Communication to the Editor

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Studies on Pharmacological Action of Choleretics by Infrared Spectroscopy

Effectiveness of infrared spectroscopy for the analyses of gallstones[1,2] and bile[3] was reported earlier and this was found to be one of the new effective methods for the study of pharmacological action of choleretics.

Infrared spectrum of dried whole liver bile of a dog has approximately definite form, having chiefly absorptions of sodium taurocholate and lecithin, and there is no necessity for making considerations about individual difference in a dog.

In the present series of work, choleretics was administered to laparotomized dog, its liver bile was collected every 30 minutes for 5~8 hours, and periodical changes in the volume of flow, amount of solid components, and quantity of bilirubin were measured. Further, changes in bile component were followed by infrared spectroscopy.

Infrared spectrum of bile is represented as the overlapped absorpions of its components and changes in bile components are observed in the infrared spectrum of the bile. Bile from dogs not administered choleretics show no changes in the amount of flow or solid components, and infrared spectrum of the bile remains the same as in ordinary dogs. Changes in the bile were followed by infrared spectroscopy after administration of 10 kinds of choleretics and following observations were made.

1) Volume of flow, amount of solid components, and infrared spectrum were measured after administration of three kinds of choleretics, sodium ursodeoxycholate[4] (I), 1-phenyl-1-propanol[5] (II), and 1-(5-norbornen-2-yl)ethyl hydrogensuccinate[6] (III), but almost no change was observed. In the case of (I), transient intensification of the lipid absorption (1738 cm^{-1}) occurred.

2) Sodium dehydrocholate (IV) is said to be a hydrocholeretic[7] and secretion of bile increases markedly 30~60 minutes after its injection but the amount of solid components increases only slightly. The absorption of sodium taurocholate (982, 951, and 917 cm^{-1}) at this stage is not proportional to the quantity of solid components, or rather, the absorption becomes extremely weak. In its stead, a substance with absorption at around 1700 cm^{-1} is secreted in a large quantity. By 6.5~7 hours after injection, the volume of bile flow is smaller than that before injection but bile components are back to normal.

3) α,4-Dimethylbenzyl camphorate diethanolamine[8] (V), magnesium bis(4-methoxy-γ-oxo-1-naphthalenebutyrate)[9] (VI), and 5-(p-methoxyphenyl)-1,2-dithiol-3(3H)-thione[10] (VII) are said to be cholaneretic, which increases secretion of natural bile but such was not found to be the case. Infrared spectrum that indicates constitution of bile components was different after administration of each of these choleretics and the common feature was the practical disappearance of characteristic absorptions of sodium taurocholate (982, 951, and 917 cm^{-1}). (V) caused no change in the absorption of lecithin (1738 cm^{-1}) but this absorption disap-

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peared in the bile after administration of (VI) and (VII). Some absorptions not observed in natural bile appeared instead, notably at around 1420 and 800 cm^{-1} by the administration of (V), at 1455, 877, and 838 cm^{-1} after (VI), and at 836 and 749 cm^{-1} after (VII).
4) Administration of 2-(1-hydroxycyclohexyl)butyric acid[11] (VIII), 1,4-O-dicaffeylquinic acid[12] (IX), or N-(*p*-hydroxyphenyl)salicylamide[13] (X) caused marked changes in the infrared spectrum of bile, showing appearance of several new absorptions (at 830 cm^{-1} after administration of (VIII), at 1396, 1261, and 1170 cm^{-1} after (IX), and at 1512, 1175, 832, and 754 cm^{-1} after (X)). Absorption of bile acid weakened in intensity but did not disappear as in the case of administration of (V), (VI), and (VII). The absorption of lecithin characteristically disappeared after administration of (IX).

The changes in infrared spectrum of bile after administration of choleretics are all characteristic to each of the choleretics and their reproducibilty has also been confirmnd. Work is now under way to elucidate the cause of these changes.

As far as the present series of experiments is concerned, none of the choleretics seemed to increase secretion of natural bile, *per se*. When a large volume of natural bile is secreted, volume of flow and amount of solid components must increase and there should not be any change in the infrared spectrum of the bile.

The method described here is an effective means in the potency assay of choleretics and this method is now being used for examination of new choleretics in this laboratory. Details of this work will be reported in the near future.

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