14 samples of butter made from infected cream and once from 11 samples of buttermilk. He was unable to isolate *Bact. abortus* from 12 samples of cottage cheese made from infected milk.

In our own experiments cream was collected by gravity separation from milk of cattle known to be shedding *Bact. abortus*. The gravity cream was not pasteurized and while still sweet was made into butter; however, commercially prepared butter is commonly made from pasteurized cream. One-half of the butter made from the cream was salted (3%) and one-half was not. The buttermilk was also collected. Guinea pigs were inoculated with these products shortly after they were made. Three such experiments were performed. *Bact. abortus* was isolated from the buttermilk, the salted and the unsalted butter. Two experiments have now been completed in which *Bact. abortus* was recovered from ice cream prepared from cream known to be naturally infected. Experiments are in progress to determine how long *Bact. abortus* will survive in butter and ice cream.

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Isolation of Mesobiliviolin from Human Feces. Its Origin and Nature.

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Coincident with the isolation of crystalline stercobilin,[1, 2] a substance resembling mesobiliviolin as first described by Fischer and Niemann[3] was regularly found in human feces.[2] Mesobiliviolin, as described by Fischer and Niemann,[3] exhibited absorption in the region of 600 and 500 mm. and showed green fluorescence with Zn acetate, the solution of the zinc salt having absorption at 626, 573, and 500. The similar substance found in feces had very similar characteristics,[2] and was consequently given the name kopromesobiliviolin.

It has now been determined that this substance still contained stercobilin as an impurity which accounted for the absorption in the region of 500 mm. as well as the green fluorescence of the zinc salt.

[1] Watson, C. J., *H. S. Z.*, 1932, **204**, 57.

[2] Watson, C. J., *H. S. Z.*, 1932, **208**, 101.

[3] Fischer, H., and Niemann, *H. S. Z.*, 1924, **137**, 292.

Their complete separation has now been effected by virtue of the somewhat greater solubility of mesobiliviolin in ether. After this separation, the violet pigment obtained possesses no "urobilin" absorption. Its hydrochloride has a more bluish violet color with 2 closely adjacent absorption bands, the narrower from 608-598 (max. 604) and the broader 594-550 (max. 575). The free substance has one broad band 587-570 (max. 578-582). On treatment with alcoholic zinc acetate the color of the solution becomes a light blue and a beautiful intense red fluorescence is exhibited. This solution has the following absorption: 625-627 (well defined, narrow), 583, max. (faint, broader).

The most highly purified substance was obtained in the following way: (1) repeated acetic ether extraction of the stool; (2) removal of the ether by vacuum distillation; (3) precipitation of fatty acids and koprosterin by dilution of the remaining acetic acid solution with a large volume of 1% HCl; (4) repeated ether extraction after making the aqueous filtrate congo negative with sodium acetate. After washing the ether with water until the latter no longer showed absorption at 500 mm., the following fractionation was carried out:

$$\text{Ether} - 1\%\ \text{HCl} - \text{CHCl}_3 - \text{soda}\ \frac{\text{acidification}}{\text{with acetic}} - \text{ether (washed with } \text{H}_2\text{O}) -$$
$$1\%\ \text{HCl} - \text{CHCl}_3 - \text{soda}\ \frac{\text{acidification}}{\text{with acetic}}\ \text{CHCl}_3.$$

After concentration *in vacuo,* the mesobiliviolin was precipitated by pouring the solution into a large volume of petroleum ether. This precipitation was repeated, and the precipitate then repeatedly extracted with dry ether, in which the substance is only a little soluble. After concentration *in vacuo,* and slow evaporation, a separation of what were apparently very small prisms took place. Because of their deep color and minute size it was impossible to positively identify the material as crystalline. The substance is not particularly stabile. Its solutions are definitely sensitive to light, and to evaporation in the presence of air. Attempts to obtain a more definitely crystalline material have not yet been successful. A material of the same appearance as that obtained out of ether may be had by adding boiling isopropyl ether to a hot chloroform solution, and further concentrating.

This substance decomposes below its melting point, exhibiting shrinking at 178-180°C., swelling and decomposition with evolution of gas at 240-245°C.

For purposes of comparison the same procedure as outlined above

was applied to mesobiliviolin as obtained by the iron chloride and heat oxidation of mesobilirubinogen (urobilinogen) according to the method of Fischer and Niemann.[3] It was found that the urobilin or stercobilin absorption band could in the same way as above be completely removed from the violet pigment, which thereafter exhibited the same absorption as that described for the mesobiliviolin out of feces, as well as strong red fluorescence with the zinc acetate, the zinc salt also having identical absorption.

Mesobiliviolin obtained by the above method *in vitro* from mesobilirubinogen has no definite melting point, exhibits shrinking from 135 to 168° at about which temperature some softening occurs without definite melting; at 230° swelling and apparent decomposition takes place. It is hoped that sufficient kopro-mesobiliviolin will soon be available to permit amalgam reduction with a view to obtaining a crystalline leukobase such as Fischer and Niemann prepared from mesobiliviolin (mesobiliviolinogen).

In the earlier literature there was occasional mention of the occurrence of bilicyanin or cholecyanin in human feces and urine.[4, 5] Fischer and Adler[6] found that certain of the blue oxidation products of bilirubin exhibit red fluorescence with zinc acetate. For this reason bilirubin was oxidized after the method of Heynsius and Campbell,[4] who originally described bilicyanin, and the blue and violet stages were compared spectroscopically with mesobiliviolin from feces and that obtained *in vitro* from mesobilirubinogen. Although the former in some instances exhibited red fluorescence with zinc acetate they were not identical spectroscopically with either of the mesobiliviolins.

[4] Heynsius and Campbell, *Pflügers Arch.*, 1871, **4**, 520.

[5] Mueller, F., *Virch. Arch.*, 1893, **131**, 106.

[6] Fischer, H., and Adler, E., *H. S. Z.*, 1932, **206**, 187.