of irradiated ergosterol, the ash of the bones was lower than in those of the control animals which had not received this addition.

It is our opinion, therefore, that very large amounts of activated ergosterol are able to withdraw calcium from the bones and that, if the dose is sufficiently large, they are able to accomplish this after the parathyroid glands have been excised. It should be emphasized, however, that this conclusion does not hold true for therapeutic doses of this drug, for it has been satisfactorily shown[3] that small amounts of irradiated ergosterol bring about a retention of calcium, as is true of cod liver oil and of direct ultra-violet irradiation.

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The "S" and "R" Forms of Hemophilus Influenzae.

MARGARET PITTMAN. (Introduced by Rufus Cole.)

From the Hospital of the Rockefeller Institute for Medical Research, New York.

During the course of an experiment with a culture of *Hemophilus influenzae,* transplants were made on a plate of Levinthal's solid transparent medium. It was observed that the culture contained 2 types of colonies. Well defined small transparent colonies resembling the textbook description of the typical Pfeiffer bacillus colony were found but in addition there were larger, slightly opaque iridescent mucoid smooth colonies. Transplants were made from the transparent colonies on Levinthal agar and all the colonies were found to be identical with the mother colony. The same was true of transplants from the opaque iridescent colonies. It was thus possible to separate 2 cultures each of which produced typical colonies. Since the largest transparent colonies were found to have a rough surface the strain producing these colonies has been called an "R" strain; and since the iridescent colonies from the other culture had a smooth surface this strain has been called an "S" strain.

This culture and 2 others from which "S" and "R" forms were subsequently isolated had been seeded many times on blood or chocolate agar and the variation in the colonies had not been noticed. Yet the difference in the colony appearance was marked when transplants were made on the transparent agar plates. Very striking was

[3] Shohl, A. F., Bennett, H. B., and Weed, K. L., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 551.

the difference when the plate cultures were held before a 100 watt electric light bulb or in the sunshine.

The morphology of the bacteria from the 2 strains has also been found to be dissimilar and characteristic for each type. This is best noticed if the stained preparation is made from a solid medium culture which is not older than 24 hours. The bacteria from the "S" culture appear as small coccobacilli that vary little in size. When preparations are made with special capsule stains there is definite indication of capsular substance, although the present methods of staining have not demonstrated a capsule that is as definite as is the capsule on the Pneumococcus. The bacilli from the "R" culture vary in length from coccobacilli to very long thread forms. The rougher the colony, the more anomalous is the morphology of the organisms; and with capsular stains we have failed to demonstrate the presence of any capsular substance.

An "S" and its corresponding "R" strain seem to be identical in their hemoglobin requirements and in their ability to form indol, to reduce nitrates to nitrites and to ferment the same sugars. To be added to these biological tests is a new one which is very interesting and probably significant. It is that both "S" and "R" strains of influenza bacilli are soluble in bile and desoxycholic acid.

At present we have 4 "S" strains. Three of these, mixed with "R" strains, were found among cultures which had been carried from 5 to 9 months. One of these cultures was from a case of meningitis, another was from the blood of a patient with pneumonia, and the third was isolated from the sputum of a case of broncho-pneumonia. Whether all the bacteria of these cultures were of the "S" form when originally obtained from the patient cannot be stated. But the fourth "S" strain, which was recently isolated from a case of broncho-pneumonia, was not associated with "R" forms.

It has been possible to change with great readiness all "S" strains into "R" strains by making daily transfers in 10% homologous anti-S serum broth. On the other hand, an "R" strain which had been converted a short time before from an "S" strain, has been reverted to its original "S" form by transferring it in anti-R serum broth.

The filtrate from a 20 hour old broth culture of the "S" strain contains a substance which is precipitable in homologous anti-S rabbit serum. Anti-R serum does not precipitate the S filtrate. And the filtrate from the "R" culture is precipitated neither by the anti-S nor by the anti-R serum. With the filtrates of the "S" strains so far isolated two distinct serological types have been observed. These

results suggest that the "S" organisms produce a soluble specific substance which may be analogous to the soluble specific substance produced by Pneumococcus.

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Filterability and Mechanisms of Excretion of Certain Dyes by the Frog's Kidney.

JEAN OLIVER AND ESHREF SHEVKY.

From the Departments of Pathology of Stanford University Medical School and the Long Island College Hospital Medical School.

This report concerns a correlation of the filterability of certain dyes with the manner of their excretion by the kidney as tested by the method of perfusion. The details of the technique of this method may be found in previous publications[1] and the results of our present experiments briefly summarized as follows:

Filtration of a series of dyes through collodion membranes gave the following average values of filterability. Phenol red, 100%; indigo carmine, 90%; toluidin blue, 60%; and neutral red, 35%. Trypan blue was unfilterable as such, but the pink component of the dye passed through the filter in small amounts, and brilliant red was entirely unfilterable.

By perfusion of either the glomerular or the tubular circulation of frogs with these dyes, it was found that phenol red and indigo carmine were eliminated chiefly through the glomeruli. Toluidin blue and neutral red on the other hand were excreted principally by the tubules. Trypan blue was excreted in the relatively short period that our experiments continued only in traces, and these traces passed through the glomeruli most readily and showed the same separation of the components of the dye as was observed in the filtration process, for the urine was pink in color. Brilliant red did not pass through either tubules or glomeruli.

Anesthesia of the tubules and repression of their function during the course of dye excretion increased the rate of elimination of phenol red and indigo carmine, but decreased the rate of excretion of toluidin blue and neutral red. When the glomeruli were damaged in such a way as to increase their permeability, both components of trypan blue escaped into the urine, which assumed the bluish color

[1] *J. Exp. Med.*, 1929, l, 15.