animals showed complete removal of both main glands, no macroscopic accessories, and no positive transplants. In 2 of these 4, the marking sutures were missing, due either to sloughing of the abdominal wall or to absorption.

Of the remaining 14 guinea pigs, 8 are still surviving in good condition, 2 for 40 days and the rest for 60 days after the removal of the second gland. These animals are active and eating, although 1 is not gaining in weight. Two were sacrificed in good condition 14 days after removal of the left gland; the autopsies showed complete bilateral ablation of the suprarenals, and numerous pin-head sized positive transplant areas, which on section showed nests of well vascularized cortical cells in glomerular formation. Four guinea pigs died from suprarenal insufficiency following removal of the left gland, the deaths occurring on the 15th, 41st, 48th, and 52nd day. Two of these animals were pregnant. The transplants in these animals had been absorbed.

Guinea pigs do not live on an average for more than 3 or 4 days after removal of both suprarenal glands. We have 8 out of 12 transplanted guinea pigs surviving from 40 to 60 days after complete removal of both suprarenal glands. These results, therefore, bring strong evidence in favor of the fact that the transplants are maintaining life.

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Purification of cultures of bacteria by means of reverse selective bacteriostatic properties of aniline dyes.

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In a recent study of the sporulation of *Bacillus anthracis,* this organism was recovered from the spleen and heart's blood of a mouse, dead of the experimental disease, in association with a small gram negative bacillus. The presence of the contaminating organism could not be detected on the agar transplants because of the overgrowth of *B. anthracis,* but it could readily be seen in smears. The observation of this gram positive spore bearing

bacillus with a gram negative bacillus presented an excellent opportunity to test the validity of the idea that in such cases purification of the cultures could be effected on the principle of reverse selective bacteriostasis. If the observations previously reported on this subject were correct, it would be possible in the case of this particular mixture of *B. anthracis* and a gram negative bacillus, to isolate either of the two organisms, in pure culture, by simply treating the mixture with one of two dyes, whose selective bacteriostatic properties, as between organisms of these two types, were known to be opposite in character.

As has been previously reported,[1] gentian violet and acid fuchsin possess these opposite selective properties. The culture of *B. anthracis* contaminated with the gram negative organism (which we may call *Bacillus X*), was therefore stroked in a heavy suspension across a divided gentian violet plate. The upper half contained dye in a 1/100,000 dilution. As had been expected, *Bacillus X* grew out in pure culture on the gentian violet half of the plate. Smears made from this culture showed practically nothing but *Bacillus X,* although an occasional individual *B. anthracis* could be seen, probably dead. An absolutely pure culture could be readily obtained by again stroking on another gentian violet plate an aqueous suspension of the growth obtained from the gentian violet half of the divided plate. It was thus very easy to rid the culture containing *B. anthracis* and *Bacillus X* of *B. anthracis* by growing it on gentian violet. The attempt was then made to rid the mixture of *Bacillus X* and obtain *B. anthracis* in pure culture. For this purpose to ½ cc. of an aqueous suspension of the contaminated culture, ½ cc. of one per cent acid fuchsin (Grübler) was added. These tubes, together with control tubes of a similar suspension containing no stain, were placed in a water bath and kept at 45 degrees for one hour. Streaks were then made on plain agar. All the controls grew well, clearly indicating that the slight amount of heat used had not injured either organism. In the tube containing acid fuchsin no *Bacillus X* appeared, a pure culture of *B. anthracis* being demonstrated by smears. It has thus been possible by the use of acid fuchsin to effect a selective purification of a contaminating culture opposite in character to that produced when the mixture was exposed to the bacteriostatic effect of gentian violet.

[1] Churchman, J. W., *J. Exp. Med.*, 1923, xxxvii, 1-10.

After these two organisms had been thus obtained in pure culture by the method of selective bacteriostasis, the selective susceptibility of each organism to the two dyes used, was then tested out. One half cc. of aqueous suspension of pure cultures of each of the two organisms were placed in test tubes. To one series of these suspensions a small platinum loop full of 1 per cent gentian violet was added, and to another series ½ cc. of 1 per cent aqueous acid fuchsin (Grübler). The gentian violet tubes were kept at room temperature, the acid fuchsin tubes at 45 degrees. At the end of an hour streaks from these tubes were made on plain agar, control inoculation of organisms which had not been exposed to dyes being always made at the same time. These experiments clearly showed that gentian violet in the strength used was entirely without effect on *Bacillus X,* though it completely inhibited the growth of *B. anthracis,* while acid fuchsin was entirely without effect on *B. anthracis,* although showing marked and often complete inhibition of *Bacillus X.*

Although I have made very clear in previous publications[2] that the bacteriostatic properties of the dyes are much more important than the bacteriocidal properties, other observers[3] who have repeated my experiments have not always borne this distinction clearly in mind. It should, therefore, be stated again that in the experiments as here described, emphasis is placed chiefly on the static properties of the dye and nothing is said as to their power actually to kill organisms. When bacteria are exposed to dyes and then streaked on plain agar, it is of course, perfectly clear that any result produced may be in part due to the inhibitive power of the dye which is carried over to the agar when the transplant is made. This fact, however, in no way invalidates the principle of reverse selective bacteriostasis which observations of the kind here reported, confirm. This principle is to the effect that in experiments done in such a way as to test the combined bacteriostatic and bacteriocidal powers of the dyes, but not so as to distinguish clearly between these powers, some of the dyes in strengths which inhibit gram negative organisms are without effect on gram positive spore bearing aerobes, while other dyes in strengths which inhibit certain gram positive spore bearing aerobes are without effect on certain gram negative organisms.

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[2] Churchman, J. W., *J. Exp. Med.*, 1912, xvi, 221.

[3] Burke, V., and Skinner, C. E., *J. Exp. Med.*, 1925, xli, 471.

The acid fuchsin used in these experiments came from a Grübler specimen which had been present for a long time in the laboratory. This particular sample of dye has been compared as regards its bacteriostatic property for *Bacillus X* with one other sample of Grübler dye and also with two samples of dye obtained from the Wills Corporation of Rochester, N. Y. 542, 45 and 524, 902. It has also been compared with neutral acriflavine. The experiments showed these substances also to possess a reverse selective power, though the sample first used (that is to say the Grübler dye) was more potent than any of the others. Certain observers[4] have reported that they have been unable to produce any static effect on bacteria by the use of acid fuchsin. The variation in the results which I obtained with different samples of this dye indicates that many substances of varying composition have probably been sold under the name of acid fuchsin and the variability in the results may be due to this fact.

The organism called *Bacillus X* was not absolutely identified. As the cultures from the animals dead of experimental anthrax, in which the contaminated *Bacillus X* appeared, were made a few hours after the death of the animals, it seemed quite likely that *Bacillus X* had invaded the blood and tissues from the intestine. The organism had the following characteristics:

Morphology	Short bacillus
Spores	None
Gram reaction	Sharply negative
Motility	5 hours, none; 24 hours, none
Gelatin	Liquefaction
Milk	No change
Litmus	Acid; no clot
Plain agar	Heavy growth
Plain broth	Heavy growth
Inulin water	No indol
Peptone water	No indol
Glucose	Acid, no gas
Mannite	No acid, no gas
Salicin	
Saccharose	
Lactose	
Russell's media	
Loeffler media	No liquefaction
Virulence	No virulence for mice

[4] Simon, C., and Wood, W., *Am. J. Med. Sc.*, 1914, cxlvii, 247.

Results of the kind here obtained might possibly be explained as due to a difference in hydrogen ion concentration of the dyes used. It seemed quite unlikely that this explanation was the correct one. The problem had already been studied and it had been found that by changing the hydrogen ion concentration of the agar used for divided gentian violet plates, the normal selective activity of this dye was in no way affected. If the pH of the agar were such that growth of the organisms was at all possible, the dye produced the expected selective result, no matter what the pH was.

In order, however, to check up this fact again, the experiments with *Bacillus X* and *B. anthracis* just described, were repeated, using—instead of the dyes—distilled water adjusted to hydrogen ion concentrations, corresponding to those of gentian violet, acid fuchsin (old Grübler) and acid fuchsin (new Grübler). These experiments were entirely negative, the fluids tested being without any effect on the growth of the organisms. The reverse selective bacteriostatic properties of these dyes are not, therefore, to be explained by variation in hydrogen ion concentration.

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Standardization of typhoid vaccine by photometric methods.

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Owing to an inquiry from Dr. Joseph W. Smith, Jr., of the Army Medical School, as to the suitability of the photomètre to estimate the strength of bacterial suspensions, a series of investigations were undertaken. Since the completion of this work, Dr. Smith[1] has published two articles on this general subject. From the result of our own experiments we are in accord with his critical statements in regard to the accuracy of the counts made by

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[1] Smith, Joseph W., Jr., *Am. J. Pub. Health*, May, 1925, 433; *J. Infect. Dis.*, 1925, xxxvii, 385.