experiments carried out on the same animals one week previously, the calcium lactate solution was administered without saponin.

The method of determining the plasma calcium content was that of Kramer and Tisdall as modified by Tweedy and Koch[1] and by Pincussen and Schimmelpfeng.[2]

The initial blood calcium values in 48 experiments, showed a mean of 11.4 mg. ± 0.069 mg. The maximum reached in 24 control experiments was 13.65 mg. ± 0.157 mg. The maximum reached in 24 saponin experiments was only 13.92 mg. ± 0.134 mg.

There was no evidence that the duration of the rise in blood calcium was effected by saponin. If the blood calcium curve can be taken as an index of calcium absorption, the influence of saponin, in the dog, appears to be negligible.

Positive effects have been reported in studies on the isolated intestine of the guinea pig,[3] on mice,[4] and on man.[5] The recent studies of Wokes,[6] however, have failed to confirm some of the earlier work.

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A Method for Estimation of Both Bile Salts and Cholesterol in Small Amounts of Bile.

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In experimental and human studies on the chemistry of the bile, it often happens that only very small amounts of bile are available in the gall bladder, as the contents of the diseased viscus are frequently very scanty. It is in these very ones that analysis of the bile may be the most important. The following method enables one to determine both the bile acids and cholesterol in a single cubic centimeter of bile.

Following the suggestion of Dr. F. C. Koch the marked affinity of petroleum ether for bile salts was utilized. The principle is that petroleum ether has the power of making a quantitative separation

[1] Tweedy, W. R., and Koch, F. C., *J. Lab. and Clin. Med.*, 1929, **14**, 747.

[2] Pincussen and Schimmelpfeng, *Biochem. Z.*, 1927, **183**, 42.

[3] Lasch, F., *Biochem. Z.*, 1926, **169**, 301.

[4] Kofler, L., and Fischer, R., *Arch. f. exp. Path. u. Pharm.*, **130**, 319.

[5] Berger, F., Tropper, E., and Rischer, F., *Klin. Wochenschr.*, 1926, **5**.

[6] Wokes, F., *J. Pharm. and Exp. Ther.*, 1931, **43**, 531.

of the bile acids and cholesterol in alcoholic solutions. The procedure is as follows:

One cc. of bile is diluted with 6 cc. of alcohol, brought to a boil and filtered to deproteinize. The filtrate is then shaken in a separatory funnel with 30 cc. of petroleum ether and extracted 3 times. The alcoholic fraction is then warmed to drive out the petroleum ether and used for the bile acid determination. The ether fraction (about 100 cc.) containing the cholesterol is placed in an ordinary 37° incubator and will evaporate to dryness in 30 to 45 minutes, as its boiling point is 25° to 65°C. It is then dissolved in chloroform, made up to 5 cc. and used for an ordinary colorimetric cholesterol estimation. The pigments remain in the alcoholic fraction.

Thirty-five samples of bile, normal and abnormal from dogs and humans have been studied for controls of this technique. In no case could any test for bile acid be obtained in the ether fraction or for cholesterol in the alcohol fraction.

In 2 cases difficulty in separating the layers in the funnel occurred. This in each case was overcome by the addition of 2 drops of water. This added water of course stays in the alcohol fraction and must of course be taken into consideration in calculation of the bile acid content.

This method had been used in our laboratory before the paper of Elman and Taussig[1] was brought to our attention. They extracted the bile with hot alcoholic 3% KOH. Perhaps owing to the lighter quality of the petroleum ether used by us (B.P. 25-65), we have found the use of either heat or alkali unnecessary. It would, of course, partly hydrolyze the bile salts so that the combined analysis could not be made.

[1] Elman, R., and Taussig, J. B., *J. Lab. and Clin. Med.*, 1931, **17**, 274.