In some of the inoculated mice there was a striking increase in the number of circulating white corpuscles. The highest count recorded in the experiments was 262,000 per cm., about 3 weeks after inoculation. The increase in cells was due to an absolute and relative increase in the number of lymphocytes, many of which were immature. In some instances, small numbers of abnormal cells were observed in the blood even when the total white count was normal.

Similar changes have resulted from the inoculation of emulsions of these experimentally produced lymphoid enlargements, and the condition has been successfully transmitted through several experimental passages. One line has now reached the sixth transfer.

Of 110 mice of strain C 58 that lived more than 10 days after inoculation, 36 are still alive, and 74 have died or been killed. Of the latter, transmission was successful in 56, unsuccessful in 10. Diagnosis is not yet complete in 8.

Fourteen animals of other strains which lived more than 4 days after inoculation with the same material, have had neither lymphatic enlargements nor leukemic blood pictures. Thirteen are still alive. One died but presented no lesions resembling leukemia.

4306

Gram Structure of Cocci.

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It has been shown[1] that when *B. anthracis* is exposed, in aqueous suspension, to certain dyes (Gentian Violet, Acriviolet, etc.) its Gram reaction is reversed; and that this reversal is accompanied by diminution in size and loss of weight of the bacterial bodies, together with increase in weight of the supernatant fluid. The existence of an outer Gram-positive cortex and inner Gram-negative medulla seemed thus to be proven. Gram-positive cocci could not be similarly changed by exposure to these dyes and it was, therefore, assumed that the Gram reaction of cocci might rest on a different mechanism from that of *B. anthracis*.

Experimental study running over the last 18 months, has shown, however, that exactly the same result as that produced in *B. anthracis* by exposure to certain dyes, may be produced in certain of

[1] Churchman, J. W., *J. Exp. Med.*, 1927, xlvi, 1007.

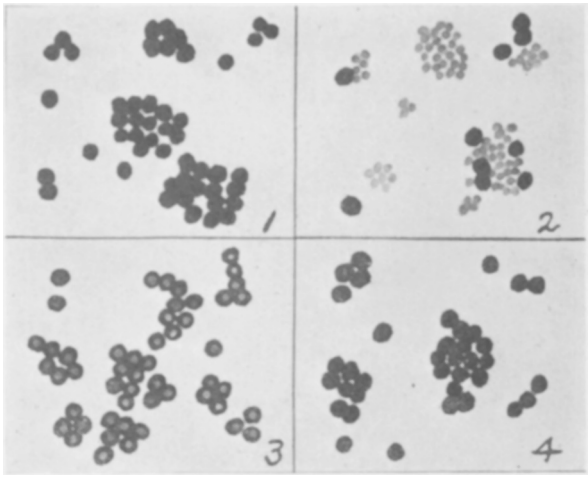
the cocci by exposure to a temperature of 52° Centigrade for a period of 10 hours or so. (Figs. 1 and 2.) Aqueous suspensions of many strains of *Staphylococcus aureus* when treated in this way become entirely Gram-negative. They also diminish in size, but the organisms are too small to measure accurately with a filar micrometer so that the amount of loss in diameter cannot be exactly stated. When centrifugated in Hopkins tubes, the organisms which have been exposed to 52° occupy less than half the space taken by the controls. They also lose about 50% in weight. This loss in weight is accompanied by a corresponding, though not an exactly identical, increase in the weight of the supernatant fluid.

The reaction does not occur in all strains of cocci. For example, the Gram reaction of *M. freudenreichii* is entirely unaffected by exposure to 52° C. On the other hand, the *Staphylococcus aureus* with which most of the experiments here reported were done—a virulent and typical organism isolated from a human being—behaved with great constancy in this respect after certain of the puzzling factors of inconstancy met with in the early experiments had been determined and taken into account. These factors were, strength of bacterial suspension, amount of suspension, and possibly the shape of the vessel in which the suspension was placed. A large series of experiments showed that constant results could be obtained with this and a number of other strains if, (a) not too strong a bacterial suspension were used, and (b) not too large an amount of bacterial suspension. Thus, when .05 cc. of suspension A* of *Staphylococcus aureus* were placed in a pipette and exposed over night in an incubator at 52° C., absolutely constant results were obtained. Complete or nearly complete reversal of Gram reaction, with marked diminution in size, always occurred. On the other hand, if 50 cc. of suspension B† were placed in a 100 cc. Florence flask and similarly exposed to 52° C., reversal of Gram reaction and loss in size and weight frequently failed to occur. The reversal of Gram reaction by exposure to 52° C. could also with constancy be entirely prevented by the addition to the bacterial suspension of 2 parts of Sodium Carbonate. (Fig. 4.)

That reversal of Gram reaction is not simply a death phenomenon is proven by the fact that if *Staphylococcus aureus* be killed in other ways—for example, by boiling—reversal is not produced; and by the further fact that though exposure of *M. freudenreichii* to 52° C. kills this organism, it does not affect its Gram behavior.

* Suspension A contained about 325,000,000 organisms per cc.

† Suspension B contained 20,000,000,000 organisms per cc.



1. Gram stain (Burke's modification) *Staph. aureus*. Control
2. Same stain after organisms have been exposed to 52° over night. Majority of organisms are now Gram negative and greatly reduced in size.
3. Effect of a pink center and purple periphery (similar to Gutstein picture) when Staphylococci are stained with methyl-violet and examined with bright illumination.
4. Gram stain of *Staph. aureus* which has been exposed, in sodium carbonate suspension, to 52°. No reversal or change of size (*Cf.* e Fig. 2).

The gain in weight which occurs in the fluid portion of bacterial suspension in which reversal of Gram reaction has been produced, is accompanied by the appearance of a positive ninhydrin test, and (if large amounts of suspension be used and the supernatant condensed by evaporation) of a positive Millon. It is hard to escape the conclusion from all the evidence that, when Gram reversal is brought about by exposure of cocci to 52° C., substance actually leaves the surface of the organisms whose Gram reaction has been reversed and passes into the fluid in which they are suspended.

By these studies, 3 grades of Gram positivity have thus been established: 1. *M. freudenreichii* remains positive after exposure to 52° and after exposure to the dyes mentioned. 2. Many cocci which show no change when exposed to the dyes mentioned are reversed in Gram reaction by exposure to 52°. 3. *B. anthracis* and many other spore-bearing aerobes become Gram negative either when exposed to the dyes mentioned or subjected to 52° C.

That the evidence thus advanced for the existence of an outer Gram positive cortex and an inner Gram-positive medulla is quite different in character from that advanced by Gutstein[2] for an ectoplasm and endoplasm ought to be sufficiently clear. Gutstein's

[2] Gutstein, N., *Centralbl. f. Bakt.*, 1924, i, 223.

evidence was tinctorial in character. Whatever interpretation be put upon his findings, it is certain that a picture identical with that obtained by Gutstein's staining method can be obtained without the use of any pink dye at all. If staphylococci be lightly stained with methyl-violet alone, the excess of dye taken off with alcohol applied for a couple of seconds and the smear examined with bright illumination, the organisms appear to have a pink center and a purple periphery exactly as described by Gutstein. (Fig. 3.) The same picture can be obtained by examining anthrax bacilli in a hanging drop to which a small amount of methyl-violet has been added. The axes of the organisms appear pink—their peripheries purple. This can hardly be interpreted as a tinctorial demonstration of ectoplasm and endoplasm; it is due entirely to the effect of bright light on the purple color.

In the experiments here recorded, the evidence offered for the existence of a Gram-positive bacterial cortex consists in the demonstration that this cortex can be removed by chemical and physical means and that its removal can be proven by a loss of weight in the bacterial bodies and by a gain in weight in the fluid in which they are suspended. It is also definitely shown that, although the cortex has been Gram-positive, the medulla which remains after its removal is Gram-negative.

4307

Characteristic Progressive Changes in the Hypertension Roentgenogram and Electrocardiogram.

ARTHUR M. MASTER.

One hundred fifty-two private cases of hypertension were followed for periods of between 3 and 9 years. Of these, 90% were patients with essential hypertension. Teleoroentgenograms, electrocardiograms and blood pressure readings were taken at intervals of 6 months or longer. Definite progressive changes took place leading to a characteristic shape, size and position of the heart and aorta on the roentgen-ray picture and also leading to definite changes in the size and shape and sign of the Q R S-T waves in the electrocardiogram. These final changes are specific for long standing cases of hypertension.

The characteristic x-ray film in hypertension shows a sthenic or hypersthenic chest; a hypertrophied left ventricle, usually a concen-