observations). Although islets of resistant lymphoid cells were found, regeneration of the tumor apparently began with proliferation of the stroma (reticular) cells. Non-leukemic spleen and lymph nodes of the test animals were histologically normal 72 hours after the third injection of colchicine.

Summarizing, colchicine therapy in a transplanted malignant lymphoid tumor lengthened survival time appreciably in 7 of 14 animals, but did not permanently suppress tumor growth. The reticular stroma cells of the tumor were most resistant to therapy and probably are "malignant" elements in the lymphoid growths of mouse leukemia. "Malignant" lymphocytes proved to be more susceptible to colchicine action than normal splenic and lymph node lymphocytes in the treated tumor-bearing animals. Thymic lymphocytes were, however, killed by colchicine treatment. It is emphasized that colchicine administration is in no sense considered a cure for malignant lymphoid growths, but a drug which may be used to advantage in the experimental histological investigation of such tumors. So far colchicine has been found to hasten death in those animals where lymphoid neoplastic disease was systemic.

## 9933 P

## Quantitative Separation and Estimation of Various Porphyrins in Biological Materials.

J. T. BRUGSCH AND ANCEL KEYS.

*From the Division of Biochemistry, Mayo Foundation, Rochester, and the Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.*

We extract the porphyrins from urine, bile and feces by prolonged extraction with acetic acid-ether (Fink and Hoerburger,[1] Fikentscher,[2] Van den Bergh and others,[3] Brugsch[4]). Uroporphyrin is lost but, according to Fischer and Zerweck,[5] this may be neglected except in extremely rare cases. The porphyrins are taken up by 5% HCl from the crude ether extract. Purification involves repetition of the transfer to ether and back to HCl. Phylloerythrin

1 Fink, H., and Hoerburger, W., *Z. physiol. Chem.*, 1931, **202**, 8.

2 Fikentscher, R., *Biochem. Z.*, 1932, **249**, 257.

3 Van den Bergh, A. A. H., Grotepass, W., and Revers, F. E., *Klin. Wschr.*, 1932, **11**, 1534.

4 Brugsch, J. T., *Z. ges. exp. Med.*, 1935, **95**, 471.

5 Fischer, H., and Zerweck, W., *Z. physiol. Chem.*, 1924, **137**, 242.

requires 10% HCl. Total porphyrin is estimated in 5% HCl by the intensity of the red fluorescence excited by ultraviolet light. Pure copro- or hematoporphyrin solution is used for standardization. Recovery of known amounts of pure porphyrins in 12 series of determinations averaged: coproporphyrin, 100%; hemato-porphyrin, 104%; mesoporphyrin, 97%; protoporphyrin, 103%.

Quantitative fractionation of the porphyrins is done by successive extractions of the purified ethereal solution with 1 volume 0.25% HCl to 3 volumes ether. Three such extractions remove all the coproporphyrin (and hematoporphyrin which, however, does not occur naturally), 62% of the mesoporphyrin, 10% of the protoporphyrin, and about 49% of the deuteroporphyrin.

These results on pure porphyrins apply also to mixtures. Quantitative estimates of copro- and protoporphyrin in known mixtures are precise within ±3% of the total; similar mixtures of copro- or proto- with mesoporphyrin were separated with an error no greater than ±6%.

With the foregoing mixtures, and with biological materials which can be treated as 2 component mixtures of porphyrins, the calculation is:

(1) Total porphyrin = A + B.

(2) $B = M/(1-y)^n$, where $n$ is the number of extractions required to remove A completely, $M$ is the amount of porphyrin remaining after $n$ extractions and $y$ is the distribution coefficient in extraction number $n + 1$.

(3) $y = r_n/M$, where $r_n$ is the amount of porphyrin extracted in extraction number $n + 1$.

There is rarely any important error in treating urine and bile as 2-component systems of copro- and non-coproporphyrin; feces may also be so treated but in some cases the error may exceed 10% of the coproporphyrin. Results are satisfactory with 5 to 200 micrograms of total porphyrins.

Five hundred analyses of urines from 142 normal individuals show that the 24-hour output of normals never exceeds 100 gammas of porphyrin, and that less than 10% are over 60 gammas. At least 95% of the urine porphyrin is normally of the coproporphyrin type.

In human bile 60 to 85% of the porphyrin is of the coproporphyrin type. We did not find phylloerythrin in human bile. In ox bile we found only 10 to 20% coproporphyrin; about 30% was phylloerythrin and nearly half of the total porphyrin was of the pyrroporphyrin and other intermediate types.

In human feces with either negative or questionable benzidine

reaction, coproporphyrin type accounted for, on the average, 30 to 40% of the porphyrins. Feces with a positive benzidine reaction (free hemin) averaged only 18% coproporphyrin type, reflecting a great increase in protoporphyrin. Acholic feces (biliary obstruction) showed zero to 3% coproporphyrin in the patients studied.

In all feces we found a considerable amount of porphyrin of the meso type similar to Schumm's[6] "sapro-porphyrin D." We also find in human feces very minute amounts of a chloroform-soluble porphyrin with a rather low HCl number; this may be what Watson[7] has termed "pseudo-deutero porphyrin."

Quantitative separation and analysis of porphyrins in the feces seems to offer a sensitive means for early detection of biliary ob-

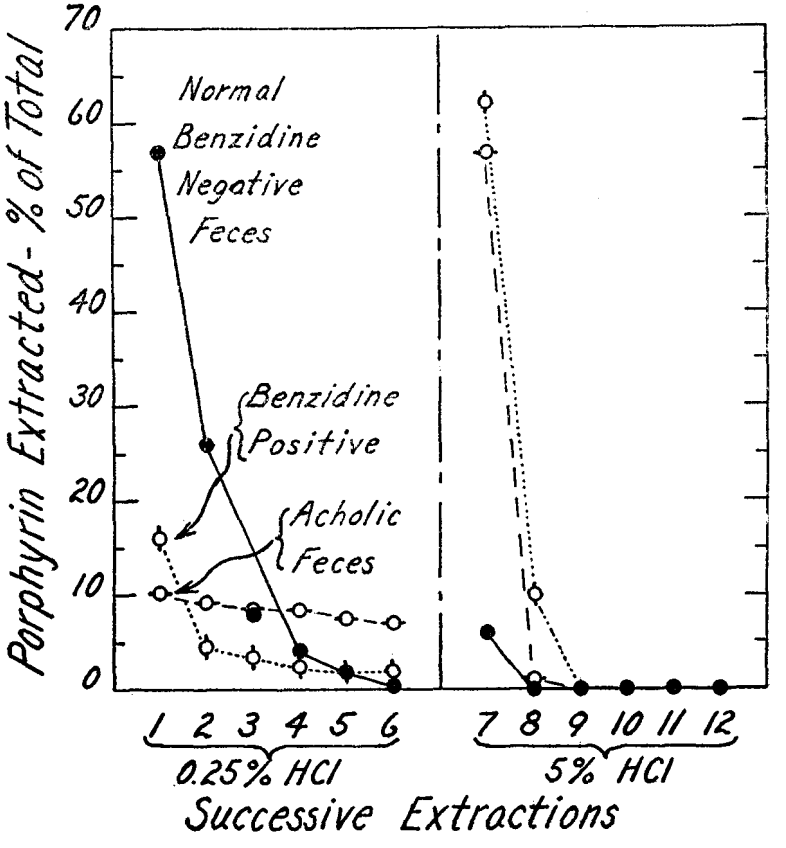


FIG. 1.

Extraction curves for porphyrins in human feces. In each extraction 1 volume of acid was used for 3 volumes of the ethereal solution.

[6] Schumm, O., *Z. physiol. Chem.*, 1927, **169**, 3, 52.

[7] Watson, C. J., *J. Clin. Invest.*, 1937, **16**, 383.

struction and perhaps for the occurrence of bleeding in the gastro-intestinal tract. Typical extraction curves from human feces are shown in Fig. 1.

9934 P

## Blood Potassium Changes as a Result of Partial Asphyxia in Dogs.

JOE DENNIS AND F. J. MULLIN.

*From the Departments of Biological Chemistry and Physiology, University of Texas Medical School, Galveston, Texas.*

This work grew out of previous studies showing that ischemia of the functioning heart leads to a marked rise in the potassium content of the coronary venous blood.[1] This report is concerned primarily with the effects produced in anesthetized dogs on clamping off the tracheal cannula for periods of from 2 to 4 minutes. Twelve dogs anesthetized with nembutal were used. In half the dogs only one asphyxial period was produced, in the others 2 or more were obtained. Blood was drawn from the external jugular vein in 10 of the dogs. In 2 dogs the chest was opened and artificial respiration administered, the blood being obtained from the superior cava in one and from the right auricle in the other. The method of Breh and Gaebler[2] was used in the potassium determinations, the analysis being completed by the diazotization procedure of Briggs.[3] The condition of the blood samples was unknown to the analyst, since they were given blind numbers when they were drawn.

The results are given in Table I. They show that during asphyxia the plasma potassium increases 22% and the whole blood potassium 16% over the normal control values. There is an increase of 43% for the plasma and 14% for the whole blood potassium during a second asphyxial period, as compared to its control taken immediately before. The second asphyxial period was produced from 10 to 20 minutes after the first one.

These figures show the increase in blood potassium during asphyxia. Data on the effects of over-ventilation are as yet inconclusive. Fenn has shown that muscular activity causes a decrease in

1 Dennis, Joe, and Moore, R. M., *Am. J. Physiol.*, in press.

2 Breh, F., and Gaebler, O. H., *J. Biol. Chem.*, 1930, **87**, 81.

3 Briggs, A. P., *J. Biol. Chem.*, 1923, **57**, 351.