Absorption of Nitroglycerin from the Alimentary Tract of the Rabbit

By ROBERT A. TURNER

Nitroglycerin, either free or contained in controlled-release granules, when administered through the alimentary tract of the rabbit, caused dilatation of the vascular bed of the ear.

THE ABSORPTION of nitroglycerin from the alimentary tract, except the mouth, has been questioned for many years. In the directions for the use of pharmaceutical products containing nitroglycerin, it is stated that the tablet ought not to be swallowed but should be retained in the mouth and situated beneath the tongue or against the cheek. Statements in the pharmacological literature are similar. For example, Salter (1) mentions that nitroglycerin escapes decomposition by the gastric juice, but the rate of absorption is so slow that large doses are required. Sollmann (2) remarks that nitroglycerin is materially more potent when it is absorbed from the tongue than when it is swallowed. He adds that, after being swallowed, it is absorbed into the portal circulation and destroyed by the liver.

In this paper evidence is presented that nitroglycerin is absorbed from the alimentary tract of the rabbit. It appears that in this species, at least, a considerable quantity of the absorbed substance is not destroyed by the liver before it can exert its vasodilatative action

MATERIALS AND METHODS

White New Zealand rabbits were assigned to groups of eight. Each group comprised four animals of each sex, which ranged in weight from 2.05 to 2.45 Kg. Most of the animals weighed 2.2 Kg.; the mean was 2.27 Kg.

Two materials were used in essentially a four-point assay. A powder containing 10% nitroglycerin and 90% β-lactose served as the control. Controlled-release granules, containing 2.4% nitroglycerin served as the unknown.[1] The nitroglycerin in these granules is released over a period of hours when the granules are suspended in solutions simulating digestive juices. With the use of the rotating-bottle method of Souder and Ellenbogen (3), the granules used in this study released 30.5% of the contained nitroglycerin from simulated gastric juice during the first hour, 19.9 from simulated intestinal juice during the second hour, 23.8 from the same juice during the third and fourth hours, and 18.6 from the same juice during the fifth, sixth, and seventh hours. Thus, 92.8% of the nitroglycerin was released during 7 hr.

The low and high doses of the powder were 1.00 and 2.00 mg./Kg., respectively, *i.e.*, 0.10 and 0.20 mg./Kg. of nitroglycerin; the corresponding doses of the granules were 4.2 and 8.4 mg./Kg., *i.e.*, 0.10 and 0.20 mg./Kg. of nitroglycerin. All doses were suspended in 1% gum tragacanth so that the materials would remain dispersed for administration.

After intubation of the dose into a rabbit, the animal was placed in a stock. An ear of the rabbit was observed before a source of light, so that the vascular bed of the ear was apparent. Thus, when dilatation occurred, it could be recognized at once because the whole vascular bed became deeply colored. The onset of the dilatation and its duration were determined by observations of the ear every few minutes.

RESULTS

The results are presented in Table I.

The computed oral ED_{50} of the nitroglycerin powder was 1.1 mg./Kg., equivalent to 0.11 mg./Kg. of free nitroglycerin. The computed oral ED_{50} of the controlled-release granules was 4.2 mg./Kg., equivalent to 0.10 mg./Kg. of nitroglycerin. The threshold dose was found by graph to be 0.09 mg./Kg. for either material.

DISCUSSION

The results show that nitoglycerin, administered by intubation into the stomach of the albino rabbit, caused peripheral dilatation of the blood vessels. Therefore, it appears that nitroglycerin so administered was absorbed through the alimentary tract and conveyed to the periphery. The effect was produced by controlled-release granules as well as by free nitroglycerin. The results with the granules and with the free nitroglycerin appear to be sufficiently similar, so that a bioassay of the granules could be obtained by comparing their effects with those of nitroglycerin according to the method described.

TABLE I.—RESULTS

Animal No.	Response to 0.1 mg./Kg.[a] Degree	Response to 0.1 mg./Kg.[a] Duration, min.	Response to 0.2 mg./Kg.[a] Degree	Response to 0.2 mg./Kg.[a] Duration, min.
Nitroglycerin Powder (Control)				
1	None	0	Strong	65
2	Slight	20	Strong	71
3	None	0	Strong	57
4	Slight	37	Strong	77
5	None	0	Strong	68
6	Slight	34	Strong	85
7	None	0	Strong	66
8	None	0	Strong	77
Mean		11.4		70.8
Nitrong Granules (Unknown)				
1	Slight	55	Strong	167
2	None	0	Strong	77
3	Slight	47	Strong	158
4	Slight	40	Strong	190
5	None	0	Strong	150
6	None	0	Strong	155
7	None	0	Strong	160
8	Slight	41	Strong	137
Mean		22.9		149.2

[a] Of nitroglycerin.

Received August 11, 1964, from the Biological Science Laboratory, Division of Foster D. Snell, Inc., Bronx, N. Y.
Accepted for publication October 27, 1964.

[1] The granules were identical to those used in the preparation of Nitrong controlled-release tablets and were supplied by U. S. Ethicals, Inc., New York, N. Y.

While these results do not indicate what effects may occur in the human, they do show that nitroglycerin is effective in the rabbit when ingested. The possibility of buccal absorption is eliminated because none of the material touched the surfaces of the mouth. Incidentally, these results indicate that most of the nitroglycerin contained in the granules was released.

It is interesting that the effect of the nitroglycerin contained in the granules was more prolonged than the effect of the equivalent amount of free nitroglycerin, shown by a comparison of the results from the groups given nitroglycerin powder and those from the groups given the granules. To produce the prolonged vasodilatation, the nitroglycerin must have been released gradually from the granules. It is possible that if small quantities of nitroglycerin are released in the alimentary tract over a period of hours, the degradation by the liver may be less than that found when a quantity is released at once. The hypothesis would account for the marked and prolonged dilatation observed when the nitroglycerin source was the granule, contrasted wth that observed when the source was free nitroglycerin.

REFERENCES

(1) Salter, W. T., "A Textbook of Pharmacology," W. B. Saunders Co., Philadelphia, Pa., 1952, p. 311.
(2) Sollmann, T., "A Manual of Pharmacology," W. B. Saunders Co., Philadelphia, Pa., 1957, p. 631.
(3) Souder, J. C., and Ellenbogen, W. C., DRUG STANDARDS, 26, 77(1958).

Phosphorus-Nitrogen Compounds IV. Some 2-Aminopyridine Derivatives

By LINDLEY A. CATES and NOEL M. FERGUSON

Seventeen phosphoramidates and phosphoramidothionates containing 2-aminopyridine moieties were synthesized as potential antineoplastic agents.

THE PREPARATION of phosphoramidates and phosphoramidothionates has been extended in this report to include 2-amino and C-5 substituted 2-aminopyridines. The synthesis of related compounds containing *p*-toluidine moieties was described in an earlier paper in this series (1).

Synthetic work similar to the P-N compounds herein reported has been previously carried out by Russian investigators. Arbuzov and co-workers (2) prepared dialkyl *N*-2-pyridylphosphoramidates by the reaction between dialkyl phosphorochloridates and 2-aminopyridine. These investigators also attemped the synthesis of the dimethyl and diethyl esters of *N*-2-pyridylphosphoramidothionic acid and obtained uncrystallizable masses in each case. Compounds III and IV (Table I) indicate the preparation of the dimethyl and di-*n*-propyl esters of the 5-methyl homolog of this acid. Zhmurova and Kirsanov (3) earlier synthesized diphenyl *N*-(5-nitro-2-pyridyl)phosphoramidate (compound XV) by means of a different procedure, *i.e.*, the reaction between triphenyl *N*-phenylphosphorimidate and 2-amino-5-nitropyridine.

The phosphorochloridate-amine method was employed for the synthesis of the compounds summarized in Table I. Thus, compound XV was prepared by refluxing diphenylphosphorochloridate and 2-amino-5-nitropyridine in reagent dioxane according to previously described procedures (1, 4).

The 2-aminopyridines employed in the synthesis of these P-N compounds are congeners of 6-aminonicotinamide, because the carbamyl radical in the 3-position is replaced by a hydrogen, bromine, or chlorine atom and by a methyl or nitro group. The antimetabolic activity of 6-aminonicotinamide (5, 6) and its value as an anticancer agent and drug adjunct has been well established (7–11). A number of pyridines related to those used in this report have been investigated for antileukemic activity (12), and an absence of good inhibitory effect was noted in mono-substituted derivatives, such as 2-aminopyridine and 3-picoline. This latter compound was also shown to be inactive by Goldin *et al.* (13). Most antineoplastic activity appears to reside in 2,5 disubstituted pyridines, and the cytotoxicity of such derivatives is exemplified by the effect of 2-amino-5-nitropyridine against trichomonal infections (14). It has also been reported that derivatives of 3- and 5-nitropyridine and pyrimidine inhibit *T. vaginalis in vitro;* whereas 5-nitropyridines and pyrimidines with a single substituent at C-2 inhibit both *in vitro* and *in vivo* (15).

In those pyridines exhibiting antitumor activity, however, toxicity for the host has been a limiting factor in therapy. One approach to increasing selective cytotoxicity has been the incorporation of alkylating agents in P-N compounds (*e.g.*, cyclophosphamide) and is based on the enzymatic influence of phosphamidases, which occur in higher concentration in neoplastic cells. The compounds reported in this paper represent a further attempt to include potential antimetabolic moieties in similar phosphoramide structures.

Samples of the compounds listed in Table I have been submitted to the Cancer Chemotherapy National Service Center for preliminary evaluation.

EXPERIMENTAL

Syntheses.—The phosphoramidates and phosphoramidothionates (Table I) were prepared by standard methods involving the reaction between

Received September 3, 1964, from the College of Pharmacy, University of Houston, Houston, Tex.
Accepted for publication October 14, 1964.
This investigation was supported by research grant CA-06820-02 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.