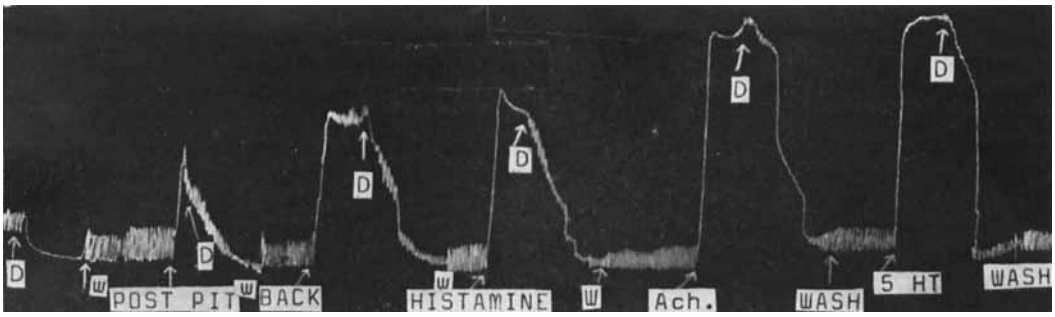

Fig. 3—Effect of muricatin on isolated rabbit ileum. Key: D, *drug (0.5 mg.).*

The blood pressure lowering property of muricatin is a characteristic response, which is partially blocked by atropinization. But this lowering of blood pressure persists even after spinal transection at the C_2 level. Therefore, it can be inferred that the lowering of blood pressure may be due to a myocardial depressant property of muricatin as evidenced by the observations on auricular and ventricular contractions.

The drug shows its benignity toward other organ systems of the body except a direct potent spasmolytic effect on gastrointestinal smooth musculature. In general, the pattern of relaxant and spasmolytic activity of muricatin was similar to that of papaverine. The difference was that the former was a weaker relaxant and spasmolytic compared to the latter. It therefore appears that the mechanism of action of muricatin on ileum is a papaverine-like, direct, nonspecific musculotropic action.

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Testing of Tablets with Prolonged Action. Enzyme Activity During the Modified Half-Change Method

By W. A. RITSCHEL and H. ORTH

The enzyme activities of pepsin, pancreatic lipase, trypsin, and amylase were studied. It was found that the activity of the enzymes in freshly prepared artificial gastric fluid and in artificial intestinal fluid decreases during heating from room temperature to 37° and by keeping at 37°. To maintain proper enzyme levels during an 8-hr. interval when testing tablets with prolonged action, a modified half-change method is suggested. A survey of enzyme levels when using this modified method is given.

MANY SUBSTANCES used in tableting as excipients and fillers, such as zein, starch, stearates, and others, are digestible by gastrointestinal enzymes. Therefore, the possible effect of enzymes on disintegration and/or drug dissolution of oral tablets in *in vitro* tests must be taken into consideration, particularly in the case of procedures of several hours' duration applicable to sustained-release medication. In prolonged-release medication forms the drug is often embedded in synthetic fats such as glyceryl monostearate (1), glyceryl myristate (1), glyceryl palmitostearate (2), *etc.* Such glyceryl fatty acid esters are hydrolyzed by pancreatic lipase (3). The enzymatic hydrolysis depends on the length of the carbon chain of the fatty acids (4). The liberation of the active principle embedded in the tablet follows a characteristic inverted curve, because beside diffusion of the drug, digestion of the tablet base material takes place after 3 hr. when optimal pH conditions are reached for the enzyme activity (5). Gelatin, used as a binder in wet granulation as well as in direct compression, is liquified by trypsin (6), while casein (7) and peptides used for coatings (8) are digested. Amylose, suggested as a dry binder for direct compression (9), is digested by amylase. Gelatin, casein, and other peptides are also digested by pepsin (10).

Studying the stability of gastrointestinal enzymes in artificial gastric and intestinal fluid, it was found that the activity of the enzymes was partly destroyed during the time needed to heat the test solutions from room temperature to 37°, and while

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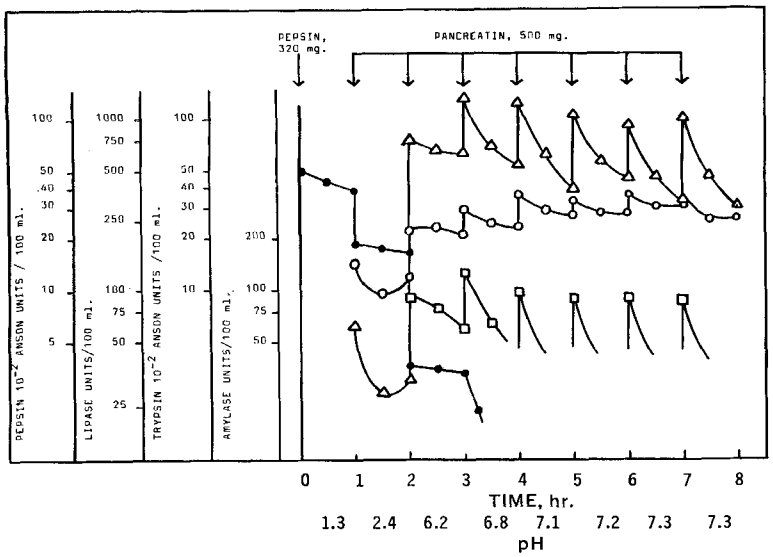

Fig. 1—Semilog plots for the ferment activity of gastrointestinal enzymes during testing with the modified half - change method at 37°. Key: ●, pepsin; △, lipase; ○, trypsin; □, amylase.

keeping artificial gastrointestinal test solutions at 37°. Loss of pepsin activity was 20% within 1 hr.; loss of lipase activity was 68% within 30 min. and 84% within 1 hr.; loss of trypsin activity was 50% within 1 hr.; and loss of amylase activity was more than 50% within 10 min. Based on the results obtained with lipase, it was felt that utilization of the principle of the so-called "half-change method" (11), in order to bring about uniformity of enzymatic activity during the course of the test period, was desirable.

EXPERIMENTAL

Materials—*Artificial Enzyme-Free Gastric Fluid*—Sodium chloride, 0.2%, and 0.7% hydrochloric acid in water. One hundred milliliters of the artificial enzyme-free gastric fluid at 37° was used in each test; 320 mg. of pepsin was added; pepsin activity was determined 30 and 60 min. thereafter.

Artificial Enzyme-Free Intestinal Fluid—Predried sodium phosphate, ($Na_2HPO_4 \cdot 2H_2O$), 0.805%, 0.165% sodium biphosphate ($NaH_2PO_4 \cdot 2H_2O$) in water. Fifty milliliters of the artificial enzyme-free intestinal fluid at 37° was used in each test.

Modified Half-Change Method—After a 60-min. interval, half of the volume of the artificial gastric fluid was withdrawn and substituted with 50 ml. of artificial enzyme-free intestinal fluid at 37°. At hourly intervals thereafter, again half of the fluid was withdrawn and substituted with fresh, prewarmed 50-ml. portions of artificial intestinal fluid. Immediately after each addition, 500 mg. of pancreatin powder was added. The test period was extended for 8 hr.

The activities of enzyme lipase, trypsin, and amylase were tested immediately after addition of each portion of pancreatin to the test solution, 30 min. thereafter, and just before the next addition.

Pepsin and trypsin were analyzed according to Anson (12); lipase activity was determined by the method described by Lazo-Wasem (13). For the estimation of amylase, a method described in the documentation of a manufacturer of pancreatin (14) was used; the method was based on the dextrinication point.

RESULTS AND DISCUSSION

The enzyme levels of the gastrointestinal enzymes during an 8-hr. run of the modified half-change method are shown in Fig. 1. Additions of pepsin and pancreatin are indicated by arrows; the different pH values of the test solution are also indicated in the figure. The change from pH 2.4 to pH 6.2 in the third hour is due to the effectiveness of the buffer substances in the artificial intestinal fluid.

It may be noted from the illustration that the pepsin level falls approximately 20% during the first hour at 37°. On substitution of half of the volume of the artificial gastric fluid by enzyme-free artificial intestinal fluid and addition of 500 mg. pancreatin, the decrease in pepsin activity corresponds to the level of dilution, but after the second substitution of the test solution by enzyme-free artificial intestinal fluid and subsequent addition of pancreatin in the third hour, remaining pepsin activity is negligible. The trypsin levels remain relatively constant during the whole test period, while lipase activity decreases to about 50% of initial between pancreatin additions. The expected amylase level could be found only after the second addition of pancreatin. The hourly additions of pancreatin result in short-lived measurable amylase levels.

Testing with the modified half-change method at a lower temperature to improve enzyme stability is not feasible since optimum enzyme activity depends on temperature and is correspondingly decreased at room or cooler temperatures.

The results obtained indicate that, when testing oral depot tablets, the dissolution or disintegration of which is dependent on gastrointestinal enzymes, it is necessary to use a method such as the "modified half-change method" in order to obtain uniformly effective enzyme levels.

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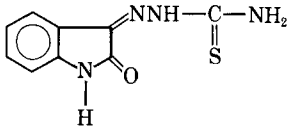
Thiosemicarbazones Derived from Indanedione-1,3

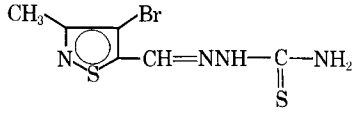
By RAJENDRA S. VARMA and W. LEWIS NOBLES

A series of thiosemicarbazones derived from indanedione-1,3 has been synthesized. Preliminary biological screening data are provided.

ANTIVIRAL (1–7) and tuberculostatic (8–14) activity has been reported for thiosemicarbazones of different carbonyl compounds. Further work on the thiosemicarbazones led to the discovery of the antiviral activity of isatin-3-thiosemicarbazone (I) against the pox group of viruses in human and type 2 polio in ERK cells (15). A number of monocyclic thiosemicarbazones derived from nicotinaldehyde, isonicotinaldehyde, and 2- and 3-thenaldehydes have also exhibited high antiviral activity (5). 4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (II), when given orally, was found to protect mice infected intracerebrally with neurovaccinia (15). Thiosemicarbazones derived from substituted pyrrolidine-2,3-diones demonstrated protection against experimental influenza infection in mice (15).



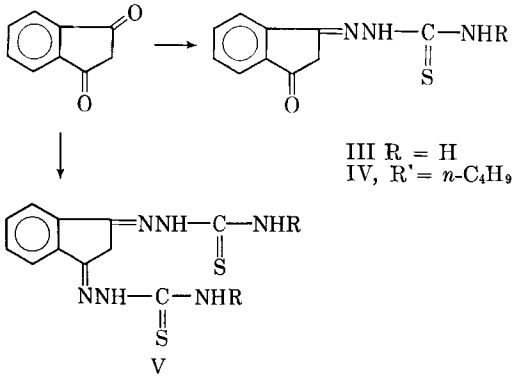
I



II

In view of the antiviral, antituberculous, and antitumor (16, 17) activity demonstrated by certain thiosemicarbazones, the synthesis and biological evaluation of compounds III, IV, and V appeared to be of interest (Scheme I).

Biological Evaluation—Several of the compounds described in this report have been subjected to preliminary antiviral and antibacterial screening procedures. The method with respect to the antiviral screening involved the use of the plastic panel and agar diffusion techniques and, in general, the



III R = H
IV, R' = n-C_4H_9

Scheme I

compounds were screened for possible activity against poliomyelitis type II, *Herpes simplex*, measles, and parainfluenza 3(HA-1) viruses.

In connection with the antibacterial screening, filter paper disks (6.35 mm.) saturated with 2 drops of a suspension of the test compounds (20 mg./ml. in alcohol or water) were tested with various organisms by the use of the agar diffusion method.

Indanedione-1,3-*n*-butylthiosemicarbazone demonstrated activity against the poliomyelitis type II virus as did the indanedione-1,3-methyl dithiosemicarbazone. The other compounds were inactive in this preliminary antiviral screen.

Indanedione-1,3-allyldithiosemicarbazone demonstrated a zone inhibition of 7.5 mm. diameter in the antibacterial screening against *Klebsiella pneumoniae* ATCC 8052; the other compounds reported were inactive against a variety of Gram-positive and Gram-negative organisms utilized in this *in vitro* screening procedure.

EXPERIMENTAL[1]

Indanedione-1,3-thiosemicarbazone (III)—Indanedione-1,3 (0.05 mole) was dissolved in 50 ml. of hot ethanol. To this solution a slurry of thiosemicarbazide (0.05 mole) in 50 ml. of ethanol was added. Instantaneous condensation occurred resulting in the separation of a yellow solid. The reaction mixture was heated for 10 min. and allowed to cool to room temperature. The product was removed by filtration and crystallized from dimethyl-

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The authors are grateful to Dr. Lyman Magee and Mr. William Bing, Department of Biology, University of Mississippi, for the antiviral and antibacterial screening data.

[1] All melting points were taken in capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. The infrared spectra were determined using a Perkin-Elmer 137 model spectrophotometer.