1951

A method of testing enteric coatings using the white rat

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A METHOD
of
TESTING ENTERIC COATINGS
using the
White Rat

by

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B.S., Montana State University, 1947

Presented in partial fulfillment of the requirement for the degree of Master of Science

Montana State University

1951

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ACKNOWLEDGEMENT

The author wishes to make grateful acknowledgement of the valuable aid and assistance given by Gordon H. Bryan, Curtis H. Waldon, Ph.D. and Charles R. Lyons, M.D., in the course of this study.

And to my brother, Mark D., for his assistance in conducting the experimental work.

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INTRODUCTION

A number of investigators have used various methods for testing enteric coatings. One of the first methods was devised by Toplis[1] who suspended a tablet in artificial gastric juice and timed the period of disintegration. The artificial gastric juice was first used by him and has also been used in many of the later methods of testing coatings in vitro.

Husa and Magid[2] developed a method employing tablets of calcium sulfide and methylene blue. If there was regurgitation of hydrogen sulfide, the tablet in all probability, had disintegrated in the stomach but if such was not the case and the urine was colored, the tablet had passed into the intestine before disintegrating. No results either way gave evidence that the coating was not satisfactory from any standpoint.

The use of tablets of barium sulfate in combination with sodium salicylate[3] was one of the first instances employing fluoroscopic methods. The barium sulfate tablet could be followed with a fluoroscope, determining the position of disintegration in correlation with the time noted when the urine gave a positive test for salicylate.

In another investigation, barium sulfate tablets were given along with a teaspoonful of barium meal.[4] The meal faintly outlined the stomach and the tablets were still visible, allowing them to be followed by radiograph until they disintegrated.

Wruble,[5] in his work, devised a disk which held test tubes containing the artificial gastric juice and which revolved through a constant temperature bath at twelve revolutions per minute. He later made a study[6] of the pH of the gastric and intestinal juices and the emptying rate of the stomach, using the above method for testing coatings on the basis of his new findings.

Worton, Kempt, Burrin and Bibbins[7] used barium sulfate in vivo, following the tablets with X-ray and then comparing their results to those obtained in an in vitro method. This test was based on time of disintegration in test tubes containing artificial gastric juices. The limits imposed by these workers were that the tablet should begin to break in six hours and be completely disintegrated in eight hours.

Goorley and Lee[8] used Wruble's[9] method for their in vitro tests and correlated them by fluoroscopic methods. To give better results based on stomach emptying time, capsules were given before meals.

Maney and Kuever[10] tested their coatings by putting the tablets into small containers of artificial gastric juice which were moving at 16 oscillations per minute in a constant temperature bath. They substantiated their results by X-ray methods in vivo.

Apparently the first attempt to use radio-active substances was reported in 1942.[11] In this experiment, tablets of radio-active sodium chloride were followed with a Geiger counter with fair results.

Thompson and Lee[12] used what they called a bathwheel which rotated at 1 to 2 revolutions per minute through a constant temperature bath. Small vials containing the artificial gastric juice[13] in which the tablets were immersed were held to the wheel by pressure. The method is not too difficult and gives good results.

Hodge, Forsyth and Ramsey[14] conducted quite extensive research with X-ray technique. They showed that coatings can be tested in humans with good results using the barium sulfate tablets.

THE PROBLEM

Statement of the Problem. It was the purpose of this study to develop a method of testing enteric coatings by using the white rat.

Importance of the Study. With the more recent concept of enteric coatings, such as the one showing that intestinal enzymes[15] may be a factor in the disintegration of these coatings, it is important that a different method be used in testing them. This procedure must be one that utilizes the action of enzymes on enteric coatings. The present in vivo method of using human patients is not feasible for most preliminary investigations.

PRELIMINARY INVESTIGATION

Some preliminary investigation was necessary prior to the undertaking of the problem. This work was divided into five phases, which were as follows:

Finding A Suitable Dye. It is known that if methylene blue is given to the human patient, it can be detected in the urine as it imparts a bluish tinge to it. When the same dye was given to the rat, this was not the case. No positive results were obtained with any of the following dyes: fuchsine, eosin bluish, gentian violet, congo red and indigo. Phenylazo-a, a-diaminopyridine monohydrochloride, however, was found to be very effective in giving the urine a bright orange color. This dye was used in the rat and methylene blue in the human patients. All dyes were given to the rats both by mouth and intraperitoneally.

Developing X-ray Technique. A series of X-rays were taken following the course of barium sulfate pills which had been given to rats and tablets which had been given to humans. It was necessary to determine the proper settings on the X-ray machine so that the positions of the pills or tablets could be determined and also the degree of disintegration.

Coating of Pills and Tablets. Because of the small number of pills and tablets to be coated, the regular size coating pan was too large. For a coating pan, a glass rose bowl with a diameter of four inches was used with good results. The coating material was applied with a small size vibrating paint sprayer. Each successive coat was dried with hot air using a small hand hair dryer. A representative sample of each batch was tested for improper coating by putting the sample in water for twenty-four hours. If no evidence of rupturing was noted, then the coating was considered satisfactory; otherwise, they were given another complete coating.

The Coatings. Two comparatively new coatings were tried. The coatings used were cellulose acetate hydrogen phthalate and cellulose acetate stearate.

Formulas:

I.	Cellulose acetate hydrogen phthalate	10.0
	Ethyl acetate	50.0
	Alcohol, 95%	50.0
II.	Cellulose acetate stearate	20.0
	Ethyl acetate	75.0
	Alcohol, 95%	75.0

The approximate weight of the coating on each pill or tablet was 200 mg.

Method of Administration. In the human patients, the tablets were taken orally followed by a glass of water. The rats were given the pills while under light anesthesia. The tongue of the anesthetized rat was pulled out and the pill was placed on the back of the tongue. It was then pushed into the stomach with a small glass rod. The rat was then placed in a separate cage until the ether had dissipated and observed to see whether or not the pill had been regurgitated. The method was very successful. Pills were used for the rat because it was not feasible to make tablets small enough which would coat easily in small numbers. The pills were approximately four millimeters in diameter since ones much larger would have passed from the stomach with difficulty.

EXPERIMENT

Ten human patients and ten white rats were selected at random. The human patients were college students. The rats were of the Denver variety, Wistar strain, weighing approximately 250 grams. In both cases no alteration was made in their diet with the possible exception that the human patients were asked to abstain from alcoholic beverages during the experiment. The human patients were identified by using their three initials. In the case of the rats, the ears were slit in accordance with the number assigned to the rat. The rats were kept in individual cages.

In this experiment the human patients were used as a check against the results obtained from the rats. As this was to be a problem of developing a method of testing enteric coatings using the rat, which in turn would be taken by human patients, it was necessary to note whether or not the human patient and the rat would give the same results. If they did give the same results, then future testing using the rat alone would be sufficient, and particularily so in preliminary investigation.

To determine comparative kidney function and average excretion time, each member of both groups was given a pill containing the respective dyes. In this

case, the pills were uncoated.

The human patients all took their pills at the same time and then reported the time when they first noted a bluish color in the urine. White papers were put under the cages of the individual rats and observed every fifteen minutes for the bright orange color. The average time for the humans was 4.73 hours and for the rat, 1.88 hours.

After a period of at least three days, the same types of pills were given but were coated with cellulose acetate hydrogen phthalate. The waiting period was necessary to insure that there had been complete excretion of the dyes. Times and conditions were noted as before in the case of the uncoated pills. The average time elapsed before detection in the human urine was 7.7 hours and in the rat, 5.1 hours.

Because the dye was detected in the urine when the coated pill was given, it is quite evident that the coating disintegrated. It was then necessary to determine whether or not the coating disintegrated in the stomach or in the intestinal tract. Due to the increase in average time over the uncoated pill, it would seem that disintegration had taken place in the intestinal tract. To verify this, the human patients and the rats were given tablets and pills respectively, containing

barium sulfate and coated with cellulose acetate hydrogen phthalate. The first X-ray was taken about two hours prior to the average elapsed time with the dyes. If needed, a second picture was taken and if necessary, a third, at two hour intervals until proper results were obtained. In both the human and the rat, the coating disintegrated in the intestinal tract and not in the stomach. In one human patient and one rat, the pill or tablet never did disintegrate during the time it was under observation.

The same procedure was used with a second coating, cellulose acetate stearate. No coloration from the dye was noted in the urine of either the human patient or the rat. Upon X-raying the human patient, it was found that the coating and tablet were still intact upon entering the large intestine. In the rat, there was no evidence of disintegration after a period of ten hours. It was apparent that the second coating did not disintegrate in either the stomach or in the intestinal tract.

SUMMARY

The procedure followed for testing an enteric coating using the white rat consists of using a group of normal white rats. Pills containing phenylazo-a-, a-diaminopyridine monohydrochloride are given and the time is noted when the dye first appears in the urine. During the time of experimentation the rats are kept on a normal diet. After three days, the time needed for the dye to be completely excreted, the rats are given the same type of pill coated with cellulose acetate hydrogen phthalate and cellulose acetate stearate. Again the time is noted when the dye appears in the urine. If no color appears, the coating is unsatisfactory, as would be true if the color appeared at the same interval as the blank. If the dye is detected approximately three hours after the time noted for the blank, the coating is considered satisfactory, as it disintegrated in the intestinal tract.

This method of testing enteric coatings is based on enzymatic deterioration which seems the most feasible because it is almost impossible to make artificial juices comparable to the intestinal secretions or contents.

CONCLUSION

A method of testing enteric coatings based on enzymatic disintegration in the gastro-intestinal tract of the white rat has been devised. The method is easily performed and seems to be practical because in vivo conditions are employed.

TABLE I

Methylene Blue Tablets Uncoated

Human	Given	Noted in Urine	Total Time Elapsed
	PM-5/17	PM-5/17	
DBL	2:00	8:15	6:15
MS	"	6:45	4:45
JHW	"	6:45	4:45
RTC	"	7:30	5:30
CFS	"	5:15	3:15
HLJ	"	6:00	4:00
BIS	"	8:15	6:15
EHD	"	7:45	5:45
HUS	"	5:00	3:00
WEC	"	5:45	3:45

Comments: These uncoated tablets were as a control and to get an average time for the excretion of the dye.

TABLE II

Methylene Blue Coated with Cellulose Hydrogen Acetate Phthalate

Human	Given PM-5/21	Noted In Urine PM-5/21	Total Time Elapsed
DBL	1:30	12:00	10:30
MS	"	7:00	5:30
JHW	"	9:30	8:00
RTC	"	9:30	8:00
CFS	"	10:00	8:30
HLJ	"	8:00	6:30
BIS	"	9:30	8:00
EHD	"	10:15	8:45
HUS	"	7:30	6:00
WEC	"	8:30	7:00

Comments: In most instances the patients felt that they had noticed the color in the urine 3 to 4 hours earlier but were not absolutely positive until the times indicated. It should be noted that had they urinated earlier, the total elapsed time might have been shorter.

TABLE III

Methylene Blue Coated with Cellulose Acetate Stearate

Human	Given	Noted in Urine	Total Time Elapsed
	AM-5/29		
DBL	7:00	After 24 hours there was no evidence of the dye in the urine of any patient.	
MS	7:30		
JHW	7:00		
RTC	7:00		
CFS	7:30		
HLJ	7:30		
BIS	7:00		
EHD	7:30		
HUS	7:00		
WEC	7:00		

TABLE IV

Barium Sulfate Coated with Cellulose Hydrogen Acetate Phthalate

Human	Given	Noted in X-ray		Total Time Elapsed
	AM-5/24	5/24		
DBL	6:00	10:15 IS	1:00 DSI	7:00
MS	7:15	10:00 LS	1:30 DSI	6:15
JHW	6:00	10:00 LS	1:45 DSI	7:45
RTC	6:00	10:15 DSI	1:15 DSI	7:15
CFS	6:30	10:15 LS	1:45 DSI	7:15
HLJ	7:15	10:15 ISI	1:30 ISI	6:15
BIS	7:00	10:20 LS	1:30 DSI	6:30
EHD	7:00	10:20 LS	1:30 *	6:30
HUS	7:15	10:25 LS	1:00 DSI	5:45
WEC	8:00	10:30 IS	1:15 DSI	5:15

* No trace after 4 X-rays, so assumed to have passed through.

LS --- had left the stomach
DSI -- disintegrated in the small intestine
IS --- intact in the stomach
ISI -- intact in the small intestine

TABLE V

Barium Sulfate Coated with Cellulose Acetate Stearate

Human	Given	Noted in X-ray	Total Time Elapsed
	AM-5/29	5/29	
DBL	7:00	1:15 lesi	6:15
MS	7:30	1:55 lesi	6:25
JHW	7:00	1:15 lesi	6:15
RTC	7:00	1:20 lesi	6:20
CFS	7:30	2:00 lesi	6:30
HLJ	7:30	1:35 lesi	6:05
BIS	7:00	2:05 lesi	7:05
EHD	7:30	1:25 lesi	5:55
HUS	7:00	1:50 msi	6:50
WEC	7:00	1:30 lesi	6:30

The tablets were given before breakfast and found intact in the areas noted.

lesi --- lower end of the small intestine
msi ---- middle of the small intestine

TABLE VI

Phenylazo-a-,a-diaminopyridine monohydrochloride Pills Uncoated

Rat	Given	Noted in Urine	Total Time Elapsed
	PM-5/10	5/10	
1	2:30	4:00	1:30
2	10:15	11:15	1:00
3	12:45	3:00	2:15
4	12:50	3:00	2:10
5	12:55	3:00	2:05
6	1:00	3:00	2:00
7	1:50	3:30	1:40
8	1:55	3:00	1:05
9	2:00	4:30	2:30
10	2:05	4:30	2:35

This uncoated pill served as a control and also it indicated average excretion time of the dye in the urine.

TABLE VII

Phenylazo-a-,a-diaminopyridine monohydrochloride Coated with Cellulose Acetate Hydrogen Phthalate

Rat	Given	Noted in Urine	Total Time Elapsed
1	Pills were given between 9:50 and 10:05 to all rats on May 22.	7:00	9:00
2		2:00	4:00
3		3:00	5:00
4		2:00	4:00
5		1:00	3:00
6		2:00	4:00
7		3:00	5:00
8		2:00	4:00
9		7:00	9:00
10		2:00	4:00

TABLE VIII

Phenylazo-a,a-diaminopyridine monohydrochloride Coated with Cellulose Acetate Stearate

Rat	Given	Noted in Urine	Total Time Elapsed
1	Pills were given between 8:10 and 8:30 to all rats on May 14.	*	
2		*	
3		*	
4		*	
5		*	
6		*	
7		*	
8		*	
9		*	
10		*	

* There was no evidence of the dye in the urine at the end of twenty-four hours.

TABLE IX

Barium Sulfate Coated With Cellulose Acetate Hydrogen Phthalate

Rat	Given	Noted in X-ray		Total Time Elapsed
1	Pills given between 9:15 and 9:30 to all rats on June 20.	4:15	DSI	7:00
2		3:15	DSI	6:00
3		6:30	DSI	9:15
4		3:30	DSI	6:00
5		6:30	DSI	9:00
6		6:15	ISI	9:00
7		6:30	DSI	9:00
8		6:30	DSI	9:00
9		6:30	DSI	9:00
10		3:45	DSI	6:30

DSI --- disintegrated in the small intestine
ISI --- intact in the small intestine

TABLE X

Barium Sulfate Coated With Cellulose Acetate Stearate

Rat	Given	Noted in X-ray	Total Time Elapsed
1	Pills were given between 10:45 and 11:00 to all rats on June 24.	X-rays were taken between 9:00 and 9:15 on June 25.	10:00
2			"
3			"
4			"
5			"
6			"
7			"
8			"
9			"
10			"

In all cases the pills were found intact after the noted elapsed time.

TABLE XI

Urinary Excretion of Dye* in Hours

Type of Coating	Number		Human	Rat
Uncoated**	10	Av	4.73	1.88
		SD	1.17	0.242
		SE	0.37	0.077
Cellulose Acetate Hydrogen Phthalate	10	Av	7.7	5.1
		SD	1.95	1.74
		SE	0.616	0.563
Cellulose Acetate Stearate	10		***	***

* Humans - Methylene Blue
Rats - Phenylazo-alpha,alpha-diaminopyridine monohydrochloride

** Control

*** The coating did not allow disintegration as there was no dye detected in the urine

TABLE XII

Disintegration Time In Hours*

Type of Coating	Number	Human	Rat
Cellulose Acetate Hydrogen Phthalate	10	Av 6.5 SD 1.48 SE 0.49	7.9 1.37 0.46
Cellulose Acetate Stearate	10	**	**

* Determined by X-rays as the pills or tablets were made of Barium Sulfate.

** No disintegration

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