influenza virus. This cannot be judged finally since comparable data are not available for the hemagglutinative reaction. The fact that the absolute relationship of the enzymatic activity to the particle number and to infectivity is not fully understood does not detract from the usefulness of the test.

Use of the smallest possible volumes for the test is necessary since the chicks yielded on bleeding about 1.5 to 2 ml of plasma and even a minute drain on the circulating blood frequently leads to death of the chick before the test can be completed. It happens that with the capillary tubes employed, the volume of plasma from the best chickens was only 5 λ or 10 λ. The range of activity measurable could be extended by the use of 10 λ volumes, but in practice this has not seemed desirable.

*Summary.* A micro procedure has been designed for the rapid testing, in 5 λ volumes, of the plasmas of chickens with erythromyeloblastic leukosis for their capacity to dephosphorylate adenosine triphosphate. Since this enzymatic activity is related both to the number of virus particles and to the infectivity of the plasmas, the test provides the means either for the measurement of those qualities or for the selection of chicks possessing the qualities in the desired degree. There is a wide variation in this enzymatic activity among the individuals of the diseased chick population. For practical application to the study of erythromyeloblastic leukosis, the test is comparable with the hemagglutinative reaction with the influenza virus. The test in its present form would not be useful in the diagnosis of erythromyeloblastic leukosis which can be accomplished much more easily by blood smear. No experiments have been made to learn whether the reaction might occur with the plasma of birds with visceral lymphomatosis which is much more difficult to diagnose in the intact bird.

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## Tubeless Gastric Analysis by Use of Insoluble Salts with Exogenous Ions.* (20391)

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Tubeless gastric analysis was introduced by Segal, Miller and Morton(1). The principle was based upon the appearance in the blood, urine and/or saliva of a non-toxic exogenous indicator ion after its release from an insoluble indicator compound by the action of the hydrogen cations of gastric juice. It was also suggested that the special indicator ion might be a dye, a radioactive material or any easily detectable substance. Extensive experience with the oral administration of an insoluble indicator compound† comprised of a cation exchange resin with the quininium cation as the specific exogenous indicator ion, has proved the efficacy of this technic(2-6). *In*

* Supported by a Special Medical Research Fund.

† Marketed as Diagnex by E. R. Squibb & Sons, New York.

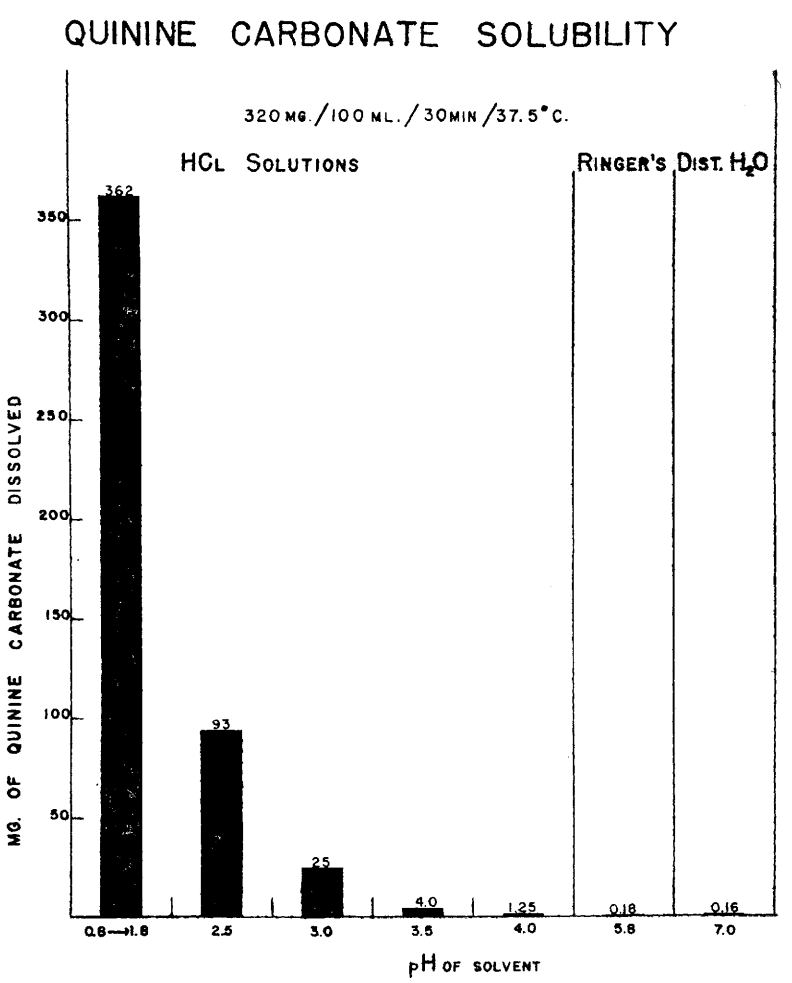


FIG. 1.

*vitro* and preliminary clinical investigations of other insoluble indicator compounds prepared with ion exchange resins and various special exogenous indicator ions as methylene blue, serenium, pyridium, fluorescein have also been reported(7). Similarly, tubeless determination of gastric acidity by the oral administration of insoluble salts such as calcium carbonate and magnesium pyrophosphate containing Ca 45 and P 32, respectively, as labeled endogenous indicator ions has been described by Maurer and Zimmer(8). Obviously, the detection of tagged endogenous ions in such compounds is not generally applicable.

The present study is concerned with the use of quinine carbonate as an example of an insoluble salt with a special exogenous indicator ion which by its solubility in hydrochloric acid permits the detection of free gastric acid without subjecting the individual to intubation. *Quinine carbonate* $CO_3(C_{20}H_{23}O_2N_2)_2$ is an amorphous white substance with a molecular weight of 674.39 and containing 96.14% of quinine. It is practically insoluble in water and Ringer's solution and soluble in hydrochloric acid. One milliequivalent of the available quininium cations of this compound should react with 1 mEq of the hydrogen cation of hydrochloric acid to form 1 mEq of quinine hydrochloride. Studies in this laboratory have shown that from 0.3% to 1.4% (average 1%) of the quinine of quinine hydrochloride administered orally will be excreted in the first 2 hours' urine and from 0.8% to 2.2% (aver-

age 1.6%) in the first 3 hours' urine. Hence, if the quinine carbonate administered orally were available to react with all of the gastric hydrochloric acid secreted per unit of time, a quantitative assay of the quinine excreted in the urine 2 or 3 hours after the oral administration of quinine carbonate should afford an approximation of the hydrochloric acid secretion per unit of time. This is at best an approximation since the orally administered quinine carbonate and the gastric juice may both pass into the duodenum before complete stoichiometric reaction can occur, and since the excretion range of quinine varies from 0.3 to 1.4% in the first 2 hours' urine. Clinically, this should suffice to differentiate between degrees of acidity and achlorhydria, if sufficient quinine carbonate can be administered orally.

*Methods.* A) *In vitro* experiments. The following technic was used to test the solubility of quinine carbonate in hydrochloric acid of varying hydrogen ion concentration, in Ringer's solution and in water. To 32 mg of quinine carbonate in a test tube were added 10 ml of the solution to be examined. Temperature was kept constant at 37.5°C and the mixture was stirred with a slow stream of air for 30 minutes. The solution was then filtered through Whatman No. 5 filter paper and 5 ml of the filtrate were placed in a 60 ml capacity separatory funnel. 1 N NaOH was added until the pH was greater than 11. Fifteen milliliters of ether were then added and mixed gently. Enough ether was then withdrawn to allow 8.2 ml to remain in the funnel. Five milliliters of 0.1 N $H_2SO_4$ were next mixed with the 8.2 ml of ether. The acid solution which now contains the quinine was drawn off and diluted with the appropriate amount of 0.0015 N $H_2SO_4$ to read on the Lumetron photofluorometer (standard 2 $\mu$g). The percentage of error is $\pm$10%.

*Results.* Fig. 1 reveals results of the test of the *in vitro* solubility of quinine carbonate multiplied by 10 to show the extent of solubility of 320 mg of quinine carbonate in 100 ml of the solution tested.

B) *In vivo* experiments. *In vivo* experiments were performed by administering orally different doses of quinine carbonate mixed in about 60 ml of water. Five hundred milligrams of caffeine sodium benzoate were used as the gastric stimulant for individuals in whom the gastric secretory response to caffeine had been determined previously, either by the usual intubation technic or by the quininium indicator compound tubeless method(2-6) on a day other than that of the quinine carbonate test.

The *first group,* consisting of 24 individuals who secreted at least a normal amount of free hydrochloric acid per hour after caffeine stimulation and seven achlorhydric patients, were given a dose of 120 mg of quinine carbonate. Urine was collected before and 2 hours after oral administration of the compound. The quinine was extracted from the urine by a simplified modification(4,7) of the Kelsey-Geiling ether-sulphuric acid technic(9) and determined quantitatively with the Lumetron photofluorometer as described in the *in vitro* tests. For clinical purposes, micrograms of quinine less than 160 can be estimated semi-quantitatively by comparing the fluorescence with appropriate standards using a Blak-Ray or other type of lamp as the ultraviolet source (4,7). To a *second group* consisting of 33 individuals who secreted free hydrochloric acid to at least a normal extent, 3 who secreted hydrochloric acid in the low range, 10 achlorhydrics and 2 individuals with gastrectomies, 320 mg of quinine carbonate was administered orally. A *third group* made up of 7 acid secretors, one low acid secretor, 3 achlorhydrics and 2 gastrectomized individuals received 640 mg of quinine carbonate.

With one exception the 320 and 640 mg groups had urine collected before, 2 hours, 3 hours and 5 hours after the oral administration of the quinine carbonate. In one hyperacid secretor, one low acid secretor and one achlorhydric (P.A.) patient, additional urines were collected at 8, 15 and 24 hours after the oral administration of 320 mg of quinine carbonate. In one gastrectomy patient, urine was collected 2 hours and 10 hours after the oral administration of 640 mg of quinine carbonate.

In the acid secretors of the group receiving 120 mg of quinine carbonate, the average amount of quinine in the first 2 hours' urine was 72 $\mu$g with the range from 0 to 192 $\mu$g.

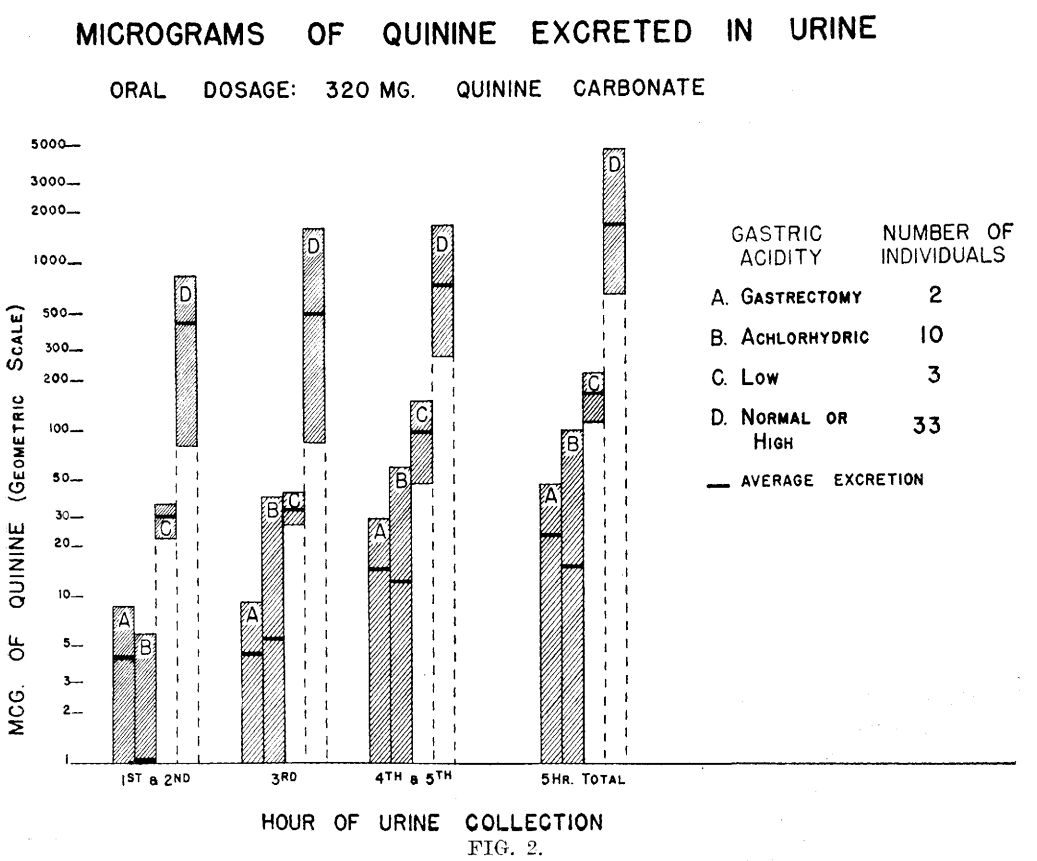


FIG. 2.

All 7 of the individuals with achlorhydria failed to excrete any quinine.

Fig. 2 demonstrates the results in the group receiving the 320 mg dose. The acid secretors in the normal or higher range excreted an average of 437 μg of quinine in the first 2 hours' urine as compared to 72 μg excreted in the comparable group receiving 120 mg. The amount of quinine in the 2 hours' urine varied from 80 to 840 μg. The average quinine excreted in the first 2 hours' urine was 30 μg in the low acid secretors and 1.1 μg and 4.3 μg in the comparable urines in the achlorhydric and gastrectomy patients, respectively. Slightly larger amounts of quinine were excreted in the third hour and in the fourth plus fifth hour periods.

In one P.A. patient the amounts of quinine in the 8th and 15th hour urines after 320 mg of quinine carbonate were zero in both collections and 114 μg in the total 24-hour urine. In the one low acid secretor it was 36 μg, 27 μg and 285 μg in comparable urines; in the one high acid secretor, 999 μg, 780 μg and 3,726 μg, respectively, were found in the urines.

The average μg of quinine excreted in the group which received 640 mg of quinine carbonate were as follows:

| | Time of urine collection | | |
|---|---|---|---|
| Gastric acidity | 2nd hr | 3rd hr | 5th hr |
| Normal or high | 1030 | 1070 | 2170 |
| Low | 54 | 156 | 120 |
| Achlorhydric | 0.6 | 2 | 23 |
| Gastrectomy | 0 | 0 | 6 |

In one gastrectomy patient who received 640 mg of quinine carbonate, no quinine was excreted in the first and second hours' urine and only 23 μg were found in the total 10-hour urine.

The data obtained show that the determination of the amount of quinine in the first 2 hours' urine excretion after the oral adminis-

tration of 320 mg of quinine carbonate can serve to detect the presence or absence of free gastric hydrochloric acid without subjecting the individual to intubation. Under proper conditions with this dose of quinine carbonate the differentiation between a low and at least a normal acid secretor seems feasible merely by assaying the 2-hour urine for quinine by the simple semi-quantitative technic previously described(4,7).

Experimentation with larger doses of quinine carbonate, if tolerated, or with other comparable insoluble salts with exogenous indicator ions, may make it possible to differentiate approximately between normal and excessive output of hydrochloric acid per unit of time. It is obvious that the time of urine collection can be changed to include the night excretion if desirable, since in achlorhydric and gastrectomized patients practically no difference was noted in the amount of quinine excreted in the 2-, 5- and 8-hour urine collections.

*Summary.* 1. The rationale of the use of insoluble salts with special exogenous indicator ions to determine gastric acidity without intubation has been described. 2. Theoretical consideration and *in vitro* and *in vivo* experiments have demonstrated that by determining the amount of quinine in the first 2-hour urine excretion after the oral administration of 320 mg of quinine carbonate, the presence of free hydrochloric acid from a low to a normal or higher range or its absence can be estimated without subjecting the individual to intubation. 3. Further experimentation with larger doses of quinine carbonate, if tolerated, or with comparable insoluble salts with various exogenous indicator ions, may make it possible to differentiate approximately between normal and high gastric hydrochloric acid secretion without intubation.

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## Molybdenum Toxicity in *Lactobacillus leichmannii.* (20392)

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Molybdenum has been shown to be toxic for cattle(1-3), rats(4-6), and rabbits(7). Neilands, Strong and Elvehjem(4) found that whole liver substance overcame molybdenum toxicity in rats on an alcohol-extracted casein diet. Since this diet would be somewhat deficient in vit. $B_{12}$, it was thought that this vitamin might be involved in the correction produced by whole liver. A microorganism requiring vit. $B_{12}$, *Lactobacillus leichmannii* 4797, was chosen to expedite a study to determine if molybdenum produced a growth inhibition reversible by vit. $B_{12}$.

*Experimental. Lactobacillus leichmannii* 4797 was obtained from the American Type Culture Collection. The medium used and the procedure followed was that described by Peeler, Yacowitz and Norris(8), with the