of undetermined (non-cystine) neutral sulfur which amounted to 19% of the extra sulfur. The analyses again indicated that additional cystine and not cysteine was being excreted.

These experiments tend to show that the metabolism of cystine may be quite different from that of cysteine and that these 2 compounds are not as interchangeable in intermediary metabolism as has been assumed generally. They further show that the metabolic behavior of an amino acid may vary markedly depending upon whether it is catabolized as a free amino acid (cysteine) or in combined form as a peptide (glutathione). The experiments also indicate that methionine may be metabolized via cysteine. The experiments thus indicate the possibility of a conversion of methionine into cystine. The experiments by Jackson and Block[2] and by White and Lewis,[3] which have established a metabolic interrelationship between methionine and cystine perhaps should be interpreted in this way.

The finding that cystine is excreted following the administration of cysteine indicates the possible rôle of the kidney in the oxidation reduction mechanism of the SS-SH system.

It appears that the cystine which the cystinuric excretes in the urine may be derived, in part or in whole, from that portion of the protein sulfur which is present in the protein molecule in the form of methionine. Further experiments will be necessary to determine to what an extent cystinuria is primarily a disturbance in methionine metabolism.

The possibility of a disturbance in glutathione synthesis in cystinuria should be considered. This view would be in keeping with the aspects of intermediary protein metabolism indicated in a previous publication.[4]

7123 P

Cultivation of European Type of Typhus Rickettsia in Presence of Live Tissue.

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The cultivation of Rickettsia presents considerable difficulties. Successful cultivation, with demonstrable rickettsia in cells, has been

[2] Jackson, R. W., and Block, R. J., *J. Biol. Chem.*, 1932, **98**, 465.

[3] White, A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **98**, 607.

[4] Brand, E., and Harris, M. M., *Science*, 1933, **77**, 589.

reported in tissue cultures by Wolbach and Schlessinger,[1] and Zinsser and Batchelder.[2] Pinkerton and Hass,[3] and Nigg and Landsteiner[4] were the first to obtain positive subcultures through several successive generations, the latter in flask or Maitland cultures. The last named authors worked with strains producing scrotal lesions in guinea pigs and used the tunica vaginalis infiltrated with rickettsia as the infective material. Pinkerton and Hass, though presumably working with a European strain, report the production (though irregular) of scrotal lesions in guinea pigs.

Our attempts to cultivate a European strain* by the methods used by Pinkerton and Hass or Nigg and Landsteiner failed to give positive cultures. Brain as well as tunica tissue was used as infective material but no rickettsia grew out. We, therefore, changed the procedure and used infected lice intestines for starting the culture.

In the experiments reported below successful initial cultures as well as subcultures were obtained by using infected lice intestines as infective material and following in all other respects the procedure employed by Nigg and Landsteiner. Apparently the positive outcome of these experiments is due to the use of heavily infected lice tissue, containing large numbers of rickettsia, to initiate the culture.

The technique was briefly as follows: Infected lice, 8-10 days after having been infected by the Weigle technique, were sterilized by treatment with alcohol and the guts removed aseptically into a drop of saline in a sterile hollow slide. Normal guinea pig tunica was macerated in the drop together with the infected louse gut and the mixture allowed to stand for 15-30 minutes. Small amounts of this material were then placed in small rubber-stoppered flasks containing 3 cc. tyrode-serum (2.5 cc. tyrode solution and 0.5 cc. fresh guinea pig serum). At first the cultures were placed at 37°C., but subsequently they were kept at 30°C. At the latter temperature cultures as well as transplants were obtained. The following typical protocols give the procedure and the results obtained thus far:

Experiment 1. Normal guinea pig tunica was macerated with 3 intestines of heavily infected lice and allowed to stand for half an hour. The minced tissue was distributed in small flasks containing

[1] Wolbach, S. B., and Schlesinger, M. J., *J. Med. Res.*, 1923, **44**, 231.

[2] Zinsser, H., and Batchelder, A. P., *J. Exp. Med.*, 1930, **51**, 847.

[3] Pinkerton, H., and Hass, G. M., *J. Exp. Med.*, 1931, **54**, 307.

[4] Nigg, C., and Landsteiner, K., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 3.

* Obtained from Dr. Breinl's laboratory. This is a typical European strain giving characteristic brain but no scrotal lesions. Scraping of the tunica failed to reveal rickettsia.

2.5 cc. Tyrode solution and 0.5 cc. fresh guinea pig serum and placed at 37°C. After 8 days a heavily infected endothelial cell was found in one of the flasks. The culture failed, however, to infect a guinea pig.

Experiment 2. Cultures were set up as above on 16/6 and 19/6 respectively and incubated at 30°C. After 8 and 9 days, respectively, fairly large numbers of rickettsia were found within cells as well as free near ruptured endothelial cells. After 13 days two of the positive cultures were mixed, the tissue ground, mixed with fresh minced tunica from a normal guinea pig and allowed to stand 30 minutes. The subcultures were set up as above and incubated at 30°C. Fourteen days later rickettsia were found in endothelial cells in the subculture. A guinea pig was infected with material from the positive cultures and after an incubation period of 12 days it developed a typical infection. A passage was made to another guinea pig which came down after 8 days and lice infected with the brain emulsion of this animal showed after 7 days numerous rickettsia in the epithelial cells of the intestine.

A successful transplant was, therefore, made from the first culture and the subculture was viable and infective 27 days after the initial culture was made. It should be noted that a suspension of rickettsia —infected louse intestines in serum tyrode solution without guinea pig tunica loses its infectivity for guinea pigs in 2 days at 30°C. Moreover, since the endothelial cells of the tunica in the cultures were found heavily packed with rickettsia it is evident that growth must have taken place. It seems reasonably certain that the European type of Typhus rickettsia can be grown in flask cultures containing guinea pig tunica and incubated at 30°C.

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Spectral Absorption of Purified Tuberculin.

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The characteristic spectral properties of normal proteins and their derivatives stimulate similar studies with material showing special

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