1:1,000 dilution of a stock solution which contained 22 mg. of the active fraction per cc. Hemorrhage was produced regularly by this dose, which contained 0.0022 mg. of active material.

8606 P

Chemical Treatment of Tumors. IV. Properties of Hemorrhage-Producing Fraction of *B. coli* Filtrate.

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The active fraction obtained from *B. coli* filtrate by the method described in the preceding paper was dissolved in water and evaporated to dryness *in vacuo*. A white residue was obtained, largely crystalline in nature. Strong heating produced some charring, but much of the crystalline residue was unaffected indicating that a considerable portion was inorganic in nature.

On repeated treatment of the active fraction with methyl alcohol, most of it (82%) went into solution. The methyl alcohol solution was evaporated to dryness and taken up in water, but most of it now failed to dissolve. However, addition of sodium hydroxide effected solution. Acidification with hydrochloric acid produced a heavy, flocculent precipitate which dissolved again on further addition of alkali. This solution was neutralized and tested on tumor-bearing mice; it was completely inactive as far as production of hemorrhage was concerned.

The methyl alcohol-insoluble portion (18%) dissolved readily in a small volume of water, with formation of a permanent foam. The presence of surface tension reducing material had been noted early in the purification, and its occurrence paralleled the presence of the active agent in the course of the fractionation. A stock solution was prepared containing 1 mg. per cc. of this fraction. This solution produced hemorrhage in mouse tumors in doses of 0.2 cc. of a 1:500 dilution, *i. e.*, the minimum hemorrhage-producing dose contained 0.0004 mg. of active material.

Upon evaporation to dryness *in vacuo* the active solution gave a residue which was largely non-crystalline. A comparatively small amount of crystalline material was present in which 3 crystal types were noted; the bulk of the residue consisted, however, of a film of non-crystalline glassy material.

The above-described stock solution of the active fraction gave a negative biuret test and a strongly positive Molisch test. It is interesting to note that the partially purified Shwartzman-active substances obtained by Apitz[1] also gave a negative biuret and a positive Molisch test.

The absorption spectrum of the active fraction was examined by Dr. E. Lorenz; no selective absorption was noted.

Since the most striking immediate effect of this agent on tumors is the production of severe hemorrhage, the reaction of the vascular system to this agent is of interest. Dr. V. Menkin[2] of the Pathology Department of the Harvard Medical School tested its effect on the permeability of capillaries of normal tissue; no increase in permeability was detected.

The purified fraction, in both concentrated and dilute solution, retained its activity after storage for 8 weeks in the cold room.

8607 P

Differential Bile Acid Analysis in Various Pathological Conditions.

HENRY DOUBILET AND RALPH COLP. (Introduced by Louis Gross.)

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By means of a method previously described,[1] bile from normal and diseased gall bladders and from biliary tract fistulae was analyzed for bile acids conjugated with taurine and with glycine, cholic acid, deoxycholic acid, total bile acids, and free bile acids. Duodenal drainage material was analyzed for cholic acid, deoxycholic acid and total bile acids. Some of the results are summarized in Table I.

A perusal of Table I shows that in a series of 24 cases of chronic cholecystitis (No. 2, Table), the average figure for the percentage of cholic acid in relation to the total bile acids was 28% as compared to the normal of about 50%. The average figure for the ratio of free bile acids rose from a normal of about 10% to 25%. This deviation in the ratio of the different bile acids is more marked in the gall bladder bile in acute cholecystitis. In 14 of

[1] Apitz, K., *J. Immunology*, 1935, **29**, 343.

[2] Personal communication.

[1] Doubilet, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 86.