with 10^{-9} mg of cell material per milliliter but not with 10^{-10}. According to the actual count, 10^{-7} mg of cell material should contain 6 viable cells; it is, of course, possible that one or more cells would be found even in the higher dilutions which would account for the growth recorded for the 10^{-9} mg ml suspension.

When a medium is inoculated with increasing concentrations of cells, there is a corresponding decrease in the lag period of growth, but all of the resulting cultures finally reach the same degree of density. On the other hand, if the inoculum is held constant and the nutrient value of the medium is varied, quite different results are obtained. An increase in the amount of growth is found to parallel enrichment of the medium. This is accompanied by a shortening of the lag period.

The effect of nitrogenous materials on the rate of growth of *M. tuberculosis var. hominis* H37Rv is presented in Table II. The incubation time required for the initiation of growth in a medium containing ammonium citrate was 10 times that required in one containing casein, a vegetable extract and serum-albumin. The difference in the amounts of growth was even more striking, the more complex medium giving 20 times more abundant growth. The rate and amount of growth are dependent not only on the number and concentration of nutrients but on the nature of the nutritive material. Tryptophane in a concentration of 0.1% is a better nutrient than 0.2% asparagine for all of the strains of mycobacteria.

Having established the normal rate of growth of mycobacteria, it was now possible to evaluate the effect of streptomycin upon the growth of mycobacteria in the presence of different sources of energy. Regardless of the source of nitrogen or of the amount of growth supported by the medium, little difference was found in the amount of streptomycin required for bacteriostasis, as shown in Table III. The only exception is found in the ammonium citrate medium; limited effectiveness of streptomycin is possibly due to the high acidity of this medium resulting from the growth of the organism.[7] Similar results were obtained with *M. avium* and the human pathogenic varieties.

Summary. A simple, safe, and accurate method is described for the quantitative estimation of the growth of *Mycobacterium tuberculosis* based upon turbidimetric measurements in Dubos' medium.

This procedure can be applied to measuring the rate and amount of growth of *M. tuberculosis* in various media, as affected by the presence of streptomycin.

[7] Waksman, S. A., Bugie, E., and Schatz, A., *Proc. Staff Meetings of Mayo Clinic*, 1944, **19**, 537.

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Preparation of an Anti-Ulcer Factor from Human Urine.*

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The preparation of crude extracts from urine (Urogastrone) and hog intestinal mucosa (Enterogastrone) which possess antigastric secretory and antiulcer activity in dogs, such as the Mann-Williamson and the Pavlov preparation has been reported in the literature. Two methods have been applied to urine for the preparation of Urogastrone or Urogastrone-like substances. Necheles[1] has prepared an inhibitor of gastric secretion and motility by treating normal human urine with ammonium sulphate and fractionating the resulting precipitate between vary-

* The authors are indebted to Mr. and Mrs. Francis E. Fowler and members of their family whose generosity has made these studies possible.

[1] Necheles, H., Hanke, M. E., and Fantl, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 618.

ing concentrations of alcohol. Gray, Wiecyrowski and Ivy[2] used a benzoic acid adsorption method for the preparation of an antisecretory substance from normal male urine, and Friedman *et al.*[3] have found an inhibitory substance in normal female urine by using the same method of adsorption. An examination of the methods elicits little information regarding the chemical properties of the extracts except that they are water soluble and insoluble in organic solvents such as ethyl ether, benzene, and petroleum ether.[4] The further characterization of these extracts has been greatly retarded due to the lack of a convenient assay method.

The Mann-Williamson dog which has been generally used in testing for antiulcer properties of various preparations, entails considerable time, expense, and experience which greatly limits its usefulness. For an excellent discussion regarding the present status of these antiulcer "hormones" see the reviews of Ivy[5] and Sandweiss.[6]

With the recent availability of the rat assay method[7,8] in which extensive gastric ulceration can be produced in the rat in the short period of 7-9 hours following ligation of the pylorus, the investigation of antiulcer factors has been continued. A fraction has been prepared from human urine which when given to rats in sufficient quantity at the time of pyloric ligation prevents the formation of the gastric ulcers. The method consists essentially of adsorbing the activity on carbon from clarified urine at neutral pH, eluting the activity from the carbon with acidified aqueous-acetone at pH 2, and precipitating the activity from the eluate by increasing the acetone concentration. This method has been used continuously in this laboratory over a period of 6 months without obtaining an inactive preparation.

Experimental. The urine used in these studies was obtained over 24-hour periods from male and female patients who were in the clinic mainly for diagnostic purposes. Most of this urine was collected in the early morning hours and processed within 8 hours. The urine, which was collected in 2.5 liter bottles and preserved with chloroform, was decanted from the chloroform before carrying out the initial clarification with Celite-545.

The Assay procedure used in these experiments has been mentioned elsewhere.[8] The method consists of using male rats 8 weeks of age or female rats 10 weeks of age weighing 120 to 150 g. The animals are fasted for 48 hours before operation in individual cages with wide wire mesh bottoms, supported well above the dropping paper. Water is allowed *ad libitum*. For the operative procedure the rats are anesthetized with ether. Through a short midline incision extending downward from the xiphoid process, the duodenum is grasped lightly with ring forceps and a ligature of linen thread is placed exactly at the pyloric sphincter. The incision is closed with Michele wound clips and the wound is covered with a thin coating of flexible collodion. At the time of the operation the material to be tested for antiulcer activity is administered intravenously to the test group and physiological saline is given to the control group. A minimum of 6 to 10 rats should constitute a test group and an equal number is used for the control group. Eight or 9 hours after tying off the pylorus the animals are sacrificed. The abdominal cavity is opened, when the animal is under ether anesthesia, and the esophagus is grasped with a hemostat. The stomach is then freed of mesentery and removed. A small slit is made on the greater curvature of the distended stomach for collection of the gastric juice. The stomach is finally cut along the whole length of the greater curvature and after a brief rinsing in physiological saline, is stretched out with the aid of pins on beeswax for examination

[2] Gray, J. S., Wieczorowski, E., and Ivy, A. C., *Science*, 1939, **89**, 489.

[3] Friedman, M. H. F., Recknayel, R. O., Sandweiss, D. J., and Patterson, T. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 509.

[4] Gray, J. S., Wieczorowski, E., Wells, J. A., and Harris, S. C., *Endocrinology*, 1942, **30**, 129.

[5] Ivy, A. C., *Fed. Proc.*, 1945, **4**, 222.

[6] Sandweiss, D. J., *Gastroenterology*, 1945, **5**, 404.

[7] Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., and Siplet, H., *Gastroenterology*, 1945, **5**, 43.

[8] Pauls, F., Wick, A. N., and MacKay, E. M., *Science*, 1946, **103**, 673.

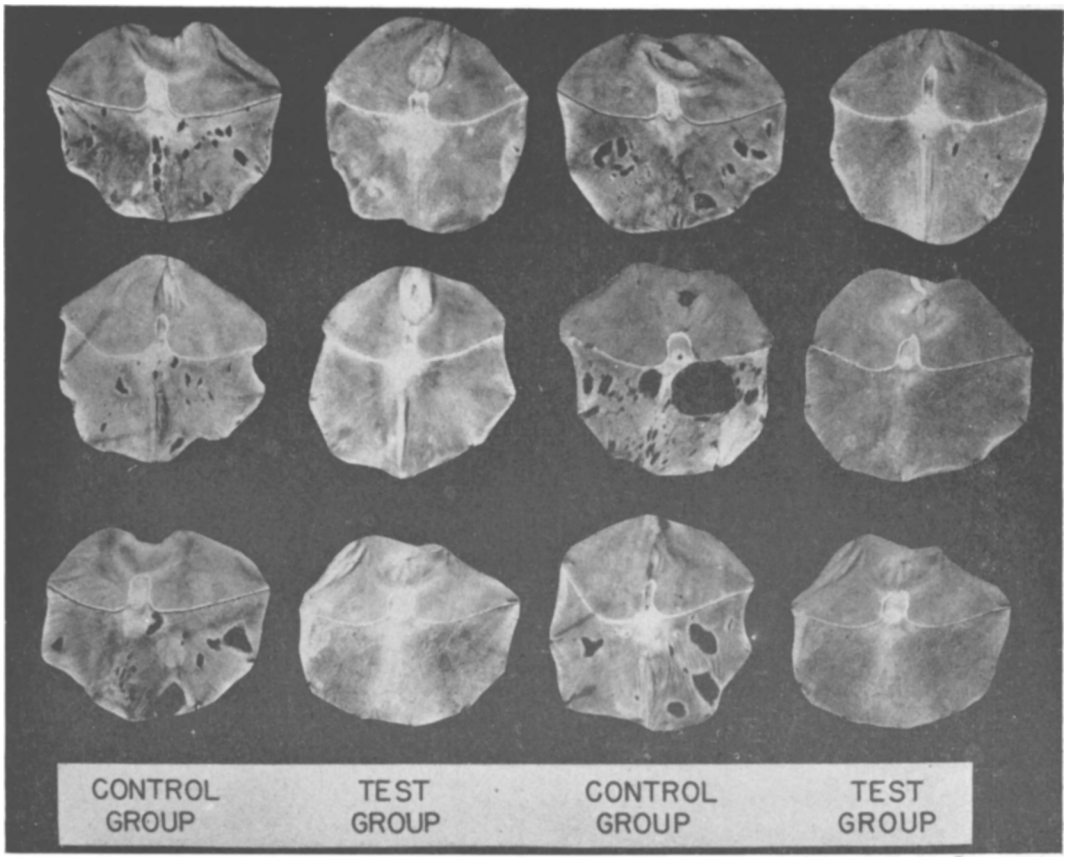


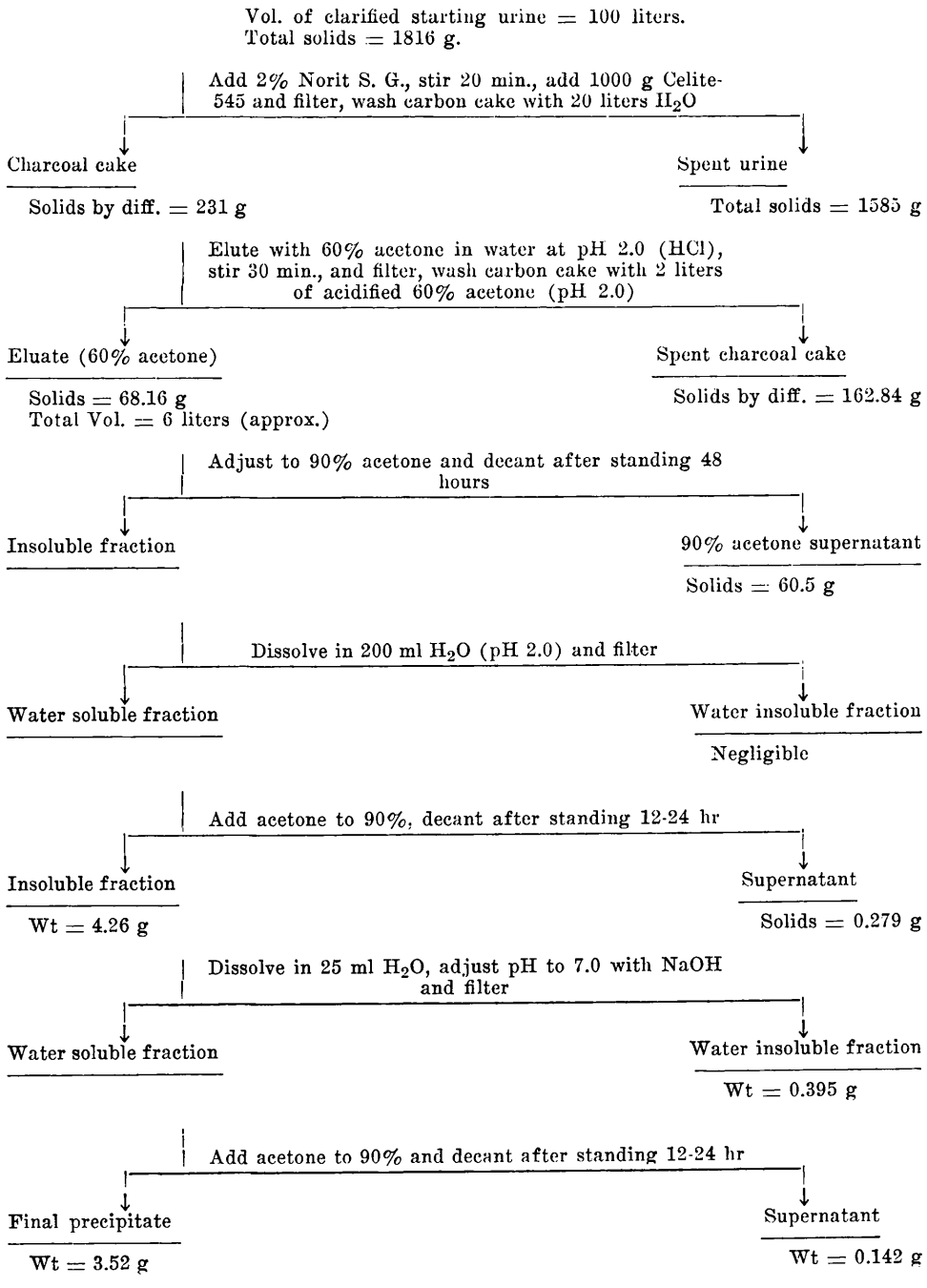
FIG. 1.

of the ulceration.

The steps for the preparation of the anti-ulcer substance are described for 100 liters of urine. Together with the distribution of solids they are outlined in detail in the accompanying flow sheet. The routine used in this laboratory is to pool all of the urine each morning and to clarify it by filtration with the aid of 1% Celite. The pH of the clarified urine has ranged from 6.0 to 6.5 and no further adjustment is made before the addition of the carbon. The percent Norite S.G. has been found to give satisfactory results for the adsorption of the activity. After the suspension is stirred for 20 minutes, the carbon is collected in large Buchner funnels. Celite is added to the mixture before filtering in order to facilitate the filtration procedure. The charcoal cakes thus obtained each day are stored in 4°C cold room until the end of the week at which time they are combined for the elution of the active substance. The elution is carried out in 60% acetone by volume in water at pH 2.0. The water content of the carbon cake, which is readily determined by weighing, is taken into account when adjusting to the 60% acetone concentration. Additional amounts of 60% acetone in water are added if necessary to prepare a thin slurry for efficient stirring. Although one elution is carried out in these experiments, a second and third elution can be used if so desired. The final product, after the neutralization and the subsequent filtration, can be obtained by the lyophile process or by increasing the acetone concentration as shown in the flow sheet.

Results. The preparation obtained in this carbon-acetone method will prevent complete-

FLOW SHEET FOR THE ISOLATION OF AN ANTI-ULCER EXTRACT FROM URINE.



ly the formation of gastric ulcers in our rat-assay method. The quantity of material required to inhibit the ulceration has varied in different preparations from 50 to 100 mg/rat. Fig. 1 shows the stomachs removed from 6 control rats which received injections of physiological saline, and 6 test animals which received 50 mg of the urinary product intravenously at the time of pyloric ligation. The control group all show marked ulceration, while those in the test group receiving the antiulcer substance show a marked diminution or a complete absence of ulceration. Such clear cut results are not always obtained, especially when the minimal active dose of antiulcer material is being investigated. For such cases we have been recording the gastric ulceration by an arbitrary scale which ranges from "clear" or "zero" to "4 plus" ulceration.

Discussion. The chemical nature of the urinary antiulcer factor described here has not been determined, although the carbon-acetone method, which we have used for its preparation, is similar to that used for the isolation of the basic substance streptomycin.[9] A discussion of the chemical relationship between Urogastrone, Enterogastrone, and the urinary "carbon-acetone" material and proof that the antiulcer activity of the "carbon-acetone" produced extract is due to a single chemical entity must wait until further purification can be carried out.

Our urinary line product is approximately 5% ash and contains 5-6% nitrogen. The neutralized material is insoluble in organic solvents which are immiscible with water. The product obtained by acidifying a solution of the antiulcer factor to pH 2.0 and drying by the lyophile process is soluble to the extent of approximately 1 g per 100 ml methyl alcohol at room temperature. This fraction has greater activity than the starting material or the methyl alcohol insoluble fraction. The antiulcer activity of the crude line product can be more readily increased by fractionating with aqueous-acetone. In a 5% aqueous solution of starting material, the fraction precipitated by increasing the acetone concentration from 30 to 60% contains 30% of the solids and approximately 60% of the starting activity.

The antiulcer factor is fairly stable to alkaline hydrolysis. When 800 mg is dissolved in 20 ml of 0.1 N NaOH and refluxed for 24 hours, approximately 50% of the activity is retained. In contrast, when an equal quantity of the material is dissolved in 20 ml of 0.1 N HCl and refluxed for one hour, most if not all of the activity is destroyed. Shorter periods of acid hydrolysis have not been studied although the acid solution darkens rapidly after the start of refluxing.

We are unable at the present time to estimate the concentration of the antiulcer factor normally occurring in the urine. Assays carried out on the clarified and spent urine at maximum tolerable levels (200 mg/rat) have shown no reduction in gastric ulceration. The "90% acetone supernatant" which constitutes 85 to 95% of the solids in the "Eluate" has slight activity at 100 mg/rat. This fraction has an ash content of 28% and since we have observed that it is quite toxic at therapeutic doses, we are hesitant in attributing much importance to this fraction.

No attempt can be made at this time to speculate on the mechanism of the antiulcer effect exerted by the material prepared from urine. Ulceration may be reduced in extent or entirely prevented by a reduction in the secretion of gastric juice, impaired peptic activity of this juice or an enhancement of the resistance to ulceration which is inherent in the gastric mucosa.

Summary. A neutral extract has been prepared from normal human urine by adsorption on charcoal and elution thereof with 60% acetone in water at pH 2. This material, when given to rats at the time of pyloric ligation, prevents the development of the gastric ulceration which normally occurs with this procedure.

[9] Vander Brook, M. I., Wick, A. N., De Vries, W. H., Harris, R., and Cartland, G. F., *J. Biol. Chem.*, 1946, **165**, 463.