at which temperature kappa can multiply faster than the animals. Those which contain at least one particle of kappa produce progeny with the full number and so yield killer clones. Those containing no particles remain sensitive and yield sensitive clones. This provides a means for calculating the number of kappa particles present in the original killer animal. The number of kappa particles present, on the average, in the progeny of a killer animal subjected to the expansion technique may be calculated by the approximate relation $P_0 = e^{-m}$, where P_0 is the proportion of individuals with no particles, m is the mean number of kappa particles per animal, and e is the base of natural logarithms (*1*).

If inactivation of kappa particles has occurred in animals exposed to nitrogen mustard, one can measure the extent to which this has occurred by comparing the mean number of particles in the progeny of exposed animals to that in the progeny of normal killer animals subjected to the expansion technique.

This comparison between animals exposed to nitrogen mustard (0.20 mg/ml) and animals exposed to buffer alone (controls) for a period of 7 min was made in experiment 3. A fresh solution of nitrogen mustard hydrochloride in phosphate buffer (pH 6) was made up twice as concentrated as was desired for the exposure. One drop of this solution was used immediately by adding it to an equal-sized drop of buffer containing the animals in a depression slide. The two drops were mixed quickly by agitating the slide, and after 7 min the animals were removed, washed three times in fresh culture fluid, isolated, and placed at 33.8° C for rapid fission. The exposed animals underwent seven fissions, and the controls underwent eight fissions. The results of carrying out these procedures on seven exposed and nine control animals from the same clone are given in Table 2.

TABLE 2

EFFECT OF NITROGEN MUSTARD ON THE NUMBER OF KAPPA PARTICLES IN KILLER ANIMALS

Animal No.	No. isolated and tested	Fraction sensitive	Mean No. of kappa particles
Exposed			
1	127	0.724	0.323
2	118	0.797	0.227
3	115	0.765	0.267
4	127	0.874	0.135
5	118	0.780	0.248
6	118	0.797	0.227
7	117	0.667	0.405
			Avg 0.261
Control			
1	233	0.236	1.44
2	99	0.222	1.42
3	98	0.092	2.39
4	98	0.143	1.95
5	98	0.122	2.10
6	94	0.106	2.24
7	27	0.148	1.91
8	29	0.138	1.98
9	24	0.167	1.79
			Avg 1.91

From the proportions of sensitive animals produced in the two series shown in the table, one may calculate that the average mean particle number for the progeny of the control animals was 1.91, whereas that for the exposed animals was 0.261. Since the controls went through one more fission than the exposed animals, the control value of 1.91 particles per animal must be doubled to make the value comparable to that found in the animals exposed to the action of nitrogen mustard. The percentage of kappa remaining after exposure is then $\frac{.261 \times 100}{2 \times 1.91} = 6.8\%$.

Experiments carried out using different lengths of exposure to nitrogen mustard indicate that the longer the exposure, the greater the percentage of kappa particles inactivated.

This procedure cannot be placed on an accurate quantitative basis at the present time because the effects of temperature alone on kappa have not been thoroughly investigated. There should be a way to correct for the effects of temperature after these have been studied in detail.

The results reported here were confirmed with the aid of the light microscope, using the methods of Preer (*3*, *4*). The cytoplasm of killer animals contains Feulgen-positive particles which are not found in sensitive animals, and which parallel the behavior of kappa. The numbers of these particles exhibited by animals exposed to nitrogen mustard were much smaller than the numbers found in untreated animals. There then seems little doubt but that the staining reaction associated with kappa and its activity as regards the killer character are destroyed by the action of nitrogen mustard.

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A New Method for the Study of Submicroscopic Spaces

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Proceeding with their research on the optical properties of osseous substance, Dallemagne and Melon (*2*) attempted to obtain diagram curves of structural double refraction of the organic constituent of bone. Since the results, which will be published, have shown anomalies in the general aspect of the diagrams, we have been looking for a different technique for scanning the submicroscopic

FIG. 1. Mineralized bone embedded in methyl methacrylate (polarized light).

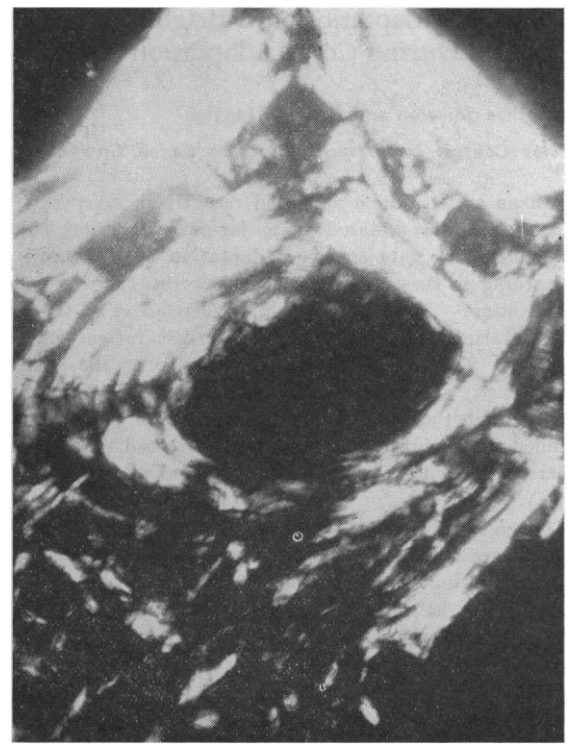

FIG. 2. The same material after treatment by hydrochloric acid, i.e., the "bone ghost" (polarized light).

spaces occupied by the organic substance in bones.

Slices of dry bone (200–300 μ thick) were sawed, then boiled (290° C) in anhydrous glycerol containing 6% KOH, until no trace of organic substance remained, according to Gabriel's method (*3*).

The bone slices were then carefully washed with distilled water, dried, and embedded in methyl methacrylate according to the technique of Coudert and Baud (*1*).

The preparations immersed in the pure monomer were placed in a vacuum until no more air bubbles escaped. Afterwards the preparations were introduced into slightly polymerized methacrylate and left for 12–24 hr at 56° C in airtight containers in the presence of benzoyl peroxide; the polymerization of the plastic was then accomplished.

The Plexiglas block was abraded on both faces by a grindstone until the embedded specimen reached the surface; it was then polished.

Examined under the microscope, both in ordinary and polarized light, the object showed, of course, the usual picture of mineralized osseous substance. Between crossed Nicols, it showed a retardation corresponding to impregnation with a medium having a refractive index of 1.490, which is the refractive index of polymerized methyl methacrylate (Fig. 1). The specimen was then immersed in a solution of normal hydrochloric acid and kept there until no more gas escaped, thus showing that all the carbonate was converted into chloride and that the mineral substance of bone had completely disappeared.

In fact, after this treatment, we cannot detect traces of bone when the plastic block is dissolved in acetone. However, such preparations, free from the initially embedded mineral substance, still look like ordinary osseous slices. Actually, this appearance is due solely to methyl methacrylate deposited in the submicroscopic spaces of mineralized bone. Thus, we have a "bone ghost."

In polarized light, we observe a picture topographically identical with the one obtained before hydrochloric destruction of the mineral substance (Fig. 2).

On the one hand, methacrylate becomes set in the submicroscopic spaces originally occupied by the organic constituents which are liberated through deproteinization; on the other hand, the mineral particles removed by hydrochloric acid leave free spaces that can thereafter be filled with liquids endowed with a different refractive index.

By means of such casts, it is possible to establish structural double refraction curves without fear of getting results spoiled by accidental influences (swelling, alteration by reagents, etc.).

The results of our optical research on "bone ghosts" will be published in detail elsewhere.

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