TABLE IV—AVERAGE ASSAY VALUE OF GENTAMICIN BATCHES CONVERTED TO THEIR FREE BASES BY LOT AND METHOD

Lot No.	Ninhydrin Assay—By Weight, % C_1	C_2	C_1a	Ninhydrin Assay—By Activity, % C_1	C_2	C_1a	Microbiological Assay C_1	C_2	C_1a
GMC-4J-5I	42.5	37.2	20.3	36.9	40.8	22.3	42.2	39.2	18.7
60-1197-65I	43.1	34.0	23.0	37.7	37.2	25.1	41.2	34.1	24.9
60-1197-64I	35.8	35.3	28.8	30.7	38.2	31.1	36.5	32.9	30.6
GMC-4J-1-4C	31.0	41.0	28.0	26.3	43.9	29.9	29.1	40.5	30.5
60-1197-61I	38.8	37.1	24.0	33.5	40.4	26.1	38.7	37.8	23.5
60-1197-60	37.5	42.1	20.3	32.3	45.7	22.0	36.8	43.5	19.7

TABLE V—CORRELATION OF NINHYDRIN DIFFERENTIAL ASSAY WITH MICROBIOLOGICAL ASSAY FOR GENTAMICIN BATCHES CONVERTED TO THEIR FREE BASES

Component	Correlation Coefficient—Weight	Activity
C_1	0.98	0.97
C_1a	0.98	0.98
C_2	0.93	0.96

As a second step, the six samples under consideration were converted to their free bases by means of an ion-exchange resin. The identical assay was performed using factors for the ninhydrin intensities of the compounds determined on known mixtures of the free bases of all of the components (Table I). The average response for these samples by weight and activity are presented in Table IV and are compared to the results obtained in the microbiological differential assay.

The correlations between the two assays using the free base mixtures measured by weight and activity are shown in Table V. All of the correlations are significant ($p < 0.01$).

The differential ninhydrin assay which has been described for the quantitative determination of the components of the gentamicin complex is highly reproducible and easier to perform than is the microbiological method. A significant correlation exists between the results obtained in the two procedures, and this new assay should therefore be very useful in determining proportions of the three gentamicin components in mixtures of the complex.

REFERENCES

(1) Weinstein, M. J., Wagman, G. H., Oden, E. M., and Marquez, J. A., *J. Bacteriol.*, **94**, 789(1967).
(2) Wagman, G. H., Marquez, J. A., and Weinstein, M. J., *J. Chromatog.*, **34**, 210(1968).
(3) Wagman, G. H., Oden, E. M., and Weinstein, M. J., *Appl. Microbiol.*, **16**, 624(1968).

Keyphrases

Gentamicin complex—differential analysis
Paper chromatography—separation, analysis
Ninhydrin—color reagent
Integrating scanner—color intensity

Dissolution Kinetics of Drugs in Human Gastric Juice—the Role of Surface Tension

By PER FINHOLT and SISSEL SOLVANG

The kinetics of *in vitro* dissolution of phenacetin and phenobarbital in human gastric juice have been determined and compared to those in hydrochloric acid containing various amounts of polysorbate 80. The surface tension of the dissolution medium is shown to have an appreciable effect on the dissolution kinetics of the drugs studied.

DURING THE LAST YEARS, a number of rate studies on the dissolution of drugs have been conducted. An excellent review of these is given by Wurster and Taylor (1). Various dissolution media have been used, but, surprisingly enough, not that solvent, in which the dissolution process takes place *in vivo*—that is, human gastric juice.

The purpose of this investigation was to study the rate of dissolution of drugs in human gastric juice. In addition, a closer examination of the

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relationship between the surface tension of the fluid and the dissolution rate of drugs was felt needed. The reason for this was that previous studies on the rate of dissolution of drugs from powders, granules, and tablets had shown that the surface properties of the solid particles was an important factor (2, 3).

EXPERIMENTAL

Materials—The powdered drugs used were of Pharmacopoea Nordica grade. They were separated into the desired size fractions by sieving.

The gastric juice was delivered from a hospital department engaged in testing the gastric secretion of patients under examination for diseases of the stomach.[1] It would of course have been desirable to use gastric juice from healthy persons; but such large amounts were needed for the experiments that it was quite impossible to get enough from healthy volunteers.

The gastric juice obtained from each patient was collected on an empty stomach, 1 hr. before and 1 hr. after stimulation with large doses of histamine (subcutaneous injection). Immediately after collection the gastric juice was frozen at about −20° and stored at this temperature in a freezer until it was to be used. The time of storage was usually 3–4 weeks. However, the gastric juice could be stored at −20° for months, without changing properties like pH and surface tension.

When gastric juice was to be used as dissolution medium, samples from 25–30 different persons were mixed and then diluted with an equal amount of water. Water was added since powders and tablets normally are swallowed with water and the dissolution process *in vivo* thus takes place in a mixture of gastric juice and water.

Determination of Dissolution Rate—The dissolution rate was determined by the beaker method of Levy (4) with minor changes.

Procedure A—The dissolution assembly consisted of a 1-l. Pyrex beaker immersed in a constant-temperature bath adjusted to 37.5°, and a flat, straight-blade (6 cm. diam. and 1.5 cm. high) stainless steel stirrer attached to an electric stirring motor giving the stirrer a constant and easily adjustable speed of rotation.

Five-hundred milliliters of the dissolution medium was placed in the beaker and permitted to equilibrate to 37.0°. The stirrer was immersed into the middle of the liquid and rotated at a speed of 50 r.p.m. A certain amount of the drug powder (1.0 g. of phenacetin, 2.0 g. of phenobarbital, 2.4 g. of sodium phenobarbital) was gently spread over the surface of the fluid. During the test the powder would partly remain on the surface and partly settle down through the fluid. At appropriate intervals 5-ml. samples were withdrawn by means of pipets provided with short plastic tubings filled with glass wool or fritted-glass immersion filter tubes of medium porosity for filtration of the sample. The samples were analyzed and the drug concentration found was plotted *versus* time.

Procedure B—A 250-ml. beaker, a flat, straight-blade (4 cm. diam. and 1.5 cm. high) stirrer, 100 ml. of the dissolution medium, and 0.5 g. of the drug were used and 2-ml. samples were withdrawn. Otherwise Procedure B was the same as Procedure A.

Analytical Methods—*Determination of Phenacetin*—The samples withdrawn were diluted with water and absorbancies were measured at 245 mμ with a Beckman DK 2A spectrophotometer.

Determination of Phenobarbital—The samples withdrawn were mixed with borate buffer pH 9.5 and diluted with water. Absorbancies were measured at 240 mμ.

Determination of Solubility—The solubilities of the drugs were determined by using the beaker method and following the dissolution process until the dissolution medium became saturated with drug.

RESULTS AND DISCUSSION

Effect of Surfactant on Dissolution Rate—Phenacetin was chosen as model substance because of its hydrophobic properties. Polysorbate 80 was used as surfactant.

Figure 1 shows the rate of dissolution of phenacetin in 0.1 *N* HCl to which different amounts of polysorbate 80 had been added. An increase in the

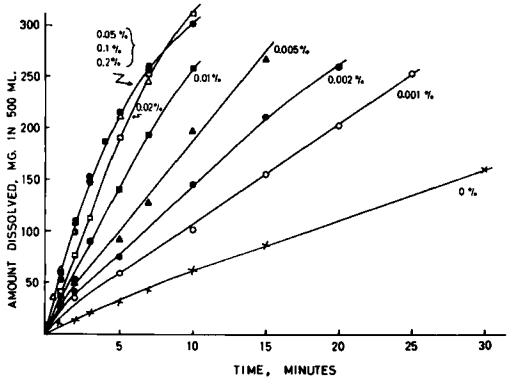

Fig. 1—Effect of polysorbate concentration of the dissolution medium (0.1 N HCl) on dissolution rate of phenacetin (0.21–0.30 mm.).

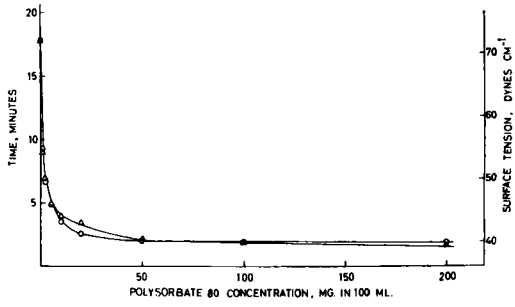

Fig. 2—Relationship between the polysorbate concentration of the dissolution medium (0.1 N HCl) and the time necessary for dissolution of 100 mg. of phenacetin (0.21–0.30 mm.); and the relationship between the polysorbate concentration of the dissolution medium and the surface tension of the same. Key: ○, time; △, surface tension.

[1] The authors want to thank Dr. J. Myren, Medical Department IX, Ullevål Hospital, Oslo, for the gastric juice and for information about the gastric secretion rate of the patients.

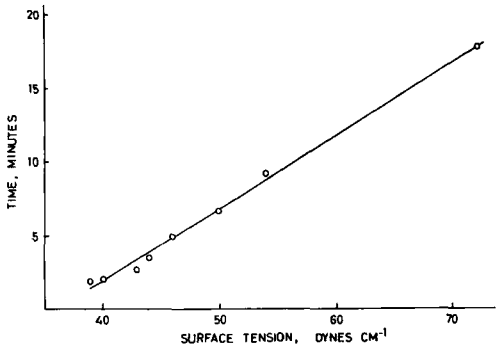

Fig. 3—Relationship between the surface tension, the dissolution medium and the time necessary for dissolution of 100 mg. of phenacetin (0.21–0.30 mm.). Dissolution media: 0.1 N HCl containing different amounts of polysorbate 80.

polysorbate concentration from 0 to 0.01% causes a significant increase in dissolution rate. Addition of further amounts of the surfactant has very little effect.

The times necessary for dissolution of 100 mg. of phenacetin in the different polysorbate–HCl mixtures can easily be picked from Fig. 1. In Fig. 2 these times are plotted *versus* the corresponding polysorbate concentrations. The same figure also shows the surface tension of the dissolution medium in relation to the concentration of polysorbate 80. The surface tension was measured at 20° with a Cenco Du Nouy tensiometer. It will be seen that the two curves are nearly superimposed. This means that a nearly linear relationship exists between the surface tension of the dissolution medium and the time necessary for dissolution of 100 mg. of phenacetin. This relationship is clearly demonstrated in Fig. 3.

The solubilities of phenacetin at 37° in 0.1 *N* HCl containing 0%, 0.001%, 0.01%, or 0.1% of polysorbate 80 were found to be 1.18 g./l., 1.26 g./l., 1.26 g./l., and 1.29 g./l., respectively. Polysorbate 80 in the concentrations used thus has a very small influence on the solubility of phenacetin in HCl. This means that the effect of polysorbate 80 on the rate of dissolution of phenacetin is due only to a small extent to its solubilizing power, but is caused mainly by its ability to decrease the interfacial tension between the substance and the dissolution medium. This result is in accordance with observations by Levy and Gumtow (5), who found that sodium lauryl sulfate enhanced the rate of dissolution of salicylic acid from compressed tablets. This effect was assumed to be due to increased wetting and to better solvent penetration into the tablets and granules as a result of the interfacial tension lowering effect of the surfactant.

Surface Tension of Human Gastric Juice—Since the surface tension of the dissolution medium seems to be a deciding factor as far as dissolution rate is concerned, it is of great interest to know the surface tension of human gastric juice. However, no values could be found in the literature. Therefore, a determination of the surface tension was made using gastric juice collected from a large number of persons under examination for diseases of the stomach. Samples of fasting as well as of histamine-stimulated secretion were tested. Table I shows the gastric secretion rate and the pH and surface tension of the gastric juice from some of the persons tested.

The results of the whole test are shown in Fig. 4, where the surface tension of the samples is plotted *versus* their pH. There is no significant correlation between these properties. The calculated correlation coefficient was less than the theoretical one at the 5% probability level. From these experiments it appears that the surface tension of human gastric juice is nearly independent of pH and secretion rate, having a value between 35 and 50 dynes cm.$^{-1}$.

Dilution of gastric juice with an equal amount of

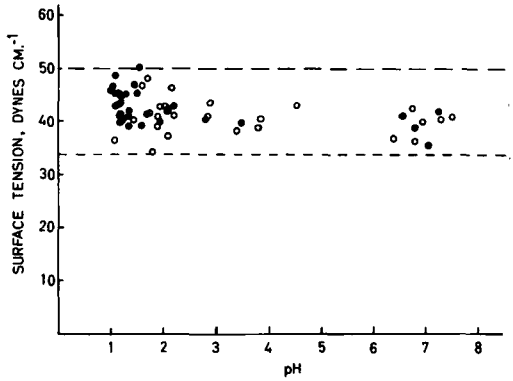

Fig. 4—pH and surface tension of gastric juice from 27 different persons. Key: ○, fasting secretion; ●, histamine-stimulated secretion.

TABLE I—GASTRIC SECRETION RATE AND pH AND SURFACE TENSION OF GASTRIC JUICE

Patient No.	Unstimulated Gastric Juice: Secretion Rate, ml. hr.$^{-1}$	pH	Surface Tension, dynes cm.$^{-1}$	Histamine-stimulated gastric juice: Secretion Rate, ml. hr.$^{-1}$	pH	Surface Tension dynes cm.$^{-1}$
1	5	7.2	—	20	2.2	42.7
2	15	3.4	38.0	100	1.2	39.5
3	25	2.5	40.1	105	1.2	37.8
4	25	6.2	40.2	15	1.6	—
5	30	5.2	38.5	110	1.3	37.5
6	35	6.3	36.5	35	6.1	38.3
7	50	1.9	40.0	230	1.1	40.3
8	60	6.8	36.1	120	1.4	40.5
9	125	4.9	44.0	230	1.2	44.2
10	130	3.8	38.5	130	1.5	46.7
11	125	1.7	42.4	165	1.4	43.2
12	180	1.6	39.0	260	1.1	40.3

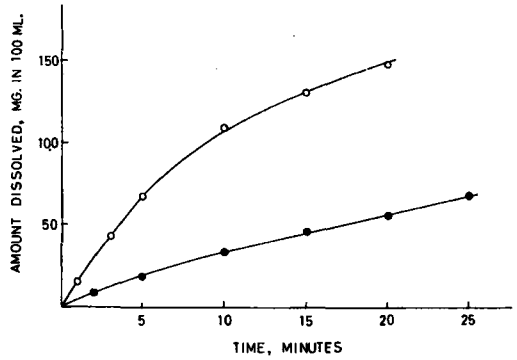

Fig. 5—Rate of dissolution of phenobarbital (0.21–0.30 mm.) in 0.1 N HCl and in diluted gastric juice. Key: ●, 0.1 N HCl; ○, diluted gastric juice.

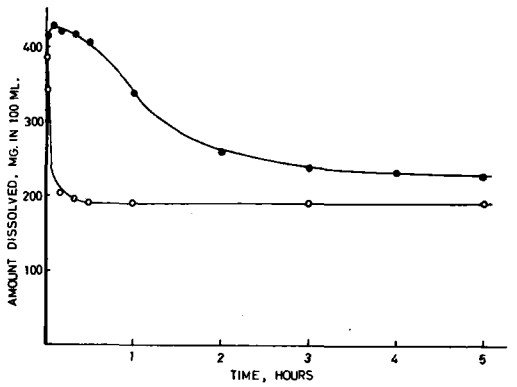

Fig. 6—Dissolution of sodium phenobarbital in 0.035 N HCl and in diluted gastric juice, and precipitation of the free acid from the supersaturated solutions. Key: ○, 0.035 N HCl; ●, diluted gastric juice.

water caused an increase in the surface tension of only 1–3 dynes cm.$^{-1}$.

Rate of Dissolution of Drugs in Diluted Gastric Juice[2]—Figure 5 shows that the rate of dissolution of phenobarbital (0.21–0.30 mm.[3]) in diluted gastric juice is substantially higher than the rate of dissolution in 0.1 *N* HCl. This was to be expected, since the diluted gastric juice has a much lower surface tension than the 0.1 *N* HCl. In addition, the solubility of phenobarbital at 37° was found to be slightly higher in diluted gastric juice (2.00 g./l.) than in 0.1 *N* HCl (1.90 g./l.).

Since phenobarbital is used in medicine not only as the free acid, but also as the sodium salt, the rate of dissolution of the salt in diluted gastric juice was determined and compared to the rate of dissolution of the salt in an HCl solution having the same pH as the diluted gastric juice. Figure 6 shows that initially nearly the same, high drug concentration is obtained in both dissolution media. However, the precipitation of the free acid in excess of solubility takes place at a much slower rate in gastric juice than in hydrochloric acid. Gastric juice obviously contains substances retarding the precipitation of the acid.

[2] A mixture of equal volumes of gastric juice and water.
[3] Powder passing through standard sieve US No. 50 (sieve opening, 0.30 mm.), but not through sieve No. 70 (sieve opening, 0.21 mm.).

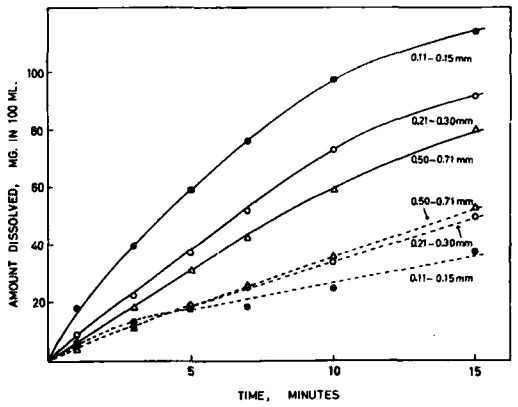

Fig. 7—Effect of particle size on dissolution rate of phenacetin in 0.1 N HCl and in diluted gastric juice. Key: – – –, 0.1 N HCl; ——, diluted gastric juice

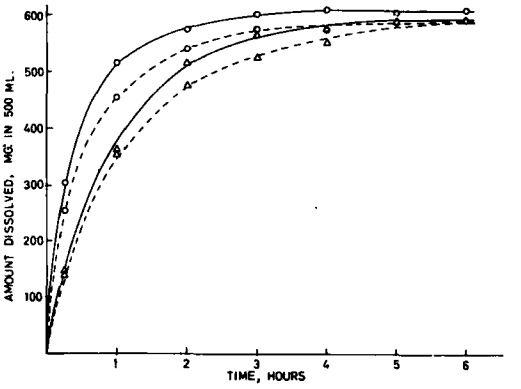

Fig. 8—Rate of dissolution of phenacetin (0.21–0.30 mm.) in diluted gastric juice and in diluted hydrochloric acid at the same pH and surface tension. Key: ——, diluted gastric juice (pH 1.6); – – –, diluted HCl + polysorbate 80 (pH 1.6); ○, surface tension 40.9 dynes cm.$^{-1}$; △, surface tension 50.7 dynes cm.$^{-1}$.

Effect of Particle Size on Dissolution Rate of Drugs in Diluted Gastric Juice—The solid lines in Fig. 7 show the effect of particle size on the rate of dissolution of phenacetin in diluted gastric juice. It is seen that the rate increases with decreasing particle size, whereas the opposite is the case when 0.1 *N* HCl is used as dissolution medium, as will be seen from the dotted lines in the same figure. These results are in agreement with earlier findings (2). Under the conditions of the experiment the rate of dissolution of hydrophobic drugs *increases* with decreasing particle size when the dissolution medium has a low surface tension, *decreases* when the surface tension is high. Figure 7 also shows that all powder fractions tested dissolve faster in diluted gastric juice than in 0.1 *N* HCl. This is due not only to the lower surface tension of the diluted gastric juice, but also to the higher solubility of phenacetin in this solvent (1.38 g./l. at 37°) compared to the solubility in 0.1 *N* HCl (1.05 g./l. at 37°).

Rate of Dissolution of Drugs in Diluted Gastric Juice and in HCl Solution at the Same pH and Surface Tension—According to Figs. 5 and 7

hydrophobic drugs such as phenobarbital and phenacetin dissolve faster in gastric juice than in 0.1 *N* HCl, mainly because of the lower surface tension of the former. The question arises whether there will be any difference between the dissolution rates in the two solvents if an HCl solut on of the same pH and surface tension as the diluted gastric juice is used. This question is important in relation to what kind of dissolution medium should be recommended for an *in vitro* dissolution test in the pharmacopeias.

The problem was studied with phenacetin as model substance. Two dilutions of gastric juice were made, one from samples of gastric juice with a relatively low surface tension (35–40 dynes cm.$^{-1}$) and one from samples with a relatively high surface tension (45–50 dynes cm.$^{-1}$). Rates of dissolution were determined using these two dilutions of gastric juice and two HCl solutions of the same pH and surface tension as the gastric juice mixtures. The surface tension of the HCl solutions was adjusted at the correct value by addition of polysorbate 80.

Figure 8 shows that phenacetin dissolves nearly at the same rate in hydrochloric acid as in gastric juice when the two media are adjusted at the same pH and surface tension. As far as this drug is concerned, an HCl solution adjusted to a surface tension of 40–50 dynes cm.$^{-1}$ is a better dissolution medium for an *in vitro* test than an HCl solution with no surfactant added. To see if this also applies to other drugs further studies are needed.

REFERENCES

(1) Wurster, D. E., and Taylor, P. W., *J. Pharm. Sci.*, **54**, 169(1965).
(2) Finholt, P., Kristiansen, H., Schmidt, O. C., and Wold, K., *Medd. Norsk Farm. Selskap*, **28**, 17(1966).
(3) Finholt, P., Højlund Pedersen, R., Solvang, S., and Wold, K., *ibid.*, **28**, 238(1966).
(4) Levy, G., and Hayes, B. A., *New Engl. J. Med.*, **262**, 1053(1960).
(5) Levy, G., and Gumtow, R. H., *J. Pharm. Sci.*, **52**, 1139(1963).

Keyphrases

Dissolution kinetics—gastric fluid
Phenobarbital—dissolution rates
Phenacetin—dissolution rates
Gastric fluid, HCl solution, compared—drug dissolution
Surfactant concentration—dissolution effect
Surface tension—dissolution effect
UV spectrophotometry—analysis

Evaluation of the Effect of Isomerization on the Chemical and Biological Assay of Vitamin D

Analysis of Fat-Soluble Vitamins X

By J. A. KEVERLING BUISMAN, K. H. HANEWALD, F. J. MULDER, J. R. ROBORGH, and K. J. KEUNING

The reversible thermal isomerization of calciferols (D) to precalciferols(P), leading to a temperature-dependent equilibrium in solutions, interferes with many chemical and biological analytical procedures for the evaluation of vitamin D products. Two quantities should be distinguished: the potential vitamin D content (precalciferol + calciferol) and the actual vitamin D content (calciferol only). Of these two, only the potential vitamin D content is of importance to the buyer of vitamin D. Biological vitamin D assays give the potential vitamin D content, provided sample and reference standard dilutions are equilibrated by heating simultaneously to be sure that all have the same P–D ratio. This equilibration is superfluous in most of the chemical determinations of the potential vitamin D content. The actual vitamin D content can be determined by chemical, but not by biological, assay.

IT IS UNFORTUNATE that the assay of vitamin D preparations still frequently gives rise to difficulties. The reproducibility of biological assays is often poor, especially when assays from different laboratories are compared. The correlation of biological and chemical determinations is often unsatisfactory.

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A source of errors which has drawn too little attention is the reversible isomerization of the calciferols in solutions to the corresponding precalciferols, forming an equilibrium mixture (1–3).

In 1931 Reerink and Van Wijk (4) observed that the physical constants of freshly irradiated solutions of ergosterol changed spontaneously. Velluz *et al.* (3) isolated the 3,5-dinitrobenzoate of the responsible intermediate in 1949 and called it