

TABLE 1
CALCIUM CONTENT OF PAIRED CHICK BONES
BEFORE AND AFTER IMPLANTATION

Rachitic host			Nonrachitic host			
Left tibia (mg Ca)	Im- planted right tibia (mg Ca)	Ratio	Left tibia (mg Ca)	Im- planted right tibia (mg Ca)	Ratio	
7.80	6.70	.86	7.85	8.12	1.03	
8.05	7.72	.96	8.90	9.45	1.06	
7.38	6.30	.85	7.35	7.40	1.01	
7.60	6.50	.86	6.80	6.85	1.01	
7.82	7.05	.90	7.52	7.38	.98	
9.05	8.10	.90	7.30	8.20	1.12	
Mean	7.95	7.06	.89	7.62	7.90	1.04

change, deposition, and solution. An attempt was made to approximate these separate quantities by implanting a bone made radioactive with Ca^{45} . In this experiment 12-day-old rachitic and nonrachitic chicks were made radioactive by injection of $1 \mu\text{c}$ Ca^{45} . The chicks were killed 48 hr later, and both tibiae removed. One was kept for reference, and the other was implanted, as above, into a 3-week-old rachitic or nonrachitic chick. Seven days later the implanted tibiae were removed, and their content of Ca and Ca^{45} was compared with their nonimplanted mates. The Ca^{45} analyses were carried out according to the method described by Migicovsky and Emslie (4).

The summarized results are shown in Table 2. It is seen that the loss and the gain of Ca in the non-

TABLE 2
EFFECT OF RACHITIC STATE ON MOVEMENT OF
CALCIUM IN IMPLANTED CHICK TIBIAE

Mean (8 values)	Implanted normal bone		Implanted rachitic bone	
	Normal host	Rachitic host	Normal host	Rachitic host
Net difference* mg Ca	0.48	-2.80	1.62	-2.76
Total loss† mg Ca	4.51	9.61	3.85	8.64
Total gain‡ mg Ca	4.99	6.81	5.47	5.88
Ratio§	0.90	1.40	0.67	1.46

* Mg Ca/implanted tibia - mg Ca/nonimplanted tibia.

† Cpm of nonimplanted tibia - cpm of implanted tibia

Mean specific activity of implanted and nonimplanted tibia

‡ Net difference plus total loss.

§ Total loss

§ Total gain

rachitic host were less than in the rachitic host, and the ratio of loss to gain was less in the nonrachitic host.

A similar experiment was conducted with rachitic and nonrachitic rats, except that nonrachitic femurs were implanted into the peritoneal cavity. The summarized results are shown in Table 3.

This technique of using the intact animal as the culture medium for a bone from another animal has

TABLE 3
EFFECT OF RACHITIC STATE ON MOVEMENT OF
CALCIUM IN IMPLANTED RAT FEMORA*

Mean (4 values)	Implanted normal bone	
	Normal host	Rachitic host
Net difference mg Ca	1.21	0.46
Total loss mg Ca	1.16	3.65
Total gain mg Ca	2.36	4.31
Ratio	.48	.84

* Calculations as in Table 2.

demonstrated that in rickets the changes in the composition of body fluid could be the cause of the rachitic lesions of bone. These lesions could arise by virtue of an increased rate of Ca solution relative to the rate of Ca deposition. The problem of how vitamin D prevents the changes in the body fluid remains to be resolved, although there is strong evidence favoring the absorption mechanism.

In addition it has been observed that after 7 days the ends of the implanted bone became encapsulated by a cellular tissue which was partially calcified. A similar observation had been made by Bull (5) with rabbit bone fragments implanted into abdominal muscle.

It appears that this cellular tissue and the implantation technique herein described could be advantageously employed in the study of the calcification mechanism. Further study along this line is in progress.

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The Critical Frequency of Taste

Margaret Hubbard Jones and F. Nowell Jones

Department of Psychology,
University of California, Los Angeles

One of the classical experiments in the field of gustation is a study reported in 1925 by Allen and Weinberg (1), which presents rather unequivocal evidence for four taste systems and their relative sensitivities, based upon the fusion frequency of electrical stimuli applied to the tongue. In the discussion of gustation in a recent important handbook (2) these results have been cited in some detail, and considerable weight has been given them. The present authors, however, encountered considerable difficulty in following Allen and Weinberg's reasoning and statistical procedures, and, in the absence of the raw data.

measures of variability, and results from more than a single subject, decided that the experiment would bear repetition.

Two attempts were made to duplicate the results. In the first attempt a Goodwin Stimulator (Model No. 3) was used, which delivered an exponential discharge with an abrupt rise time and a time constant of 0.5σ . The active electrode was a small piece of tantalum wire, doubled and drawn to a point. The inactive electrode was a small coil of tantalum wire which rested under the tongue. The anterior dorsal surface of the tongue was explored with stimuli of 0.4–3.0 v at frequencies of 100–1000 cps. Below 0.8 v no sensations were aroused, but at 0.8 v all frequencies aroused pressure or cold sensations. Sour was not in evidence until 0.9 v was reached and then was accompanied by cutaneous sensations. Sour sensations, when obtained, were either continuous or accompanied by pressure or pain which sometimes fluctuated. It would have been easy to confuse continuous sour plus discrete pressure pulses with discrete sour pulses. Similar results were obtained with a second trained subject. It was concluded that the judgment required of the subject was too difficult to permit of precise results. The first apparatus was abandoned as being unfair to Allen and Weinberg, and a second apparatus was assembled which was designed to deliver a stimulus resembling more closely the stimulus used by them.

Allen and Weinberg used mechanical control of the stimulus, whereas the present authors attempted to duplicate the essential features of their stimulus using electronic control. An audio-oscillator (Hewlett-Packard Model 200B), a square-wave generator (Hewlett-Packard Model 210A), and an attenuator (Hewlett-Packard Model 350A) were employed to deliver half a square wave, variable as to voltage and frequency. The active electrode (the cathode, as in Allen and Weinberg's study) was the same as in the first study; the inactive electrode was a double strand of tantalum wire stretched across a plastic plate on which the subject rested his tongue. A silver active electrode (similar to Allen and Weinberg's) was used in some series, with no change in results.

In the second study six subjects were used, of whom four were experienced in psychophysical judgments. They were instructed to report any and all sensations and to describe the time characteristics of any sensations experienced. As before, the anterior dorsal surface of the tongue was explored with stimuli of increasing voltage until sour was aroused. Experimentation began with 0.11 v, and the stimulus never exceeded 2.08 v. Then the frequency of the stimulus was varied from 20 to 300 cps. All subjects reported sour, seldom accompanied by other sensations, so that it was possible to observe the time course of the sour sensation. In no instance did any subject spontaneously report fluctuating or pulsing sour. When finally queried as to whether discrete pulses of sour occurred, no subject was able to observe it. For all subjects, then, sour was "fused" at

all frequencies, and it thus became impossible to obtain fusion frequencies, as reported by Allen and Weinberg.

The reasons for the discrepancy in results are not obvious. It is possible that Allen and Weinberg's subject was confused by cutaneous sensations aroused simultaneously with sour. In any event, unless their results can be substantiated by other investigators, they should not be used as evidence for four taste systems nor for the relationships among the taste systems.

References

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Skeletal Units in Protein Crystals¹

Dorothy Wrinch

Department of Physics,
Smith College, Northampton, Massachusetts

Recent estimates of the numbers of amino acid residues in various protein structures are of interest in relation to the idea that the manner in which the amino acid backbones polymerize to form the skeletons of native protein molecules may be characteristic for proteins as such (differentiating proteins from amino acid polycondensations which are not proteins) and that there may be a single skeletal type in all the individual chemical entities of proteins or a homologous series of types embodying some common constructional principle (1). For horse hemoglobin, ~580 residues are estimated (2), 6 with free α -amino groups (3); for horse (2) and whale (4) myoglobin, 146 and ~147 residues are suggested, one of each set having a free α -amino group (3,4). For ribonuclease, a complement of 100 ± 10 is proposed (5); for the trigonal insulin structure (6), three substructures, each with ~102 residues (7) (4 with free α -amino groups [8]) are diagnosed (9).

From these figures there arises the possibility of a single type of skeleton in which about 48 residues are interlocked, with or without additional residues inserted by a single terminal and acting as substituents. (The fact that such a skeleton presents itself in the cyclol system [10] will be discussed elsewhere.) The ribonuclease structure would have 2 such units, the myoglobins 3, and insulin three sets of 2 such units. For horse hemoglobin (and probably also for the many other hemoglobins with about the same mol wt) the number would be 12.

Studying the idea of a characteristic skeleton or skeletons first in the form of the postulate of a single molecular skeleton for proteins in general, we see that the description of a protein structure would

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