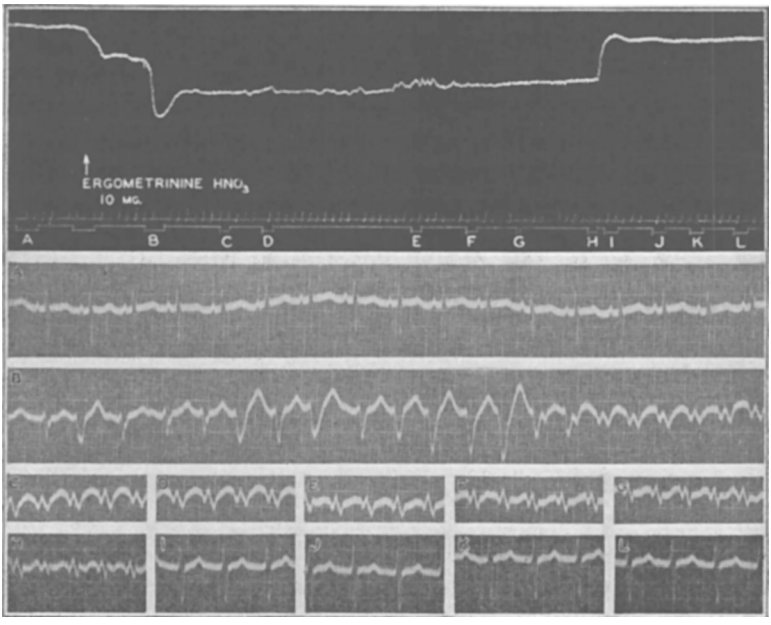

FIG. 2.

Action of Ergometrinine on Blood Pressure and Electrocardiogram. Cat No. 1372, female, weighing 2.5 kg., was anesthetized with ether. The letters marked on the electrocardiograms correspond to those on the kymographic tracing which shows from above down the carotid pressure, time, and the base line.

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8551 C

Possible Bipolar Nuclear Distribution in Bacteria.

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Variations in the staining intensity of portions of the bacterial cell so that the bacterium appeared to contain granules situated at the poles were noted while employing a Gray's flagella stain on a glucose broth culture of *Eberthella typhi*. Subsequent investigation demonstrated that with improved technic the granules could be shown in a variety of bacteria irrespective of the sugar em-

ployed but inversely related in size and frequency of appearance to the age of the culture.

A slide is prepared as for a flagella stain and a ring marked on it with a wax pencil. Two loopfuls of sterile water are placed within the ring and to this is added a loopful of the culture. This is allowed to dry without heating and a quantity of 10% alcoholic basic fuchsin sufficient to cover completely the ringed area is dropped on. After 7 to 10 minutes, during which it is kept moist with drops of 95% alcohol, the stain is washed off with water, a drop of acid alcohol placed at the periphery of the ringed area, and instantly washed off with a gentle stream of water. After drying in the air, the slide is ready for observation.

Granules were definitely demonstrated in: *Eberthella typhi, Proteus vulgaris, Escherichia coli mutabile, Shigella paradysenteriae,* Flexner (2 strains), *Sh. paradysenteriae,* Sonne, *Salmonella paratyphi, Pseudomonas fluorescens, Alcaligenes fecalis, Alc. bronchisepticus.*

Granules were apparent in *P. vulgaris* when cultivated in broth containing glucose, salicin, maltose, sucrose, mannite or lactose. At the 96-hour stage of growth the salicin culture failed to show the granules and at the 120-hour stage the mannite exhibited them but poorly. Lactose broth cultures gave poor results at the 144-hour stage and at 168 hours only glucose and maltose cultures showed the granules. At 254 hours no granules were demonstrable from any medium though viability was shown by growth on transplanting. The granules did not appear when the organism was cultured on meat-extract agar, meat-extract or meat-infusion broth, Dorsett's egg medium or glycolized blood agar. Sugar-free agar slants of *Alc. fecalis* and *bronchisepticus* did not show the staining reaction in contradistinction to lactose broth cultures of the same organisms. On an Endo plate the Sonne dysentery bacillus showed no granules while a strain of Flexner type of paradysentery bacillus did. On culturing in lactose broth the Sonne organism showed faint but perceptibly stained polar areas. An agar slant of *P. vulgaris* did not manifest the staining reaction but a maltose broth suspension which was allowed to stand overnight in the ice-box contained a few granular staining organisms. A stain made immediately after the suspension had been prepared did not show the granules. *B. cloacae, Br. abortus,* and 2 strains of *Sh. dysenteriae* did not show the granules with the technic employed. Two Gram-positive organisms, *B. mesentericus* and *B. subtilis,* proved granule-negative.

When a maltose broth culture of *P. vulgaris* was washed by centrifugalization with 95% alcohol, 70% alcohol, N/10 HCl, $CHCl_3$, or 0.85% NaCl no granules could be demonstrated. Upon similar treatment with distilled water positive results were obtained. A control stain made with the original culture showed granular staining in approximately 70% of the bacteria encountered in a random field.

When 5 cc. portions of a maltose broth culture were treated with 5 cc. of 5% NaCl, 5% phenol, and 2% tincture of iodine respectively and allowed to stand 24 hours, the characteristic staining was noted in the first instance only. That this was related apparently to the viability of the organism was shown by the fact that the NaCl culture was the only one viable. Fischer[1] reports similar staining of bacteria plasmolyzed with 2.5% NaCl solution. Such preparations responded only to 0.1% basic fuchsin among the ordinary simple stains.

Methylene blue, crystal violet, carbol fuchsin, 0.1% basic fuchsin, and malachite green stains employed on maltose broth cultures of *P. vulgaris* did not yield the granule-staining results yielded by 10% basic fuchsin.

In an attempt to ascertain the chemical nature of these deeply staining areas, iodine, Scharlach R, and the polychromatic methylene blue stains were employed. By none of these were they demonstrable and the assumption that these granules were neither starch, glycogen, fat nor volutin seemed justified. On the basis of a negative Millon's reaction and failure to reduce nitric acid, it was tentatively assumed that they contained no hydroxyphenyl proteins and were of non-reducing nature.

The granular staining was perceptible with the iron-hematoxylin technic on a maltose broth culture of *P. vulgaris*.

The presence of a sugar appears essential for the demonstration of these granules. Utilization of the sugar does not seem to be necessary as *P. vulgaris* does not utilize either mannite or salicin. These sugars are the first to lose their granulogenic powers, however. The fact that the granules can be demonstrated in a sugar broth suspension from an agar slant after standing overnight in a temperature not conducive to growth would suggest a physico-chemical relationship. It may be that the sugar molecule passes through the cellular membrane to activate the existing but unstainable granule in such a manner that it becomes receptive to

[1] Fischer, "Structure and Functions of Bacteria," Oxford, 1900.

the stain. Since the granules are demonstrable with iron-hematoxylin and strong basic fuchsin, both of which are nuclear stains, the assumption that they may be composed of nuclear material does not appear to be entirely unwarranted. Zettnow[2] based his classification of granular inclusions on staining reactions and ac-

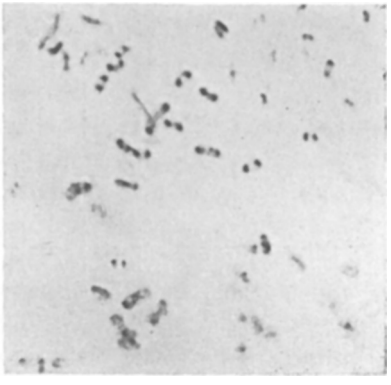

FIG. 1.
48-hour maltose broth culture *P. vulgaris* showing granules.

cording to his theory, these polar granules would fall into the category of chromatin. Since they do not appear in the killed cultures, it would seem that they are seen only in the living organism. This agrees also with their gradual disappearance from an aging culture. It may be that they are terminal concentrations of nuclear material just previous to fission and that this nuclear material is in some manner rendered especially permeable and susceptible to the chromatin stains. Considering the regularity of their appearance under certain conditions and not under others, the possibility that they may be artifacts would seem to be excluded.

[2] Zettnow, *Z. f. Hygiene*, **85**, 17.