Table 1. Test of goodness of fit for chromatid interchanges. In the columns headed "Aberrations per cell," when a number is placed between two columns, it represents the sum of the expected values for those two columns. In all chi-square tests there is one degree of freedom.

Material		X-ray dose (r)	Aberrations per cell 0	1	2	3	χ^2 test
			All interchanges				
Tradescantia microspores	Observed	120	208	76	14	2	$\chi^2 = 0.05$
	Expected*		209.4	75.3	15.3		$P = .5$
Vicia lateral root tips	Observed	200	202	76	17	5	$\chi^2 = 0.85$
	Expected*		197.7	82.5	19.8		$P = .3$–$.4$
	Expected†		193.5	89.7	16.8		$\chi^2 = 4.07$
							$P = .045$
			Symmetrical interchanges				
Tradescantia microspores	Observed	120	260	40			$\chi^2 = 0.33$
	Expected*		263.4	36.6			$P = .62$
Vicia lateral root tips	Observed	200	234	58	6	2	$\chi^2 = 0.99$
	Expected*		226.8	63.6	9.6		$P = .3$–$.4$
			Asymmetrical interchanges				
Tradescantia microspores	Observed	120	232	66	2		$\chi^2 = 0.86$
	Expected*		238.5	61.5			$P = .35$
Vicia lateral root tips	Observed	200	257	37	6		$\chi^2 = 2.75$
	Expected*		255	41.7	3.3		$P = .1$

*Expected values calculated from the Poisson formula (Eq. 2). †Expected values calculated from the binomial formula (Eq. 1) when $n = 4$.

a dose rate of 100 r/min from a G.E. Maxitron tube operated at 250 kv (peak), 30 ma, with a 3-mm aluminum filter (half-value layer, 0.441 mm Cu). Squash preparations of the microspores were made 18 hours after irradiation; they were stained in aceto-carmine and permanently mounted in Euparal. Metaphases were studied and scored for aberrations.

Vicia lateral roots were grown in continuously aerated, glass-distilled water after the peeled soaked seeds had been germinated between layers of wet cotton batting and filter paper. The roots were irradiated in air with 200 r, placed back in the aerated water, collected 24 hours later, treated in 0.2-percent colchicine solution for 2 hours, fixed in Ford's modification of Flemming's fixative, and stained by the Feulgen method. Squash preparations were made, and again metaphases were studied and scored for aberrations.

Both symmetrical and asymmetrical chromatid interchanges produced after chromosomal duplication were scored in both materials. The distributions of such aberrations were then tested for the goodness of fit to the Poisson formula of

$$e^{-m} \cdot m^r / r! \qquad (2)$$

where r is the number of aberrations in the cell, whether a cell had zero, one, two, three, or more interchanges, and m is the mean number of aberrations per cell observed in an experiment.

As may be seen in Table 1, the chromatid interchanges induced in cells of *Vicia* lateral roots are distributed according to the Poisson formula. This is contrary to the results for chromosome exchanges (*3*) or for chromatid interchanges induced close to metaphase when the chromosomes are relatively condensed (*6*).

For chromosome aberrations, however, only asymmetrical exchanges were scored since most symmetrical exchanges can not be distinguished as exchanges at metaphase. For chromatid interchanges, however, because of sister attraction of the chromatids, both symmetrical and asymmetrical exchanges can be scored. We thought, therefore, that a more accurate test would be to check the distribution of asymmetrical chromatid interchanges, which are more comparable to the aberrations scored as chromosome exchanges. Table 1 shows that both the symmetrical and asymmetrical chromatid interchanges taken separately still fit the Poisson distribution.

The chromatid interchanges in *Vicia* lateral root tip cells, analyzed for goodness of fit to the binomial distribution of Eq. 1, did not fit such a distribution when n (the number of sites in the cell) was taken to be equal to four, the number of sites expected after chromosomal duplication since the number of sites for the interphase nucleus has been calculated to be two (*5*). The results, however, show a good fit to the Poisson formula, as good a fit as the *Tradescantia* microspore data show.

The Poisson distribution obtains when there is a large number of places where an event can occur (sites) and a small probability of its occurrence. The fact that the *Vicia* data give a good fit to the Poisson formula indicates that after chromosomal duplication the number of sites where the chromosomes lie within h microns of one another is greatly increased.

It is concluded, therefore, that the number of sites where by chance the chromosomes are close enough to interchange if they are broken, increases after the chromosomes are duplicated and that the increase is not a mere doubling of the original number. The increased number of sites leads to a Poisson distribution of interchanges among cells. This is contrary to the results obtained when the number of sites is smaller, either before synthesis of deoxyribonucleic acid (*3, 4*) or after chromosomal condensation has progressed (*6*).

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Mineralization of Bacteria

Abstract. A variety of viable and nonviable bacteria became mineralized with hydroxyapatite when implanted in dialysis bags in the peritoneal cavities of rats. The microscopic pattern of mineral deposition appeared analogous to that in the formation of oral calculus. Since nonviable organisms were mineralized at an accelerated rate, bacterial metabolic processes may not be essential for mineralization.

It has been demonstrated that certain preparations of collagen undergo calcification when implanted in the peritoneal cavities of living animals (*1*). This phenomenon is now serving as a basis for in vivo studies of the mineralization process. A recent refinement in experimental technique has been the implanta-

tion of specimens enclosed in dialysis tubing. The main advantage of this procedure is that cells and proteins and other compounds of high molecular weight of the host are excluded from the implants. More adequate control is thus afforded when substances being assessed for their effects upon mineralization are incorporated in the implant.

When various collagenous tissues were implanted in rats in sealed dialysis bags, some specimens became calcified, whereas other samples of the same material did not. Further investigation revealed that the implants which mineralized had been contaminated with bacteria during preparation, and those which did not mineralize had remained sterile. Because microorganisms were thus implicated as a factor in the observed mineralization, it was of interest to determine if they would calcify when implanted alone. Therefore, studies of several individual microorganisms were undertaken.

The bacteria so far implanted include *Streptococcus salivarius, Actinomyces israelii, A. naeslundii, Bacterionema matruchotii (Leptothrix buccalis,* Kligler type) (*2*), and an unclassified oral diphtheroid, all isolated from human dental calculus. One-day-old cultures were washed, suspended in saline, and transferred into sterile bags made from 0.25-inch-diameter cellulose tubing. The sealed bags were then implanted through an abdominal incision into the peritoneal cavities of rats. Implantations were also made of bags containing similar samples of nonviable (acetone-treated or autoclaved) *Streptococcus salivarius.* Ten or more animals were used for each of the single-culture preparations tested. In addition, bags containing only isotonic saline and some containing mixtures of two strains were implanted. The host animals were sacrificed at various intervals over a 90-day period, and the contents of the bags were examined by standard bacteriological techniques, x-ray diffraction, and electron microscopy.

Viable organisms in pure culture were recovered over the entire course of the experiment from bags that had been inoculated with living cultures, but not from the bags containing acetone-treated or autoclaved organisms, nor from those containing isotonic saline only. A few preparations gave evidence of contamination.

All of the viable implants underwent calcification and the presence of hydroxyapatite was established by x-ray diffraction. The first signs of mineralization in these specimens were detectable between 14 and 26 days (Fig. 1). Specimens containing acetone-treated or autoclaved cultures of *S. salivarius* gave hydroxyapatite diffraction patterns at 6 to 8 days, whereas sterile specimens containing isotonic saline showed no hydroxyapatite for as long as 39 days. Unmineralized samples were either amorphous or gave a diffraction pattern of sodium chloride.

Analogous observations were made in smears stained by the Gram or von Kossa methods. Early calcification usually took the form of roughly spherical refractile bodies, several microns in diameter, which were noted a few days after diffraction patterns for apatite had been obtained (Fig. 2). In some specimens calcification seemed to follow the morphological outlines of individual organisms (Fig. 3). Preceding the first indications of mineralization there was a gradual interbacterial accumulation of amorphous safranin-straining material.

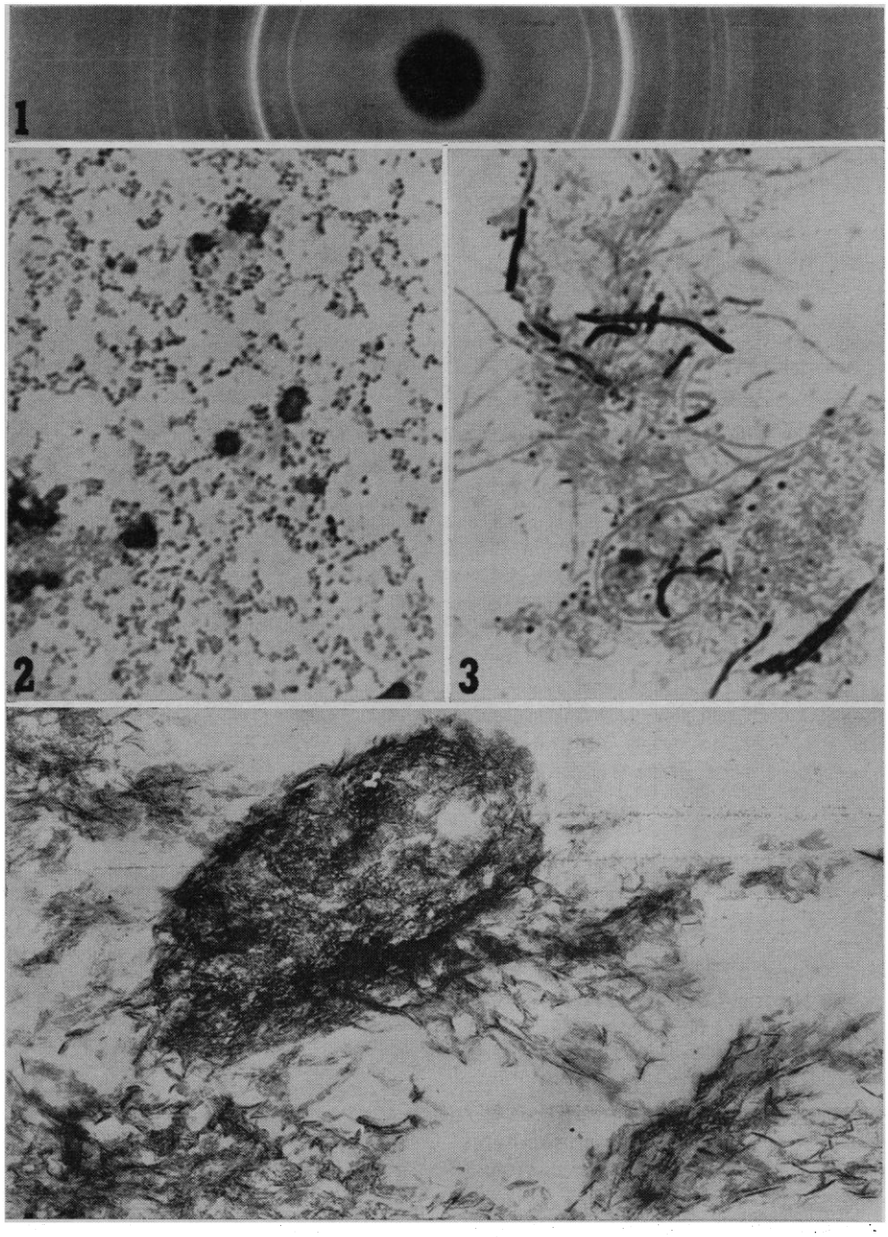

Fig. 1. Typical x-ray diffraction pattern of hydroxyapatite given by mineralized bacterial implants. Fig. 2. Darkly stained calcified bodies in a 21-day viable *S. salivarius* implant. Modified von Kossa stain (× 1100). Fig. 3. Mineralized filaments in a 14-day viable implant containing *B. matruchotii* and *S. salivarius.* Unstained organisms were not mineralized. Modified von Kossa stain (× 1100). Fig. 4. Electron micrograph of a sectioned 75-day viable implant of *S. salivarius.* Apatite crystals are evident inside and around the organism at the top of the picture (× 45,000).

Electron microscopy revealed striking similarities between the mineral deposits recovered from the bags and those obtained previously from developing human dental calculus (*3*). In both types of specimens areas were found in which mineralization occurred adjacent to and within the organisms (Fig. 4). Clusters of crystals were also found, which undoubtedly correspond to the refractile bodies seen optically.

The studies reported here indicate that a variety of bacterial implants will undergo calcification. While the presence of hydroxyapatite in biological specimens is usually but not always associated with higher forms (*4*), it is clear that even bacteria will mineralize with hydroxyapatite under proper conditions. Although the mechanism is at present unknown, the accelerated mineralization of nonviable organisms suggests that metabolic processes of the bacteria are not essential for mineralization and may indeed retard it.

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Contribution of E-Amino Groups to Ninhydrin Color Production in Proteins

Abstract. The ninhydrin color given by model compounds and by characterized proteins shows a consistent contribution from the ϵ-amino group of lysine of about 67 percent of that of an α-amino group, except in free lysine or in N-terminal lysine, where the ϵ-amino group makes a small contribution (7 to 10 percent) to the total color. This information can be applied to structure determination of N^{ϵ} peptides of lysine.

In view of the current interest in atypical linkages in proteins (*1, 2*), it seems pertinent to point out that the ninhydrin color reaction can be applied to distinguish free ϵ-amino and free α-amino groups. The color developed from ϵ-amino groups is similar in a variety of compounds studied. When the Troll-Cannan method (*3*) is used, the molar extinction is 65 to 70 percent of that of leucine. An exception occurs when the ϵ-amino and α-amino groups are in the same residue [free (*3*) or N-terminal lysine]. Here the total color is only 107 to 110 percent of that of leucine; the apparent ϵ-amino contribution is only 7 to 10 percent of that attributable to the free α-amino group (*4*).

These conclusions were drawn from studies of model compounds and of certain purified proteins (*5*) for which complete analytical data are available.

The details of the Troll-Cannan method are as follows: a sample containing 0.05 to 5 μmole of amino groups in 0.4 ml of water is heated in 1 ml of 80 percent phenol and 1 ml of 0.0002*M* KCN in pyridine. One-fifth of a milliliter of 5 percent ninhydrin in alcohol is added, and the mixture is boiled 3 to 5 minutes. The solution is cooled, diluted to 10 ml with 60 percent alcohol, and the optical density is read at 570 mμ. The method, when applied to proteins, may give a white precipitate, but this has no apparent effect on color development in the supernatant.

Table 1 shows the results obtained from a variety of compounds studied.

To obtain the molar extinction per ϵ-amino group, the extinction calculated for the α-amino groups was subtracted from the total extinction per molecule, and the difference was divided by the number of ϵ-amino groups. In the case of ribonuclease, the total color was attributed to one α- and nine ϵ-groups, since the terminal lysine contribution should be that of one α-amino group only, as found for lysine (*3*) and for lysylglycine. Similarly, in the case of lysozyme, which also possesses an N-terminal lysyl residue, the total color obtained was attributed to one α- and five ϵ-amino groups. The results are expressed in leucine color equivalents, as the absolute value for the molar extinction of leucine varies with the method used. It is noteworthy that, when the Moore and Stein method (*6*) was used on ribonuclease, the same results were obtained.

These observations have already proved helpful in assigning a structure to an ϵ-amino linked peptide of lysine (Table 1) which was isolated from a partial hydrolysate of collagen (*1, 7*).

Table 1. Ninhydrin color yields from compounds with known contents of ϵ-amino and α-amino groups.

Compound	Leucine equivalents per mole	No. of ϵ-amino groups	No. of α-amino groups	Leucine equivalents per ϵ-amino group*
Leucine	1†		1	
Lysine	1.10	1	1	0.10
Lysylglycine	1.08	1	1	0.08
N^{ϵ}-Carbobenzoxylysine	1		1	
N^{ϵ}-Acetyl lysine	1		1	
ϵ-Aminocaproic acid	0.67	1		0.67
N^{α}-Tosyl-lysine benzyl ester	0.67	1		0.67
Methemoglobin (human) (*8*)	34.3	43	4	0.70
Conalbumin (*9, 10*)	37.2	60	1	0.60
Lysozyme (*10, 11*)	4.35‡	6	1(lys)	0.67
Ribonuclease (*12*)	7.70†	10	1(lys)	0.74
Insulin (*13*)	2.67	1	2	0.67
N^{ϵ}-(Glycyl-α-glutamyl)lysine (*1*)	2.00		2	
Hydrolyzate of above (*1*)	2.87§	1	3	−0.13

* Except for lysozyme and ribonuclease, yields (column 5) are given in leucine equivalents per mole (column 2) minus the number of α-amino groups (column 4), the remainder being divided by the number of ϵ-amino groups (column 3). For lysozyme and ribonuclease, the divisor is one less than the number of ϵ-amino groups. † By Moore and Stein method, as well as Troll-Cannan method. ‡ Tallan and Stein (*14*) found .70 leucine eq per amino group, or 4.9 leucine eq per mole for lysozyme. § The color increase on hydrolysis of the peptide was less than expected, the theoretical value being 3.10.

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4. Perhaps some of the lysine is converted to compounds other than the typical blue color of the ninhydrin reaction. It is not possible to state which of the two amino groups would actually be involved in a side reaction. Therefore, the assignment made is a matter of convenience.
5. We thank Dr. R. C. Warner for samples of crystalline lysozyme and conalbumin and Dr. J. Dancis for a sample of crystalline human methemoglobin. Insulin was obtained from Armour Co.; ribonuclease was obtained from Worthington Biochemical Corp.