those involving saliva and in those involving synthetic calcifying solutions. The presence of carbonic anhydrase in bovine submaxillary gland extract has recently been demonstrated (*3*). Earlier experiments (*4*) had demonstrated that this extract was capable of initiating deposition of synthetic calculus.

To be sure, the hydration of carbon dioxide by carbonic anhydrase is virtually instantaneous, but this fact is completely irrelevant to our conclusion that carbonate (or bicarbonate) ions must be present during the formation of bone mineral. It is evident that carbonate ions must be available during the mineralization process because a carbonate-containing substance cannot be formed in the absence of carbonate ions. As we previously clearly stated: "However, when these synthetic solutions were not initially saturated with carbon dioxide and when the apparatus was enclosed in an atmosphere of carbon dioxide, the results . . . were essentially similar to those obtained with boiled saliva: that is, no significant deposit was obtained unless carbonic anhydrase was added, and addition of sulfanilamide prevented formation of a deposit."

We note that the report of Bachra and Trautz contains one principal point: they were unable to duplicate our results, and they state that our experimental conditions "were not specified in sufficient detail." There is indeed a limitation on the length of reports in *Science*, but it might have been helpful had Bachra and Trautz read with comprehension some of the statements that we did make. Thus, they could have avoided several illogical interpretations, and they surely could have obtained further information (without resorting to publication of their dubious speculations) if, indeed, they were not present when additional details were supplied at a meeting of the International Asso-

ciation for Dental Research, held from 23 to 26 March 1961 in Boston (*5*).

In summary, it should be quite apparent that their experiments do not present any evidence which vitiates either our experimental results or the conclusions which we obtained.

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Protective Effects of Human Milk in Experimental Staphylococcus Infection

Abstract. Mice were given human milk and sublethal doses of virulent *Staphylococcus aureus* subcutaneously on 7 to 14 consecutive days. When mice of this group and mice of control groups were later challenged through intraperitoneal injection of a lethal dose of the same *Staphylococcus aureus*, the death rate for the experimental group was found to be much lower than the death rate for the controls.

During the last few decades, the progressive decline in breast-feeding in technically highly developed countries has been stimulated by cultural-anthropological factors and abetted by the apparent success of "artificial" feeding of infants. The greatest benefit in artificial feeding has been achieved by following proper aseptic rules. In technically underdeveloped countries, poor hygienic conditions make breast-feeding practically essential if young infants are to survive.

The additional claim that breast-fed infants have increased resistance to disease has been often advanced, even in the recent past (*1*), but this claim has not been supported with unequivocal scientific evidence. Clinical observations on the beneficial effect of human milk in the treatment of chronic staphylococcal infection in the preantibiotic era (*2*) belong possibly in the same category.

In the studies reported here, the synergistic action of human milk and

sublethal doses of a virulent strain of *Staphylococcus aureus* on the resistance of mice to a lethal dose of *S. aureus* of the same strain have been tested. Human milk and, as controls, cow's milk and saline were given subcutaneously, not by the oral route.

Swiss albino littermates (from Huntingdon Farms, Inc.), predominantly male, 5 to 6 weeks old, and weighing 18 to 25 g, were used. The animals were given free access to Purina chow and water.

Fresh pooled human milk, obtained from the Philadelphia and Delaware Milk Banks, was used. The cow's milk was pasteurized, pooled, homogenized dairy milk. All samples of human and cow's milk were autoclaved (at 230°F, 7 lb pressure) for 10 minutes and then stored in the frozen state until used.

The strain of *Staphylococcus aureus* used was phage type 44A. The inoculum was prepared according to the method of Higginbotham and Dougherty (*3*), except that the cells were harvested from the log phase of multiplication of the culture at turbidity 60 as measured in the Klett-Summerson photoelectric colorimeter with a No. 66 red filter (*4*). The LD_{100} inoculum—1.0 to 1.3×10^8 cells suspended in 0.25 ml of 0.85-percent saline solution—was injected intraperitoneally.

The mice, in groups of ten, were given consecutive daily subcutaneous injections of material, at either the same or different sites for periods of 3, 5, 7, and 14 days, respectively.

Three groups of mice were injected daily with either 0.3 ml of human milk, 0.3 ml of cow's milk, or 0.3 ml of 0.85-percent saline. Three other groups were injected, respectively, with these materials plus a 1/100 dilution (in saline, 85 percent) of the LD_{100} of the virulent strain of *S. aureus*. Two additional groups of mice were injected, respectively, with human milk or cow's milk and, at a different site, with 0.01 LD_{100} of *S. aureus* in 0.1 ml of 0.85-percent saline. In some experiments 0.001 LD_{100} was also used with and without human milk and cow's milk.

All the mice were given an intraperitoneal injection of *S. aureus*, LD_{100}, at either 1, 7, or 14 days after completion of the subcutaneous injection schedules.

Twenty experiments were carried out over a period of more than 1 year. No protection was observed when the preparatory period did not exceed 5 days. In contrast, groups of mice pretreated

Table 1. Protection of mice, by preliminary injection of human milk or cow's milk and a sublethal dose (0.01 or 0.001 LD_{100}) of staphylococcus suspension, against a lethal dose of the same staphylococcus given intraperitoneally 1, 7, or 14 days after the conclusion of the pretreatment schedule.

Pretreatment	Deaths after injection of LD_{100}	
	No.	Percentage
Normal saline	104/114	91
Human milk, 0.3 ml	91/109	83
Cow's milk, 0.3 ml	79/109	72
Staphylococcus, 0.01 LD_{100}	78/110	71
Staphylococcus, 0.001 LD_{100}	49/61	80
Human milk and 0.01 LD_{100}, same site	13/108	12
Human milk and 0.01 LD_{100}, different sites	32/93	34
Human milk and 0.001 LD_{100}, same site	26/54	48
Human milk and 0.001 LD_{100}, different sites	26/32	81
Cow's milk and 0.01 LD_{100}, same site	55/112	49
Cow's milk and 0.01 LD_{100}, different sites	62/94	66
Cow's milk and 0.001 LD_{100}, same site	21/39	54
Cow's milk and 0.001 LD_{100}, different sites	28/32	87

with injections, at the same site, of human milk and 0.01 LD_{100} or even 0.001 LD_{100} for 7 or 14 days survived a challenge with an LD_{100} of virulent staphylococci, whether the lethal dose was administered 1, 7, or 14 days after completion of the pretreatment. Protection was also observed when human milk and staphylococci were injected in the preparatory period at different sites, but this was true only with 0.01 LD_{100}, not with 0.001 LD_{100}. Injection of cow's milk and 0.01 LD_{100} of staphylococci in the preparatory period was much less effective.

Table 1 gives the percentage of survival for the various groups, summarizing all experiments in which the challenging dose was given after a preparatory period of 5 days or more. Figure 1 portrays graphically the pro-

tective effect of pretreatment with human milk and 0.01 LD_{100} of staphylococci in three specific experiments.

The results clearly indicate that human milk given subcutaneously in combination with sublethal doses of virulent staphylococci is effective in giving mice significant protection against a challenge with a lethal dose of the same staphylococci. No protection was observed within the first days of the experiment, and none was observed when human milk was given alone. The protection lasted over 14 days after termination of the initial injections, and the pretreatment was also effective when the human milk and the sublethal dose (0.01 LD_{100}) of staphylococci were injected at different sites. These findings seem to indicate that the effect of human milk is not that of an adjuvant
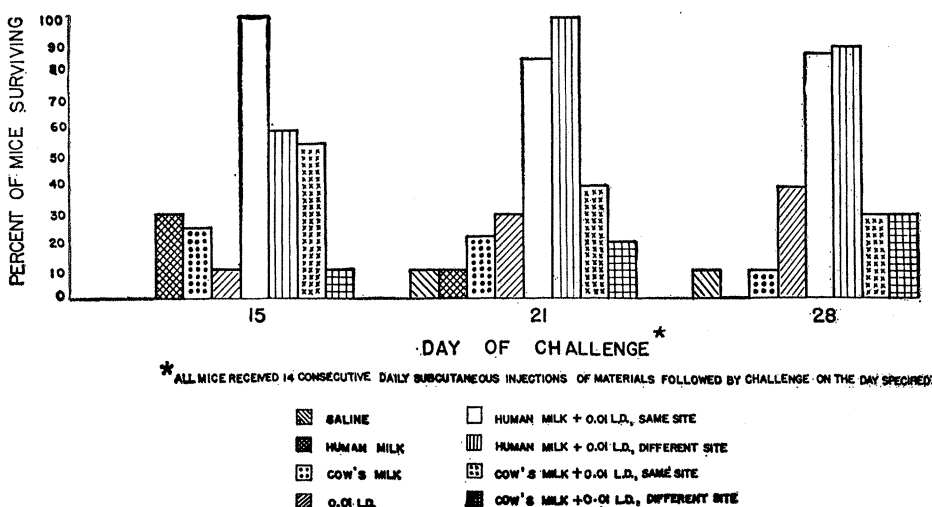

Fig. 1. Effect of prior injection, for 14 consecutive days, of milk and staphylococci on the survival of mice receiving a lethal dose of staphylococci. The day of challenge with the lethal dose is shown along the abscissa.

and is different from the protection given by lipopolysaccharides (5). It is probably based on some other immunological reaction, possibly on enhancement of specific antibody production.

Whether the slight effect observed by cow's milk given in combination with sublethal doses of virulent staphylococci is based on the same mechanism is not known at this time.

The experimental results reported here (6) are not to be considered applicable to breast-fed infants without appropriate further direct studies.

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Electrical Activity in Single Myocardial Cells of Limulus polyphemus

Abstract. Single cells of the heart of Limulus were examined in situ with conventional microelectrodes. Resting and action potentials were measured in a total of 20 animals, with 25 penetrations per heart during normal spontaneous activity. Resting-potential values averaged 45 millivolts. Action potentials showed a rapid upstroke and a prolonged plateau. A small, irregular burst of electrical activity occurred during the plateau phase, a discharge presumably associated with the "neurogenic" character of the heartbeat. There was no significant overshoot, nor was there any topographical localization of action-potential "types."

The heart of Limulus polyphemus, the king crab, is generally regarded as an example of the "neurogenic" type of cardiac tissue. This view is based on the assumption that the heartbeat does not originate in specialized muscle tissue, as is true of the "myogenic" heart but, rather, that the initiation of activity is relegated to neural elements contained in the ganglionic chain embedded on the dorsal surface of the heart.

The electrocardiogram of Limulus has been extensively analyzed, and three phases of electrical activity have been described—a fast and a slow component associated with the depolarization and repolarization, respectively, of the myocardium, and a rapid oscillatory discharge coincident with neural activity from the cardiac ganglia (1). This report describes the electrical activity associated with the heartbeat as recorded from single cells of the myocardium of Limulus.

Specimens of adult Limulus polyphemus measuring approximately 4 in. in diameter were used. The dorsal surface of the exoskeleton covering the heart area was carefully dissected free. The heart was maintained intact and in situ; natural sea water was used to bathe the tissue. In several specimens the heart was exposed by a ventral dissection to make sure that injury to the tissue by a dorsal exposure did not reduce the magnitude of the measured potentials. Findings on 20 animals, with 25 penetrations per heart, are reported. Conventional microelectrodes with tips of less than 0.5 μ outside diameter were used. The recordings were photographed from the screen of a cathode-ray oscilloscope.

The resting potential of individual cells averaged 45 mv. An action potential accompanied each beat of the heart, as shown in Fig. 1. The contour of the action potential resembles that generally described for vertebrate myocardia (2), being distinguished by a prolonged phase of repolarization, or "plateau." A distinguishing feature of the action potential of Limulus heart is the burst of an oscillatory discharge which occurs during the plateau phase. The onset of this activity can occur at the moment the action-potential peak is attained (Fig. 1B), or it can start just as the repolarization phase begins (Fig. 1A). The duration of the action potential—the time from onset to the point of 90-percent return to the original resting level—averaged 430 msec. Rise time—the time required for the potential to rise from 10 to 90 percent of maximum amplitude—averaged 14 msec.

The burst of activity which occurs after the rising phase of the action potential appears to be the neural activity of ganglionic pacemakers, described by others during external recordings (1). This oscillation occurs just after the action potential reaches its peak, but it
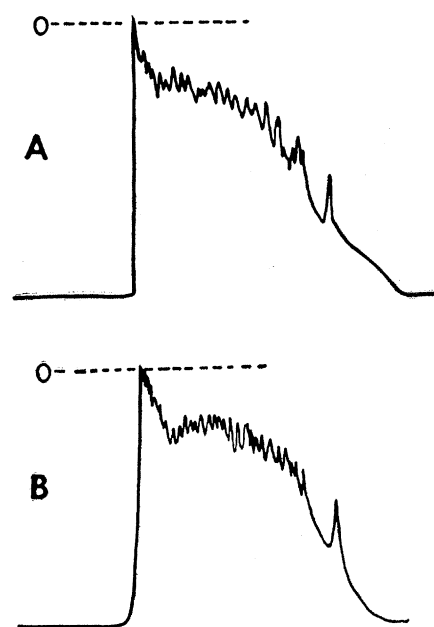

Fig. 1. Single-cell action potentials recorded from the heart of Limulus polyphemus (¼ in. on the ordinate equals 10 mv; 3/16 in. on the abscissa equals 100 msec). The ganglionic burst starts at the onset of the plateau in A and appears just after the peak of depolarization is reached in B.

may be suppressed until the onset of the plateau phase. The frequency of this discharge varies as the initial high rate decreases toward the end of the plateau. As the plateau declines, activity ceases, at about 23 mv, but it is followed by one last single discharge which has a somewhat larger amplitude than those comprising the neural burst.

The electrical activity in single cells of the myocardium in Limulus polyphemus is strikingly similar to that found in the cardiac ganglion of the lobster (3). The initial depolarization signaling the onset of the myocardial action potential reflects the synchronous discharge of pacemaker neurons within the ganglion. The persistent neuronal discharge during the plateau phase resembles the activity of "follower" neurons, the large motor neurons located in the anterior portion of the ganglion.

Each segment of the heart has been examined with an intracellular electrode, but there is no indication that there is a pacemaker component such as is found in vertebrate "myogenic" hearts. Differences in contour of the action potentials indicative of conducting or pacemaker tissue were not observed (4).

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