TABLE III. Effect of Cortisone Alcohol Upon Sulfate Conjugation by Embryonic Chick Liver Tissue *in Vitro*.*

Conc. of cortisone in medium, mg/l	No. of samples	Wt of sulfate ion esterified (μg/100 mg tissue)
0	12	4.4
50	12	5.2
100	12	4.5

* No phenol was added to the medium (7).

approximately the same degree as was the fixation. Data from a representative experiment are shown in Table I.

From the data of Table II it will be seen that tissue from healing wounds was similar to embryonic tissue in its response to cortisone. Conjugation of sulfate by liver tissue was not affected by concentrations of cortisone which completely suppressed sulfate fixation and synthesis of soluble organic sulfate by heart and skeletal muscle (Table III).

Sulfate conjugation by the liver tissue is probably a detoxication mechanism (7-9) and hence a phase of the catabolic metabolism. The fact that cortisone did not affect the synthesis of ester sulfate by liver tissue, while it did inhibit esterification of sulfate by heart and skeletal muscle tissues may indicate that different mechanisms are involved. It is possible that the soluble organic sulfates synthesized by heart and muscle are related to the connective tissue and plasma mucoids and to heparin. The decrease in the sedimentation rate of the blood of patients receiving cortisone therapy may be due to a decreased synthesis of soluble plasma mucoids by the connective tissue cells.

It is possible that the system affected by cortisone therapy is the one which synthesizes the chondroitin sulfate moiety of the mucoids. These investigations have been extended to a preliminary study of tissues *in vivo*. Similar results were obtained. These will be reported in a subsequent paper.

Summary. 1. The experiments described indicated that cortisone inhibits the synthesis of chondroitin sulfate by embryonic and wound tissues maintained *in vitro*. Graded responses were obtained with graded concentrations of cortisone in the medium. Cortisone was shown to inhibit the synthesis of soluble organic sulfate by heart and skeletal muscle; it had no such effect upon the synthesis of soluble ester sulfate by liver tissue. 2. Concentrations of cortisone which suppressed sulfate fixation, had no apparent effect upon the initial migration of fibroblasts. Cortisone had no effect upon the pulsations of heart tissue. 3. It was suggested that the palliative action of cortisone in the connective tissue diseases may be due to its inhibitory effect upon the synthesis of the chondroitin sulfate moiety of the connective tissue ground substance.

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On the Existence of a Cell Granule in a Thermophilic Bacterium.* (18572)

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In a study of a thermophilic bacterium† (1-3) we showed that most of the enzymes

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† Thermophile No. 2184 obtained from the National Canners Association. This organism has tentatively been identified by us as *Bacillus stearothermophilus* (Donk).

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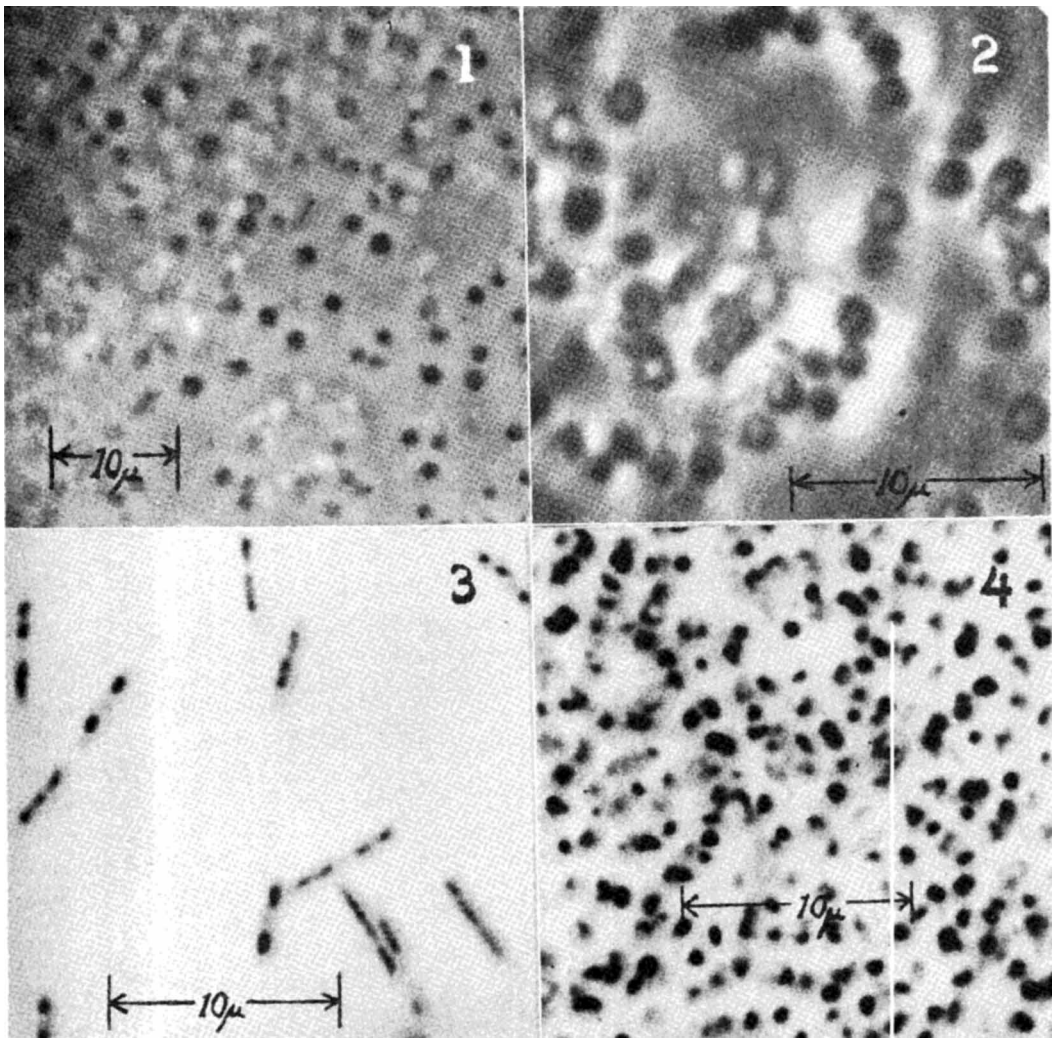

FIG. 1. Wet mount of particles liberated from thermophile N.C.A. #2184 a few minutes after treatment with lysozyme. Photographed with phase contrast microscope.
FIG. 2. Wet mount of washed red fraction photographed by phase contrast.
FIG. 3. Eight hour agar slant culture of thermophile #2184 showing granules within cells using hydrochloric acid-Giemsa stain.
FIG. 4. Red fraction stained with hydrochloric acid-Giemsa.

were associated with an insoluble fraction called the red fraction. This fraction separated on centrifugation from the lysate of the bacteria which had been treated with lysozyme. We stated that the red fraction behaved as though it were a specific particle of bacterial protoplasm. The belief has now been supported by data from several independent sources. The data supporting the conclusion follow.

The most direct evidence is that obtained with the phase contrast microscope. Fig. 1 is a picture of a saline suspension of bacteria a few minutes after they have been treated with lysozyme. The cells have disappeared leaving nothing visible but spherical bodies. These bodies can be separated by centrifugation and compose the fraction referred to as the red fraction. Fig. 2 is washed red fraction photographed at greater magnification. In both figures there is a striking similarity in the size and shape of the granules. Brown-

ian movement hampered the photography of these preparations, and, as a result, only a fraction of the total number of particles was in focus at any one moment.

The size of the particles observed in Fig. 1 and 2 is greater than the size of particles we have observed with the phase microscope in the intact bacterial cell. (No illustrations showing the granules in whole cells were attempted because of motility.) This difference in size does not mean that the released particle could not be the same as that in the cell. It has been noted before that inclusion bodies swell and change their shape once released from their native state. Mitochondria, for instance, change shape easily depending upon the medium in which they are suspended, a fact observed many years ago by Bensley and Hoerr(4) and recently explored by Harman(5).

The question may be raised whether the granules might not be spores, which this organism is capable of producing, rather than protoplasmic particles. The particles seen here are definitely not spores. Spores are highly refractile in wet mounts and are not opaque to light as are the red fraction granules. Further, the bacteria were grown in liquid media at 65°C with copious aeration, conditions which did not favor spore formation. Spores, when they are formed, can be observed by the usual spore staining technics. They appear much larger than do the granules of the red fraction. Granules, when packed together during centrifuging, have a bright red color due to cytochrome c and cytochrome oxidase. This is not likely to be a characteristic of spores.

Further evidence is gained by staining the bacteria and the red fraction. When the Giemsa stain is applied to the bacterial cell preceded by a brief treatment with N hydrochloric acid at 60°C(6), bodies can be seen which could easily be red fraction granules. Fig. 3 shows these intensely stained bodies within whole cells of thermophile No. 2184. Fig. 4 is a preparation of red fraction similarly stained.

TABLE I. Oxygen Uptake at 37°C of Red Fraction in Presence of Succinate and Other Substrates.

Preparation	μl oxygen/hr
Red fraction	12
" " + succinate (.18 M)	110
" "	0
" " + p-phenylenediamine (.018 M)	450
Red fraction + hydroquinone (.018 M)*	252
Red fraction + p-phenylenediamine + cyanide (5×10^{-4} M)	87
Red fraction + hydroquinone + cyanide (5×10^{-4} M)*	0

Warburg flasks contained the following: 1.0 ml phosphate buffer pH 7.4; .5 ml fresh red fraction preparation containing 26 to 32 mg protein; .5 ml or distilled water to make a total volume of 2.8 ml.
* Corrected for autoxidation of hydroquinone.

From Fig. 3 and 4 it follows that the liberated granules are larger than the particles appearing in the intact cells. Roughly this difference is about 2-fold, which is about what was observed with phase microscopy. It is also apparent that the granules in the stained preparations (Fig. 4) are smaller than the granules seen in the wet mounts (Fig. 2). This is due to shrinkage upon drying during the preparation of the stained mount.

Another line of evidence that the particles are specific granules comes from their enzyme activity. Like mitochondria(7), the granules are the site of considerable enzyme activity. Thus far, we have reported on malic dehydrogenase, cytochrome oxidase, and apyrase(1-3). Malic dehydrogenase, cytochrome oxidase, and cytochrome c were found to be concentrated in the granules of the red fraction. In addition to these, there are aldolase(8), adenosinetriphosphatase(9), cytochrome b and succinoxidase. Figures from a typical run for the activity of the latter two are given in Table I. The cytochrome b assay is based on the manometric method rather than on the

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spectrometric, since the strong cytochrome c bands seem to prevent observation of other cytochromes.

Of special significance is succinoxidase activity. The oxidation of succinate by molecular oxygen requires a system of enzymes and cytochromes that seems to demand an unbroken organization(10). The opinion is held by many(11) that a disturbance of such a close association as is present in the cell results in destruction of succinoxidase activity. If this be true, then any preparation retaining succinoxidase activity must have been removed from the cell as an intact unit. The succinoxidase system from most sources, by virtue of its demand for organization, is extremely sensitive to inactivation(11). We have found the system in the red fraction granule to be sensitive to drying and aging and treatment with acetone, although not especially vulnerable to heat.

Taking all of the evidence together, from phase microscopy, from staining technics, and from enzyme activity, we now feel safe in making the statement that the red fraction is a specific granule isolated from the bacterial cell. To the best of our knowledge this is the first such instance reported for bacteria.

Conclusions. The red fraction, previously isolated from a stenothermophilic bacterium, represents a specific piece of bacterial protoplasm. The evidence presented consists of photographs taken with the phase contrast microscope, photomicrographs of stained preparations of bacteria and of red fraction, and of enzymatic data. The enzymatic data rest chiefly on the presence of succinoxidase activity.

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Effect of Desoxycorticosterone on the Colon: Its Relation to the Action of Cation Exchange Resins in Man.* (18573)

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One of the actions of the adrenal gland affects the transfer of sodium from the lumen of the kidney tubule to the surrounding blood stream. Desoxycorticosterone acetate (DOCA) acts in a similar fashion. This system of sodium transfer found in the kidney is analogous to that found in the intestinal tract where sodium is again transferred from the lumen to the surrounding circulation. Previous investigation of the influence of the adrenal gland on the transfer of sodium in the latter system has indicated that the adrenalectomized animal transfers sodium across the wall of the intestine at a somewhat slower rate than normal(1-3). The present study concerns the influence of DOCA on the mucosa of the colon as indicated by the eventual excretion of sodium in the stool of rat and man.

The rat was selected because normally sufficient amounts of sodium appear in the stool to measure changes with certainty(4,5). In man, the fecal excretion of sodium is normally so small(6) that changes are often difficult to interpret. For the purpose of this study, a cation exchange resin was fed to

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