cannot act as an electron donor since it has no electrons to donate."

The demonstration that the biological activity of a molecule is related to its structural ability to exist as a resonance hybrid is of interest from many points of view. Sulfanilamide, for example, is *p*-aminobenzenesulphonamide. The isomeric *meta* and *ortho* compounds are therapeutically inactive.

REFERENCES

(1) Hoffman, Charles, Schweitzer, T. R., and Dalby, Gaston, *Ind. Eng. Chem.*, 33 (1941), 749–751.

(2) Hoffman, Charles, Schweitzer, T. R., and Dalby, Gaston, *Food Research*, 4 (1939), 539–545.

(3) Buswell, A. M., Downing, J. R., and Rodebush, W. H., *J. Am. Chem. Soc.*, 62 (1940), 2759–2765.

A New Method of Testing Enteric Coatings

By K. Lark-Horovitz and Herta R. Leng†*

It is sometimes necessary to prevent the reaction of medicaments with the gastric juice in the stomach because the substance administered may produce either irritation of the mucous membrane or the preparation may be destroyed by the chemical reaction. To avoid these effects, protective coatings, so-called enteric coatings, are used which are supposed to protect the preparation in the stomach and let it dissolve after a definite length of time in the small intestines. According to Jordan (1) 3.3% of the medicinal preparations on the market are administered enterically, therefore methods to test the efficacy of enteric coatings are of great interest.

To test the coatings in the test tube with solutions artificially reproducing the fluids in the stomach is not very satisfactory or convincing. To investigate the coatings *in vivo*, it is necessary either to make the preparations opaque, so as to produce shadowgraphs with X-rays (2, 3) or to make them self-radiating and to detect their location and behavior by following the radiation. This latter procedure has been proposed by Lark-Horovitz (4). The method is ideally suited for the problem since only a substance which is used in our everyday diet, sodium chloride, is introduced and since the amounts of radiating material are so small as to cause no harm; the lifetime of the radioactive material is so short that the same person can be used again for a test after three or four days.

EXPERIMENTAL

Sodium chloride in the form of a crystal is activated by the impact of heavy hydrogen nuclei, deuterons, which are accelerated to several million volts by one of the modern devices available in the nuclear physics laboratories, a cyclotron or high-tension machine. Both the sodium and the chlorine in the salt become radioactive. The lifetime of the chlorine is so short (half-life[1] about one-half hour) that practically one is dealing only with sodium of mass 24, which has a half-life of 14.8 hrs.

The radioactive sodium emits fast electrons and gamma (γ) rays. It is detected mostly by the effect of its γ-rays. For the detection one uses so-called counter tubes which consist of a metallic cylinder and a coaxial wire in a vapor atmosphere, usually argon and a trace of alcohol, at a few centimeters pressure. We have been using Geiger counters consisting of a Dow-metal[2] tube of 11 cm. length, 1.5 cm. width, and a thickness of about 0.01 cm. The counters are filled with an argon-alcohol mixture at about 7 cm. pressure (1 cm. alcohol, 7 cm. argon).

* Head of the Department of Physics, Purdue University, Lafayette, Ind.

† Amelia Henry Reinhardt International Fellow of the American Association of University Women.

[1] Radioactive substances decay with a certain probability and it is customary to characterize this probability by the time after which half the number of original atoms is still present. Thus radioactive chlorine of mass 38 with a half-life of 37.5 min. has a greater chance to disintegrate than sodium of mass 24 with a half-life of 14.8 hrs.

[2] Since this metal and also aluminum are difficult to obtain at present it is quite feasible to use brass counters with a thin mica window of 0.3 mm. thickness mounted over a grill so that the window will stand the difference between the atmospheric pressure and the vacuum inside.

Usually a NaCl crystal was bombarded with deuterons (of 8 million volts) for 1 microampere hour. This resulted in an initial activity for the sample used of several hundred microcuries (μC).[3] The activated NaCl was scraped from the crystal and 0.3 Gm. was put in a No. 00 gelatin capsule. This was sealed and the enteric coating applied by hand. The enteric coating was prepared in accordance with the formula by Goorley and Lee (2) of the School of Pharmacy of Purdue University and consisted of a mixture of shellac, castor oil and alcohol. To test commercial coatings several capsules of the same type which are to be used for the medicaments are filled with the radioactive material. All of the capsules are run through the coating machine in the usual way and spread out at the end of the process on a tray. Those which are radioactive were picked out with a counter. Since the counting equipment is easily portable it is possible to locate the capsules in the factory without any difficulty.[4]

The actual testing is performed in the following way: The capsule is followed with a counter tube throughout its path in the stomach and intestines. By using a second stationary counter mounted in a lead shield of $4^1/_2$-in. thickness, the distribution in the body is measured by determining the activity of the hand. Any leak of the capsule before the actual process of dissolving of the coating is detected after a few minutes by the activity measured in the hand. As long as the capsule is holding together, it can be followed from point to point with the movable counter. The experiments of Goorley and Lee (2) and Bukey and Klemme (3) have shown that tests are not reliable if the capsule is swallowed during a meal or immediately following. Therefore the experiments were always started after a fasting period of several hours. Some of the capsules remained for hours in the same position in the stomach. In one of our experiments, the capsule was not dislodged for over 10 hrs.; however, the same person tested a few days later showed an extremely short emptying time of $1^1/_2$ hrs. The average emptying time in our experiments was found to be 3 7 hrs. It is interesting to note that the process of solution of enteric coatings in the small intestines in many cases takes place extremely rapidly so that the activity in the intestinal tract suddenly spreads over a wide area and the counts in the hand increase rapidly. In some experiments, the capsule is dissolved slowly and the amount absorbed apparently increases in time.

Ordinary sealed gelatin capsules are dissolved in the stomach after 10 to 20 min. and the absorption curve as measured by the activity of the hand is the same as observed when the salt is administered in aqueous solution.

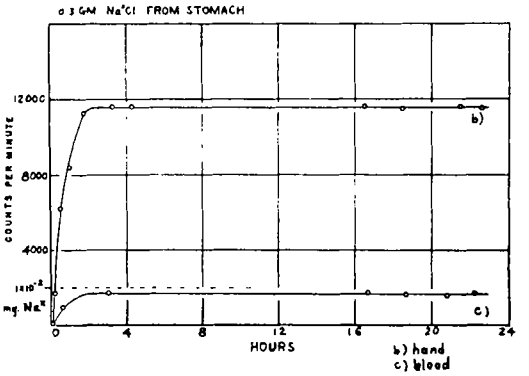

Fig. 1.—Uptake of Radioactive Sodium from the Stomach.

The number of counts per minute is plotted against time in hours indicating the uptake of radioactive sodium from the stomach. A few minutes after intake the sodium can be detected in the hand, reaches equilibrium in a few hours, and remains at this level for 3 to 4 days. Curve *b* represents the activity in the hand; curve *c* represents the activity in the blood. A typical test for enteric coatings is then performed in the following way. The movable counter is used to locate the capsule and the activity in the hand indicates any leak or break in the capsule.

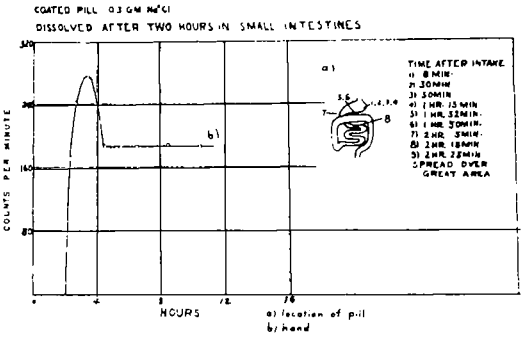

Fig. 2.—Uptake of Radioactive Sodium from the Small Intestines.

Figure 2 shows the absorption from the small intestines. Curve *b* again represents the activity in the hand; Fig. 2 (*a*) shows the location of the capsule at various times. Up to the time the capsule is intact, the activity in the hand is zero. As soon as the capsule dissolves, a rapid increase of the activity in the tissues is observed by measuring the activity in the hand, and sometimes after a period of several hours this high level recedes to the normal level as observed after intake from the stomach.

[3] It is customary to give all activities in curies by defining as equivalent to one curie a radioactive sample which emits 2.22×10^{12} counts per minute. A microcurie is then the millionth part or 2.22×10^6 per minute, a quantity which is far too small to cause any biological disturbance, but ample to be detected when distributed throughout the body (6, 7, 8).

[4] Such a portable counting equipment is easily constructed at a cost not exceeding $50 to $100, depending on the equipment used and the precision required.

Table I

	Total No. of Capsules	Dissolved in Intestines	Dissolved in Stomach	Unknown	Not Dissolved
Hand-coated capsules (radioactive method)	23	15 (65.2%)	2 (8.7%)	4 (17.4%)	2 (8.7%)
Commercial coatings (radioactive method)	10	5 (50%)	4 (40%)	1 (10%)	2 (8.7%)
Tests with X-ray method (Goorley and Lee)	137	89 (65%)	4 (3%)	...	44 (32%)

In all the experiments with Na a rapid increase followed by a decrease in activity is observed only if absorption is taking place from the small intestines. It is, therefore, possible to distinguish between the uptake of Na from the stomach and the small intestines by inspecting the activation curve alone. We believe, however, that for diagnostic purposes, such as detection of obstructions of the intestinal tract, the location of the capsule with the movable counter is of interest and importance.

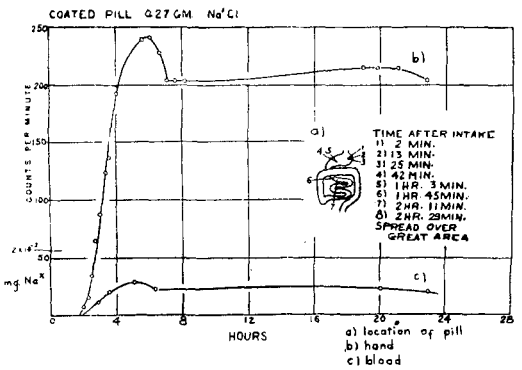

Fig. 3.—Uptake of Radioactive Sodium from the Small Intestines.

Figure 3 shows a curve obtained when a capsule does not break suddenly but dissolves slowly. Also in this case, the maximum in the absorption curve characteristic for the uptake from the small intestines is observed.

As a control of the uptake in the hand, blood activities have also been determined and are shown in figures under *c*. In this way it is possible by comparing the activities to determine whether the uptake from the stomach and from the intestines is of the same order of magnitude. If the active salt is fed from solutions an accurate determination of the concentration can be made by comparing the measured activities with the activity of the original solution. When the salt is taken in capsules, this determination is uncertain as the comparison sample has to be obtained by weighing the dry salt and it is difficult to obtain a uniform mixture of the radioactive and inactive fractions of the salt.[5] Within the limit of error, the final total uptake is the same in both cases, absorption from the stomach or from the small intestines. Corresponding to the maximum uptake in the hand, there is a maximum temporary retention in the blood.[6]

DISCUSSION OF RESULTS

Table I shows the results obtained in the tests made.

The object of these experiments has been to show the effectiveness of the method used and not to accumulate extensive data. For this reason, the number of capsules tested was small as compared with those used in the investigations of Goorley and Lee. The results, however, are in good agreement with their findings. They found that 65% were dissolved in the small intestines as compared to the 65.3% found by us. As stated in their paper, their results of 32% not dissolved represent all incomplete observations, including all cases in which the capsule does not leave the stomach in three hours. Our results give actual observations extended over a two-day period. Whenever a capsule has dissolved, the radioactivity can be observed the following day and, therefore, it is possible to state definitely whether it was dissolved or has passed through the intestinal tract undissolved. Under "unknown," we have listed cases where the capsule dissolved but so long after the intake that it was still in the stomach when the patient left and the activity observed the following day indicated only that disintegration had taken place in the intestines or in the stomach. If we add our "unknown" and "not dissolved" we obtain 26% as compared to the 32% found by Goorley and Lee.

The advantage of this method as compared with the X-ray method is the inexpensive and simple equipment. Besides it also has the advantage of detecting by the activity of the hand any leak or break in the capsule, which is impossible with the X-ray method.

In our note in *Nature* (4), for instance, we have reproduced a curve which shows an activity in the hand from the start, in spite of the fact that the capsule is holding together and can be perfectly well located. This is apparently due to a leak and possibly also to a contamination of the enteric coating during the preparation. The curve shows, first, the slow increase and the level reached due to "absorption from the stomach," and indicates later the sudden break of the capsule in the small intestines

[5] Control measurements of the comparison sample of the powder show that the different mixtures can vary as much as 10% to 15%.

[6] It is important to note that this correspondence between blood and hand values does not exist in the uptake of K. The maximum in the hand is also found, but the blood values remain constant.

and the final level reached after the total salt is absorbed.

The method also allows one, as already mentioned above, to state definitely whether the capsule has dissolved or not, regardless of whether the patient is present at the time the disintegration has taken place. It is not possible to locate the capsule so accurately with a counter as it can be located with the help of X-rays, but that is not necessary for the purpose of testing the efficacy of enteric coatings.

Our experiments show that the formula used by Goorley and Lee is superior to the one particular commercial coating which we have tested. Since we have used hand coating, it is impossible to say whether the commercial method or the composition of the coating is responsible for this difference.

It will be of interest to compare the effect which the different fillings may have on the coatings. This can be done in a simple manner by mixing active NaCl powder with the medicaments to be used. For the pharmacologist and the medical profession, it would of course be of the greatest interest if activated medicaments could be used to study the different rates of absorption from the intestines and from the stomach. This would be possible by synthesizing medicaments containing artificially radioactive atoms. Such an investigation is limited to substances which have a sufficiently long lifetime to permit the complete synthesis to be carried out comfortably. C 14,[7] P 32, S 35, Ca 41, Fe 59, As 74, Sr 89, I 131, are among the elements which could be used. We desire to emphasize, however, that in most of these cases radioactive NaCl would have to be added to make the location of the capsule possible since most of the substances mentioned have radiations too weak to be detected through the tissues.[8] If this addition were made, it would be possible to study systematically the difference in the rates of absorption in the stomach and in the intestines by introducing the medicaments in enteric coatings, locating them by the radiation of NaCl and determining from small samples of ashed tissues and the blood the amount which had been taken up.

[7] The numbers indicate the masses of the respective radioactive elements.

[8] Since the lifetime of all the substances mentioned is far longer than that of Na, these measurements can be comfortably carried out after the Na has decayed. Such experiments shall be carried out in the near future when higher currents in the Purdue cyclotron will be available.

SUMMARY

A new method has been described for testing the efficacy of enteric coatings.

1. Fillings of radioactive NaCl are used in capsules enterically coated and their location is determined by the use of a movable Geiger-Müller counter.

2. This method makes it possible to detect, not only the location of the capsule, but also any possible leak in passing through the intestinal tract by measuring simultaneously the activity in the hand.

3. Comparison of these tests with the results from X-ray investigations substantiates the findings of Goorley and Lee as to the efficacy of enteric coatings.

4. Measurements of the activity of the hand as compared with similar measurements after feeding the salt in solution show a maximum in the absorption curve which is characteristic for the uptake of activated sodium from the small intestines.

5. Applications of this method to the solution of problems of metabolism in the intestinal tract have been pointed out.

REFERENCES

(1) Jordan, C. B., JOUR. A. PH. A., 20 (1931), 930.

(2) Goorley, J. T., and Lee, C. O., *Ibid.*, 27 (1938), 379–384.

(3) Bukey, F. S., and Klemme, C. J., *Ibid.*, 28 (1931), 87–90.

(4) *Nature*, 147 (1941), 580.

(5) Crane, H. R., *Phys. Rev.*, 56 (1931), 1243–1244.

(6) Mullins, Lorin J., *Phys. Rev.*, 56 (1939), 1244.

(7) Hevesy, G., *Phys. Rev.*, 57 (1940), 241.

(8) Goudsmit, *Science*, December, 1939.