According to our observations, we assume that more than one factor essential for the rat is present in animal proteins.

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A Micro Ball Mill for the Disintegration of Bacteria

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For grinding small quantities of bacteria a centrifuge tube with a ground-in glass rod can be quite satisfactory, but it is difficult to obtain the same mechanical effects if a number of specimens must be treated simultaneously. For this reason the vibrator equipment described below was designed.

The use of 50 cps vibrations for disintegrating biological materials has been described by E. Hammarsten (*1*). The present author found the method very useful and devised an extension of the technique, which may also prove of value for grinding other biological materials besides bacteria.

The grinding was effected in glass tubes made out of ordinary pyrex glass tubing (diam, 7 mm), which was cut about 15 cm long. The pieces were bent in the middle to resemble the tube in Fig. 1. A special holder made out of brass and containing Woods metal (Fig. 2) was heated to melt the alloy (mp 65.5° C), and the tubes were then inserted in a row. When the metal had solidified, the tubes were taken out of their setting and the material in question—mostly in the form of a freeze-dried powder—was added. Up to 42 specimens could be accommodated. Nine carefully chosen glass beads of a diameter slightly smaller than that of the tube were also added to each specimen, and the tubes were plugged with rubber stoppers. They were replaced in their settings, and a brass pole covered with a piece of rubber hose was placed in the angle of the tubes and screwed tightly down so as to keep them firmly in place. The holder was mounted on the stage of a 50 cps a.c. vibrator (Type EMR6, 70 w, 200 v, Vibroverken Ltd., Stockholm) in a freezing box at −15° C. The vibrations were mainly vertical and caused the glass beads to be thrown between the walls of the tube, but owing to the slope of the branches they also developed a rotating movement (arrows, Fig. 1), which counteracted the tendency of the material to accumulate at the bottom of the tube and effected a continuous mixing of the material. Treatment for 4 hr was not accompanied by any perceptible rise in temperature and was as a rule sufficient to break up the bacteria. The treatment could be shortened by adding weighted amounts of glass powder to the specimens or by etching the glass beads and the inside of the tubes with fluorine.

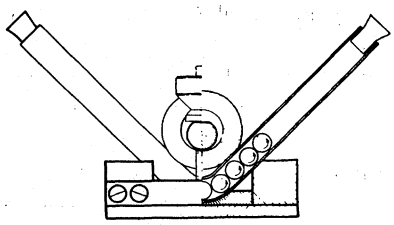

FIG. 1.

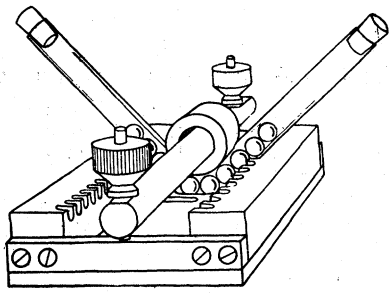

FIG. 2.

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The Purine and Pyrimidine Content of Three Strains of *Escherichia coli*[1]

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Witkin isolated a spontaneous mutant, B/r, of *E. coli* strain B which proved to be more resistant than the parent to both x-rays and ultraviolet light (*1*). These two strains, together with strain K_{12} which was found by Lederberger to show genetic recombination unusual in *E. coli* (*2*), have been analyzed for purine and pyrimidine content.

The organisms were grown in a synthetic medium, with ammonium chloride as source of nitrogen and with succinate and glutamate as source of carbon. In each case 24-hr cultures were harvested by centrifugation, and the organisms extracted with cold 7% trichloracetic acid, ethanol, and boiling ethanol-ether. The residue after being dried *in vacuo* was rendered to a powder. Approximately 10 mg of the powder was digested with $HClO_4$, the digest subjected to chromatography, and the yield of nitrogenous bases

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determined by spectrophotometry as previously described (*3*).

The results are shown in Tables 1 and 2.

TABLE 1

MICROMOLS/MG DRY WEIGHT

	Adenine	Guanine	Cytosine	Uracil	Thymine
B	.118	.150	.119	.068	.026
B/r	.127	.212	.147	.058	.026
K_{12}	.097	.158	.117	.044	.023

TABLE 2

MOLAR RATIOS

	Adenine	Guanine	Cytosine	Uracil	Thymine
B	1	1.27	1.01	0.58	0.22
B/r	1	1.67	1.16	0.46	0.20
K_{12}	1	1.63	1.21	0.45	0.24

In the resistant strain B/r, the adenine content is greater than in B by 7%, and the guanine and cytosine contents are greater by 41% and 23%, respectively. The uracil in B/r is low by 15%, whereas the thymine content is the same as in B. Thus there is approximately 56% more base in B/r than in B. Since there is no difference in thymine content, it may be assumed that this represents an increase only in pentosenucleic acid.

The survival curves for strain B with both x-rays and ultraviolet are peculiar in showing a sharp inflection at the 1% survival level (*1*). The curve for B/r in ultraviolet also seems to be a composite curve, with a change in slope at approximately 30%. From these curves it appears that 1% of the B population and 30% of the B/r population have the same sensitivity to ultraviolet, and the remaining 99% of B is 16 times as sensitive as the other 60% of the B/r. The difference in response to x-rays is less marked, B being not more than 3 times as sensitive as B/r.

The marked increase in guanine as compared with the other bases may be taken as evidence that the nucleic acid increase is due to cytoplasmic PNA (*4*) which would act as an absorbing screen around the radiation-sensitive portion of the cell, i.e., the nucleus, and thus account for the greater sensitivity of B as compared with B/r to ultraviolet light; but it seems unlikely that the additional PNA alone could account for the greater resistance of B/r to x-rays. However, since nucleic acids are capable of binding considerable amounts of metal (*7*), and since most of the ionization reaching the bacterial nucleus is produced by β-particles originating in the medium outside the bacterial cell, it is possible that the nucleic acid-metal complex may act as a β-particle filter and thus reduce the ionization actually reaching the nucleus. Alternatively, it may be assumed that the bacterial cell, like cells of higher organisms, has one phase in its life cycle that is more sensitive to radiation, this phase being analogous to one of the mitotic stages. Also by analogy with higher organisms, production of PNA proceeds primarily during the "resting stage," which is relatively insensitive to radiation. A mutation that would result in a decrease in the relative duration of the sensitive phase of the cell cycle would thus also be expected to produce an increase in the average PNA content of the bacteria. By this hypothesis the results with both ultraviolet and x-rays can be accounted for. Although Witkin has considered the change in shape of the survival curve in ultraviolet in B/r as compared with B as evidence against the "target theory," it seems clear from the above considerations that the apparent change in shape may have no bearing upon the target theory.

The molar ratios of the various bases are shown in Table 2.

Although strain K_{12} has an absolute base content similar to that of B (Table 1), the proportions of its various bases resemble those of B/r rather closely. In other words, K_{12} contains approximately the same amount of nucleic acid as B per unit dry weight of cells, but the composition of its nucleic acids and the relative proportions of each of these nucleic acids are similar to those of B/r.

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A Simplified Technique for Microelectrocoagulation

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During the course of an investigation dealing with the production of cardiovascular anomalies in chick embryos, we have developed and used successfully a technique for the destruction of minute areas of tissue. The method should be applicable to a wide range of investigations, particularly in the field of developmental research. Hilleman (*1*) used a similar technique, to obtain even smaller areas of coagulation than those required by our experiments, in destroying anlagen of pituitary glands of chick embryos. Most biological laboratories have on hand materials from which the apparatus can be assembled.

The materials needed are a dissecting microscope, a micromanipulator, microelectrodes, and a source of coagulating current. A suitable micromanipulator can be assembled quite readily by mounting an ordinary detachable-type mechanical stage on top of the rack of a compound microscope (Fig. 2. *B*). This manipulator is described in a separate publication (*2*).

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