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I hereby recommend that the thesis prepared under my supervision by Helen Stix Glazer

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II. The Effect of Atropine on the Toxic Actions of Dacryorrhetin.

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

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I. THE RELATION OF THE BLOOD PHOSPHOLIPIDS TO GASTRIC ULCERS AND STUDIES ON THE ANTAGONISM OF VARIOUS DRUGS TO THE GASTRIC ULCER PRODUCING ACTION OF BILE SALTS.

II. THE EFFECT OF ATROPINE ON THE TOXIC ACTIONS OF DACRYORRHETIN.

A dissertation submitted to the

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by

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Table of Contents.

I. The Relation of the Blood Phospholipids to Gastric Ulcers and Studies on the Antagonism of Various Drugs to the Gastric Ulcer Producing Action of Bile Salts.

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I. Introduction.

It is well known that the prevention and knowledge of the origin of a disease are often closely related. If the cause of a disease is known methods of prevention and treatment are often, though not always, suggested. For instance, the disease of dog-bite can be prevented by avoiding dogs, and most diseases caused by known bacterial agents can be prevented by avoiding contact with the offending organism or by immunization. In a similar manner the discovery of a cure or prophylaxis has often afforded a clue as to the cause of the disease. For instance, a diet including cabbage or citrus fruits was found to prevent scurvy. This eventually led to the discovery that scurvy is caused by a vitamin C deficiency. Because of this interrelationship between causative and preventive factors we have attempted to find means of preventing experimental gastric ulcers in the hope that this may throw some light on their etiology. Before discussing methods of prevention, however, we shall review the present theories of the etiology and prevention of peptic ulcers.

A. Acid Theory

The most common theory of the etiology of peptic ulcer

is that the gastric juice, especially the HCl, is largely responsible. Best and Taylor (8) state that the pepsin-HCl of the gastric juice is a dominant causative factor as shown by the following facts: (1) except in cases where the ulcer is caused by a specific disease, such as tuberculosis or syphilis, the ulcer occurs in that portion of the gastro-enteric tract where the wall is exposed to the acid action, e.g. in the lesser curvature of the pyloric region, in the lower oesophagus and cardia where the acid may be regurgitated, in the first part of the duodenum where the acid is not neutralized; (2) in cases of gastro-jejunostomy the stomal ulcer occurs at that point where the gastric juice hits first; (3) healing is encouraged when the excess acid of the juice is neutralized. They (8) also mention the hyperchlorhydria found in the majority of patients with duodenal ulcers and add that even though cases of gastric ulcer do not show hyperacidity this may be due to the resultant gastritis and does not exclude a preexisting hyperacidity.

Experiments on dogs (19, 44) show that injection or instillation of dilute HCl into the stomach will cause ulcers. Histamine, which stimulates the secretion of acid, also produces ulcers and will increase the severity of those caused by acid injection. (19)

However, Best and Taylor (9) state that "Though the

importance of acid in the production of ulcer can not be denied, this factor can not be solely concerned." For instance, (1) many people with hyperacidity do not have ulcers and there are some cases of ulcer with no free acid. Also, as mentioned above, gastric ulcers usually occur with a slight hypoacidity. The experimental work of Stalker, Bollman and Mann (72) with cinchophen shows that this chemical produces ulcers even though there is no change in gastric acidity.

It is possible that the acid in the stomach, even though not causing ulcers, does act as an important factor either in causing local erosion after some other factor has lowered the resistance of the stomach mucosa or in preventing healing. (32)

B. Neurogenic Theory

The neurogenic theory is supported by clinical and experimental observations. It is well known that the nervous "highly strung" type person is more likely to develop ulcer than the phlegmatic type. In ulcer treatment this factor is emphasized. Sedatives are given and the patient is told to avoid mental stress and strain of any kind. Most ulcer patients find that their symptoms which may have completely subsided recur if they undergo a period of worry or emotional shock. Harvey Cushing (22) found a high incidence of acute gastric ulcers following certain intracranial

operations. He also noticed a frequent association between gastro-enteric disturbances and tumors of the hypothalamus. He suggested that influences arising in the parasympathetic centers of the hypothalamus are conveyed along the vagus causing changes in the gastric mucosa, which then develops an ulcer. An association between tumors of the mid-brain and of the diencephalon and peptic ulcers has been observed (8). That these correlations are not coincidental is borne out by experimental work. Many investigators have produced gastric hemorrhage and ulcers by injuring the hypothalamus. Cushing's theory is further substantiated by the work of Beattie (4), who caused hyperemia and small erosions in the gastric musculature by stimulating the hypothalamus but prevented these changes by cutting the vagus nerve. Stimuli arising in other parts of the brain and central nervous system also seem to affect the stomach wall; extirpation of the celiac ganglia (42), section of the splanchnic nerves (42), section or removal of the vagus (5, 32), all lead to ulcer formation.

Evidence of the importance of local nerve reaction is shown by the production of ulcers in rabbits by paralyzing the motor nerve terminals and the sensory secretory plexus (6). The finding of perineuritis of the nerves adjacent to ulcers also tends to point to a local phenomenon (49).

It must be remembered however that the changes in innervation may affect other systems which may then be directly responsible for the ulcer rather than the nerves per se.

C. Circulatory Theory

The production of gastric ulcers by interference with the local blood supply supports the circulatory theory. Such interference may be produced by emboli (1), ligation (56), or by narrowing of the lumen of the vessel (56). However Alvarez (1) says that in animals in which 1/3 to 4/5 of the gastric blood supply was cut off no necrosis resulted. He mentions the possibility of collateral circulation. The production of ulcers by pilocarpine is interpreted by some as favoring the circulatory theory. Westphal (42) believes this drug causes extreme vagal irritation with resultant vascular spasm and ischemia, whereas Murata (42) believes the ischemia is due to the spasmodic contraction of the gastric musculature caused by the drug.

Hewlett (32) explains the observation that gastric ulcers in young people are usually acute while in older persons generally chronic on the basis of circulatory diseases. In the young the circulatory disturbance is usually infectious or embolic, therefore causing an acute gastric defect, whereas in older people the vascular disturbance is more likely to be arteriosclerotic, thereby causing progressive interference with

the nutrition of the stomach wall and so a more chronic lesion.

D. Endocrine Theory

Several well known facts concerning peptic ulcers suggest that the endocrine glands are important etiologic factors. The difference in sex susceptibility has often been observed. In children before puberty the ratio of ulcers in male to female is 1:1 but after puberty this ratio changes to 4:1 (63). Pregnancy causes remissions in ulcer patients. In a series of 25 ulcer patients (63) who became pregnant 52 times, only 2 had active symptoms during pregnancy and these two had abnormal births. Out of 70,310 consecutive cases (63) of pregnancy, there was only one proven case of peptic ulcer although there was a relatively high incidence of other gastro-enteric disturbances. In 1938 Sandweiss and his co-workers (62) found that antuitrin S, the anterior pituitary-like gonadotropic hormone found in great abundance during pregnancy, was of value in preventing ulcers produced by the Mann-Williamson operation. Theelin was not effective. The results with antuitrin S in human ulcer cases (63) were not so marked as in pregnancy, but he explains that the doses given were much lower than the levels maintained during pregnancy. Following this work (1939, 63) he used an extract from the urine of non-pregnant women, pre-

pared in the same way as antuitrin S and found it beneficial in dogs. The extract from the urine of ulcer patients lacked the protective factor.

Gastric ulcers are commonly associated with and aggravated by hyperthyroidism (1). Tashiro and Schmidt, (1931, 75) in our laboratories, have shown that thyroxine increases the susceptibility to ulcers produced by bile salts. They also showed (75) that thyroxine changes the type of ulcer from super-acute to one similar to the human type.

Injections of various other hormones will also cause ulcers in certain animals, e.g. pitressin (48), adrenaline (29, 30). Certain others seem to retard ulcer development (45, 25, 31, 54).

E. Miscellaneous Theories

The bacterial theory is supported by the work of Rosenow and Anderson, (60) who produced ulcers in guinea pigs by bacterial toxins; the toxins were ineffective however if neutralized. Also it has been noted that gastric ulcers often occur with various foci of infection, such as diseased tonsils, teeth, appendices. (32)

Immunological factors may also play a part. Shapiro and Ivy (68) suggest that ulcer production may be a form of Schwartzman reaction since they obtained acute ulcers in rabbits and dogs after local anaphylaxis. Kondritzer, (39) in this laboratory, found that animals which had been pre-

viously shocked were more resistant to bile salt ulcers.

Other factors which seem to be of some importance are the mechanical (43), traumatic, hereditary (1), nutritional (5), especially the vitamin factors, and chemical (47), but evidence indicates that although they may play a secondary role or retard healing, they are not a primary cause of ulcer in the majority of cases.

F. Methods of Treatment and Cure.

A study of the literature shows that the question of gastric ulcer treatment is very confused. The methods of ulcer production are extremely varied and although certain measures prevent one type of ulcer formation, they are ineffective in another. Insulin is reported as beneficial in human ulcers (31, 54), but Mimaki (45) found that it increased the number and depth of ulcers produced by histamine. Section of the vagus prevented ulcers from hypothalamic lesions (4), but there are many reports that section and removal of the vagus nerves actually produce ulcers (42).

Many widely varying methods of prevention have been tried. Dietary changes (44), fundusectomy (63), and injection of pituitary extracts (25) are just a few of the measures used.

Even with the same preventive treatment in the same type ulcer different workers disagree as to results. For instance Weiss and Aron (79) reported beneficial effects using histidine in dogs with Mann-Williamson operation.

Sandweiss et al (61) found no significant results.

Because of this confusion and the many discrepancies we do not feel that the literature on ulcer prevention is worthy of further discussion.

G. Phospholipid Factor.

The relation of blood phospholipids to gastric ulcers was first suggested by Dr. Tashiro's work (1931,74) on bile salts in the blood. He observed that the freer the bile salt fraction was of lipids, the more toxic it became. Following this Tsuruta (1931, 77), in our laboratories, found that the gastric ulcer producing property of bile salts was not affected by fatty acids but was antagonized by phospholipids. A definite protective ratio between phospholipid and bile salts was established.

Tashiro and Schmidt (1931,75) have shown that ulcer producing agents lower the blood phospholipids. They used five different agents, diphtheria toxin, streptococcus suspensions, bile salts, thyroxine, and adrenaline, and in all cases the blood phospholipid values were decreased from 11 to 51%.

On the other hand in pregnancy there is a decreased susceptibility to ulcer formation and an increase in blood phospholipid. Boyd's work (10-17) (1934-37) on the changes in blood phospholipids during pregnancy is very extensive and conclusive. He finds that the increase occurs in the plasma and that the rise is marked in those animals which

show this phenomenon. This increase of phospholipids in pregnancy is found in humans, mares, fowls, guinea pigs, and mice (78), but there is a decrease in cows and rabbits, and no significant change in dogs and rats. No explanation for this species variation is given, nor is the cause for the phospholipid changes known. Boyd mentions that an endocrine origin is one of the most popular explanations of the lipemia of pregnancy.

We know that phospholipids will prevent the ulcerogenic action of bile salts and also that ulcer producing agents cause a decrease in blood phospholipids. The purpose of this investigation was to determine if there are any substances which are neither phospholipid nor even lipid in nature which will antagonize bile salt ulcer formation and, if so, to select the most promising of these antagonizers and to determine if it causes any change in the blood phospholipid level.

II. Experimental Work.

A. Problem 1 - The Antagonism of Drugs to Bile Salt Ulcers

1. Methods.

Because bile salt ulcers have been extensively studied in these laboratories we used this means of producing ulcers in our experiments. The dosage of bile salts used was high enough to assure ulcer formation. 20mg. per 100gm. body weight is the ulcer dose, but in all our experiments we injected,

Table I

Prevention of Gastric Ulcers

Control Series - Bile Salts Alone

Number of G.P. used	Bile Salt mg/100gm. body wt.	Ulcer		No Ulcer	
		number	%	number	%
19	25.0-30.0	17	89.5	2	10.5

The problem of drug antagonism to the formation of experimental ulcers is complicated by the question whether the antagonistic agent actually prevents ulcer formation or whether it merely aids the healing processes. We shall use the term "antagonism" to mean either actual prevention or aid in healing.

2. Controls.

A series was run using bile salts alone to serve as a control for the rest of these experiments. As seen in Table I, only 2 out of a group of 19 did not get ulcers, a 10.5% variation.

3. Atropine.

Atropine was tried as an antagonizer because it prevented some of the effects of dacryorrhetin, which is an ulcer producing agent prepared in this laboratory.

Atropine sulphate was weighed and dissolved in a known quantity of sterile distilled water each day, because it has been reported that solutions of atropine deteriorate (2). The desired quantity was injected intraperitoneally using sterile technic. Doses from 0.025 to 25. 0 mg. per 100 gm. body weight were tried to see which amounts were most effective. Because of previous experience atropine was injected 10 minutes before bile salts. Later it was thought that better results might be obtained by dividing the dosage, giving half 10 minutes before and half 2 hours after the

bile salts. In this way the atropine effect would be prolonged.

As seen in Table II atropine will antagonize the production of ulcers. A divided dosage is more effective, giving 74% antagonism as compared with 47% for the undivided dose series. Large doses did not seem more antagonistic than small either when they were undivided or divided.

A small series was run on mice to see if atropine would prevent death, the toxic manifestation of bile salts in this species. In the control group, using 40mg. per 100gm. body weight, only 28.5% lived, but when atropine was given 100% of the animals survived (Table III.)

4. Pilocarpine.

Because the injection of pilocarpine produces ulcers (30) it was used first to see if small doses would increase the animals susceptibility to bile salts. Subminimal doses of pilocarpine and bile salts were given 10 minutes apart. When no aggravating effect was found (Table IV) this drug was then tried as an antagonizer. Known amounts of pilocarpine hydrochloride dissolved in sterile distilled water were injected intraperitoneally 10 minutes before bile salts. This time interval was sufficient to show the first effects of the drug, e.g. salivation. No marked antagonistic effect was found (Table V). The series was small, however, so that the results are not of much significance.

Table II

Prevention of Gastric Ulcers

Atropine and Bile Salts

Number of G.P. used	Atropine mg/100gm. body wt.	Ulcer		No Ulcer	
		number	%	number	%
	undivided				
12	.025-0.37	7	58.3	5	41.6
5	0.5	1	20.0	4	80.0
6	1.0	3	50.0	3	50.0
9	12.0-21.0	6	66.6	3	33.3
Total 32	.025-25.0	17	53.1	15	46.8
	divided				
15	0.5-0.75	4	26.6	11	73.3
11	1.0-1.5	2	18.1	9	81.8
1	25.0	1	100.0	0	0.0
Total 27	0.5-25.0	7	26.0	20	74.0
Grand Total 59	.025-25.0	24	40.7	35	59.3

Statistical analysis showed results of total series reliable, difference between divided and undivided dose series questionable.

Table III

Prevention of Death

Atropine and Bile Salts - Mice

Number of Mice used	Bile Salt mg/100gm. body wt.	Atropine mg/100gm. body wt.	Death		No Death	
			number	%	number	%
7	40.0	0.0	5	71.4	2	28.5
7	40.0	0.5	0	0.0	7	100.0

Statistical analysis showed results significant.

Table IV

Production of Gastric Ulcers

Pilocarpine and Subminimal Doses of Bile Salts

Number of G.P. used	Bile Salt mg/100gm. body wt.	Pilocarp. mg/100gm. body wt.	Ulcer	
			number	%
4	9.0	0.1-1.0	0	0.0

Table V

Prevention of Gastric Ulcers

Pilocarpine and Toxic Doses of Bile Salts

Number of G.P. used	Bile Salt mg/100gm. body wt.	Pilocarp. mg/100gm. body wt.	Ulcer		No Ulcer	
			number	%	number	%
6	25.0-35.0	0.05-1.0	3	50.0	3	50.0

Statistical analysis showed that while apparantly pilocarpine had an antagonistic action similar to that of atropine, the number of experiments was too few for the result to be significant.

5. Ergotamine.

Ergotamine was tried as an antagonizer because Ornstein and his co-workers (50) have reported that it causes a "tendency" towards an increase in blood phospholipids.

It was injected in the form of gynergen (Sandoz), a solution of ergotamine tartrate. The injections were given intraperitoneally immediately before the bile salts.

As seen in Table VI, it prevented ulcer formation in 76% of the cases, a significant antagonism.

6. Antuitrin S.

Antuitrin S., the anterior pituitary like gonadotropic hormone from pregnancy urine, was used because Dr. Tashiro (76) had received a personal communication that it might increase the blood phospholipids. Following this some preliminary experiments (76) in these laboratories showed that it exerted some antagonism to the ulcer producing property of bile salts. The results of this early work were irregular, probably due to the instability of the preparation at that time. Our work was started before the publications of Sandweiss et al (62, 63). Since their articles have appeared the importance of this hormone in gastric ulcers has become more evident.

The antuitrin S solution was injected subcutaneously over a period of days for slower absorption and a more last-

Table VI

Prevention of Gastric Ulcers

Ergotamine and Bile Salts

Number of G.P. used	Ergotamine mg/100gm. body wt.	Ulcer		No Ulcer	
		number	%	number	%
21	0.16-0.29	5	23.8	16	76.2

Statistical analysis showed results significant.

ing effect. In all but one case 10 rat units per 100gm. body weight (on the basis of daily weight) was given daily (except Sunday) for varying lengths of time. In that one case a daily dose of 5 rat units per 100gm. body weight was given. The bile salts were injected immediately after the last antuitrin S injection. In 3 cases a second injection of bile salts was given after a lapse in antuitrin S treatment.

Adequate amounts of antuitrin S prevented ulcers (Table VII). We are not sure, however, if adequacy is dependent on the size of the dose or the length of treatment. In view of Sandweiss's statement (63) that he got no evidence of larger doses being more effective it is probable that the length of treatment is more important. With the prolongation of treatment the percent of antagonism rose from 28.5 to 77.7% (Table VII). It was also found that the antagonistic effect seemed to last for at least 7 days but not for 35 days (Table VIII).

7. Pregnancy.

In humans pregnancy seems to protect against ulcers. Because of this and because there is a marked rise in blood phospholipids during gestation in guinea pigs (15), we tried to determine if pregnancy will antagonize bile salt ulcers in guinea pigs.

In these experiments bile salts were injected during the first quartile of pregnancy in 2 cases, and, in

Table VII

Prevention of Gastric Ulcers

Antuitrin S and Bile Salts

Number of G.P. used	Antuitrin R.U/100gm body wt.	Ulcer		No Ulcer	
		number	%	number	%
5	10-100	3	60.0	2	40.0
8	100-200	5	62.5	3	37.5
9	200-330	2	22.2	7	77.7
Total 22	10-330	10	45.4	12	54.5

Statistical analysis showed total results questionable, but results using over 100R.U. significant.

Table VIII

Prevention of Gastric Ulcers

Antuitrin S and Bile Salts - Prolonged Effect

Number of G.P. used	Antuitrin R.U/100gm body wt.	Days since final Ant. injection	Ulcer		No Ulcer	
			number	%	number	%
1	320	7	0	0.0	1	100.0
2	320	35	2	100.0	0	0.0

the third case, during the latter period of gestation.

There seemed to be an antagonism exerted if bile salts were injected early in pregnancy, but the effect was doubtful if both early and late term animals were included (Table IX). With such a small series, however, these results can not be regarded as conclusive.

B. Problem II - Changes in Blood Phospholipids.

Since we were able to antagonize ulcer formation by non-phospholipid substances we proceeded to the second problem. Do antagonizers change the blood phospholipids? As antuitrin S seemed the most promising of the drugs used, we studied the phospholipid levels in the blood of guinea pigs which had been treated with this hormone.

I. Methods.

Samples of blood from guinea pigs were taken by cardiac puncture. No anesthetic was used lest it affect the phospholipid levels. The blood was heparinized and centrifuged using calibrated centrifuge tubes to take account for any deviations in the ratio of plasma to cell volumes. Determinations were made on the plasma as the changes in phospholipid values usually occur here (18, 69).

2cc. of the heparinized plasma was pipetted off and slowly added to about 20 cc of alcohol-ether mixture (3 parts absolute methyl alcohol to 1 part absolute ether),

Table IX

Prevention of Gastric Ulcers

Pregnancy and Bile Salts

Number of G.P. used	Period of Pregnancy	Ulcer		No Ulcer	
		number	%	number	%
2	1st Quartile	0	0.0	2	100.0
1	4th Quartile	1	100.0	0	0.0
Total 3	1st and 4th Quartile	1	33.3	2	66.6

Statistical analysis showed total results questionable, but results of early pregnancy only significant.

which was rotated to keep the particles of plasma well broken up. This was vigorously shaken and allowed to stand overnight. It was then filtered through Schleicher fat-free filter paper, washed with the same alcohol-ether mixture and made up to 50cc.

25cc. portions were taken (representing 1cc. of plasma) and evaporated to dryness after the addition of a drop of capryllic alcohol to prevent foaming. The lipid phosphorus was then determined according to the Fiske-Subarrow method (27) as modified by Schmidt (66). 2.5cc. of 5N H_2SO_4 was added to the evaporated portion and the solution heated on a sand bath until charring appeared complete and there were no more white fumes. Then 0.5cc. concentrated HNO_3 was added and the heating continued until all HNO_3 fumes disappeared, and the white fumes reappeared. The tube was cooled, 10cc. distilled water, 2.5cc Molybate III (2.5% $(NH_4)_2MoO_4$ in water) and 1cc. reducing agent (1-amino 2-naphthol 4-sulfonic acid) added. This was made up to 25cc. with distilled water and allowed to stand 15 minutes. The standard was made up simultaneously in the same manner except that Molybdate I (2.5% $(NH_4)_2MoO_4$ in 5N H_2SO_4) was used instead of Molybdate III. The standard and experimental solutions were compared in a micro-colorimeter. Calculations were made according to Beer's Law. Blanks were run on the alcohol-ether mixture. The phospholipid values were computed from the phosphorus values by multiplying by the factor 23.5 (37).

Table X

Effect of Antuitrin S on Blood Phospholipids

Phospholipids in Plsama as Lecithin

G.P.	Before Treat.	After Antuitrin S Treatment 10 Rat Units/100gm.body wt./day					
		8 Days		15 Days		26 Days	
No.	mg.%	mg.%	% incr.	mg.%	% incr.	mg.%	% incr.
#76	48.64	66.51	36.7	68.15	39.8	57.81	18.8
#77	49.35	77.32	56.6				
#78	54.05	64.15	18.7	73.79	36.5	59.69	10.4
#79	44.65			61.81	38.4	58.98	32.1
#80	48.88						
#81	58.75	58.75	0.0			63.16	15.2
Mean	50.72	66.68	31.4	67.91	33.8	59.91	16.1

Statistical analysis showed increase significant.

Table XI

Phospholipids in Total Blood Volume and Doses of

Bile Salts Antagonized by Phospholipid Increase

Ant. S. Treat.	Body Wt. gms.	Blood Vol.* cc.	Phospholipids Conc. mg.%	Phospholipids Total Blood mg.	Phospholipids Total Incr. mg.	Bile Salt mg/100gm. body wt. antag. by Pl. incr.	Bile Salt mg/100gm. body wt. injected	Ulcer
G.P.#76								
Before	353	26.1	48.6	12.6				
8 Days	370	27.6	66.5	18.3	5.7	15.3		
15 Days	401	29.8	68.1	20.3	7.6	19.0		
26 Days	510	38.0	57.8	21.9	9.3	18.5	20.0	-
G.P.#77								
Before	385	28.3	49.3	13.9				
8 Days	430	32.0	77.3	24.7	10.8	25.0	died before inj.	
G.P.#78								
Before	374	27.0	54.0	16.4				
8 Days	410	30.5	64.1	29.2	12.8	31.0		-
15 Days	423	31.3	73.8	36.4	19.9	47.0		
26 Days	540	40.2	59.7	38.0	21.6	40.0	24.7	-
G.P.#79								
Before	352	26.1	44.6	11.6				
15 Days	414	30.6	61.8	18.9	7.3	17.6		
26 Days	530	39.5	60.0	23.7	12.1	22.0	25.0	-
G.P.#81								
Before	396	29.1	58.7	17.1				
8 Days	370	27.6	58.7	15.9	decr.			
26 Days	560	41.7	63.2	26.4	9.3	16.6	25.3	-

*Blood Vol." = body wt. (gms.) X $\frac{0.08}{1.06}$

2. Results.

There was a definite increase in the plasma phospholipids after 8 days of treatment. This increase continued until sometime between the 15th and 26th day when the phospholipid concentration decreased (Table X). If, however, account is taken for the increased blood volume which accompanies the increased body weight it is seen that the total amount of phospholipid in the circulating blood was still increasing (Table XI).

III. Discussion.

Will any one of the existing theories of ulcer etiology explain the antagonism exerted by all the drugs which we used? Atropine and antuitrin S have been reported by some workers to decrease gastric acidity (21, 35), but others have found no change (3, 57, 26, 63.). The effect of ergotamine has not been reported.

Discussion of the action of these drugs on the nervous system is complicated by the fact that their effects vary with the dosage, species of the animal used as with the "neurotonic condition of the animal and the functional state of the organ affected", (20). However, generally speaking, atropine is a parasympathetic inhibitor, while ergotamine paralyzes the sympathetics (3, 70). Antuitrin S has not been studied in this regard.

The same discrepancies occur when the circulatory system is considered. Generally atropine dilates blood vessels, ergotamine constricts (3, 70), and the effect of

antuitrin S is not known.

In a like manner it is impossible to explain the antagonistic effect of all these drugs on the basis of any of the other common theories. Because of these inconsistencies it is improbable that the antagonism of these drugs is dependent on any of these factors.

Before postulating the theory that the phospholipids prevent ulcer formation it is necessary to see if the various conditions which influence ulcers affect phospholipids.

As stated earlier many substances which produce ulcers decrease phospholipids. Various bacterial agents such as diphtheria toxin and streptococcus suspensions cause ulcers and also decrease blood phospholipids (75). Friedman (29) and McCann (42) produced ulcers in rabbits and guinea pigs using adrenaline, which according to numerous authors decreases blood phospholipids (33, 24, 53). Pitressin, which forms ulcers in rabbits and rats (7) also decreases phospholipids (58). Bile salts are known to cause gastric ulcers (67) and to reduce the phospholipids (75). Vitamin B deficiency has been reported to produce ulcers (23) and also to decrease blood phospholipids (38).

On the other hand, lecithin, which will reduce the incidence of bile salt ulcers (77), not only is itself a phospholipid, but, according to Osoda (51), increases the lecithin of the blood. The increased blood phospholipid

in pregnancy and the decreased susceptibility to ulcers has already been discussed. Antuitrin S increases resistance to ulcers (63). Laskowski (40) found that gonadotropic hormone from the pituitary or from mare serum raised the blood phospholipids in resting and laying hens. He says however that the gonadotropic hormone of urinary origin does not change the blood phospholipids, but he gives no tables nor any account of his methods of preparation.

A difference in sex susceptibility to gastric ulcers has been observed both in experimental animals and in humans (1, 77). In guinea pigs (75) and fowls (41) the blood phospholipid is higher in the female than in the male. No comparisons have been reported yet in humans. It is possible therefore that this sex difference in ulcer susceptibility is on the basis of phospholipid values.

The so called "ulcer age" is from 20 to 40 years. This is very interesting in view of the statement by Stearns and Warweg (73) that the phospholipids reach a maximum in early childhood and maintain that level throughout early adolescence and one by Parhon, Ornstein and Sibi (55) that lecithin values in a group of individuals 18 to 48 years old were lower than those in a 60 to 85 year old group. Page et al (52) found no significant difference between the ages of 20 to 90 years. However their observations were made on men. The other authors do not state the

sex of their groups. Kaufman and Muhlback (34) found the lecithin values in women after menopause to be higher than those in normal or castrated women.

The sex hormones may influence the levels of blood phospholipids. If so we can then explain the observation that the incidence of ulcers in children is independent of sex while in adults ulcers are four times more common in men than in women. The marked decrease in the occurrence and symptoms of ulcers during pregnancy also conforms with this explanation as here there is a rise in blood phospholipids. The work of Lawrence and Riddle (41) on fowls presents an interesting correlation. They noted that the phospholipid content of the plasma in males and in non-laying females was much lower than in laying females.

In our experiments we found that atropine, ergotamine, antuitrin S, and early pregnancy antagonized bile salt ulcers. The only previous work on the effect of the drugs on blood phospholipids is that of Ornstein et al (50). The changes they report were so slight and irregular that we do not regard them as significant. The effect of pregnancy on blood phospholipids has been determined by Boyd (10-17). He found a marked rise in the blood phospholipids in pregnant guinea pigs (15).

We found significant increases in plasma phospholipids following Antuitrin S (Table X). This was especially

true if the amount of phospholipid in the total blood volume was considered (Table XI). Tsuruta (77) has shown that a phospholipid : bile salt ratio of 1:10 prevents bile salt ulcers in guinea pigs. Although the normal values of phospholipid seem high enough to antagonize the amount of bile salts injected, it must be remembered that this phospholipid may already be used in antagonizing the bile salts which normally occur in the body. It is important therefore to see if the increase in phospholipids was sufficient to antagonize the amount of bile salts injected. We computed the amount of phospholipid in the total blood volume, and, on the basis of the animal's weight and Tsuruta's ratio, found what dose of bile salts the increase in phospholipids would antagonize. The results are shown in Table XI. In guinea pig #76 the dose of bile salts injected could be antagonized by the increase in phospholipid. This animal died of peritonitis; there were no ulcers. Guinea pig #77 died following blood sampling so that no bile salt injection could be given. Guinea pig #78 had an increase great enough to antagonize more than the dose given, while the increase in guinea pig #79 would antagonize just slightly less than the amount given. Neither of these animals showed any toxic effects from the bile salts. Although guinea pig #81 did not have an increase great enough to account for the protection against the bile salt injection it is noteworthy that the

original concentration of bile salts was unusually high. It is possible that this case is one of that small group which is naturally resistant to bile salt ulcers. This resistance may be due to an unusually high blood phospholipid level.

IV. Summary.

1. Atropine, ergotamine, antuitrin S and early pregnancy antagonize bile salt ulcer production in guinea pigs.
2. None of the existing theories of ulcer etiology explain this antagonism.
3. A review of conditions influencing ulcers suggests the theory that blood phospholipids are a factor in ulcer prevention.
4. Pregnancy has previously been shown to increase blood phospholipids. Our determinations show that antuitrin S also increases blood phospholipids.
5. Blood phospholipids are a factor in ulcer prevention.

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The Effect of Atropine on the Toxic Actions of Dacryorrhetin.

I. Introduction.

The preparation and properties of dacryorrhetin prepared from muscle have been reported previously (5). A synthetic dacryorrhetin has also been prepared now in this laboratory. Details of this work will appear elsewhere (4). A toxic dose of this product, like that of natural dacryorrhetin, causes secretion of "milky tears", saliva, gastric ulcers, and death when injected into guinea pigs. We have found that the production of bloody tears in rats by natural dacryorrhetin can be prevented by atropine (6). We have also found that the production of gastric ulcers and death in guinea pigs by bile salts can be prevented by atropine (6). Because of this we studied the effect of atropine on guinea pigs treated with dacryorrhetin.

II. Experimental Work.

In all our experiments we used male guinea pigs. The injections were made intraperitoneally using sterile technic. Dosage was computed on the basis of body weight. The animals were kept on a regular stock diet.

A. Natural Dacryorrhetin

With dacryorrhetin prepared from beef heart muscle, as low as 38mg. per 100 gm. body weight caused saliva and milky

tears (Table I). Atropine (1.0 mg. per 100 gm. body weight injected 5 minutes before dacryorrhetin) with comparable or even higher doses of dacryorrhetin always antagonized the saliva and tear production (Table II). The effect of atropine on gastric ulcers and death caused by natural dacryorrhetin was not studied.

B. Synthetic Dacryorrhetin.

At the time these experiments were conducted the method of obtaining natural dacryorrhetin was not perfected so that there was great variation in the toxicity of the product. Because of this and because of the length of the procedure necessary to extract natural dacryorrhetin, the synthetic form was used in the following experiments.

The synthetic dacryorrhetin was prepared by Miss Badger in these laboratories. According to Dr. Tashiro and Miss Badger (4) the pure synthetic form will produce gastric ulcers in guinea pigs in doses of 20γ per 100 gm. body weight, bloody tears in rats in doses of 10- 15γ per 100 gm. body weight when injected intraperitoneally. The product used in this work was impure, 1/10th as active as the pure judged on the basis of bloody tear formation. The impurity was largely urea. Since this substance has no effect on ulcer production (4) and since there was little pure synthetic dacryorrhetin available, we used this product.

With the impure synthetic dacryorrhetin as low as 0.2 mg.

Table I

Natural Dacryorrhetin--- Control Series

Guinea Pig		Dacry.	Toxicity	
No.	Wt. gms.	mg/100gm. body wt.	Saliva	Tear (milk)
966	229	38.4	+	+
963	175	45.7	+	+
965	227	46.7	+	+
964	220	50.4	+	+
974	260	49.0	+	+
962	174	51.8	+	+
971	253	50.2	+	+
972	268	68.6	+	+
973	265	72.4	+	+

Table II

Natural Dacryorrhetin and Atropine

Guinea Pig		Dacry.	Atropine	Toxicity	
No.	Wt. gms.	mg/100gm body wt.	mg/100gm body wt.	Saliva	Tear (milk)
968	266	50.0	1.0	-	-
967	250	51.6	1.04	-	-
969	250	59.6	1.0	-	-
975	266	75.0	0.97	-	-
966	259	77.0	1.0	-	slight
970	263	100.0	1.0	-	-
965	255	101.0	1.0	-	-

per 100 gm. body weight produced saliva and milky tears. Larger doses (0.25- 0.31 mg. per 100 gm. body weight) produced gastric ulcers, either hemorrhagic spots or definite lesions, lung infarcts, and death within three hours (Table III). Atropine (0.5 mg. per 100 gm. body weight 10 minutes before and 2 hours after dacryorrhetin) antagonized the toxic manifestations of dacryorrhetin even though higher doses of dacryorrhetin were used. In 17 experiments none of the animals had milky tears or died, only one had salivation. In the 8 animals purposely killed 24 hours after injection only two showed ulcers (Table IV).

Table III

Synthetic Dacryorrhetin --- Control Series

Guinea Pig		Dacry.	Toxicity				
No.	Wt. gms.	mg/100gm body wt.	Saliva	Tear	Death	Ulcer	Lung Infarcts
1432	716	0.195	+	clear	-		
1433	839	0.20	+	milk	-		
1427	740	0.20	+	milk	40"	+	multiple
1435	788	0.247	+	?	1½hr	+	multiple
1434	979	0.25	+	milk	2hrs	+	-
1428	754	0.25	+	milk	3hrs	+	multiple
1421	787	0.31	+	milk	35"	+	multiple

Table IV

Synthetic Dacryorrhetin and Atropine

G.P. No.	Wt. gms	Dacry. mg/100gm body wt.	Atropine mg/100gm body wt. before	Atropine mg/100gm body wt. after	Toxicity Saliva	Tear milk	Death	Ulcer	Lung Inf.
1415	558	0.30	0.5	0.5	-	-	-		
	557	0.32	0.56	0.5	-	-	-		
	562	0.30	0.5	0.5	+	-	#	+	small
1416	797	0.30	0.5	0.5	-	-	-		
	787	0.30	0.56	0.5	-	-	-		
	831	0.30	0.5	0.5	-	-	#	-	-
1417	770	0.30	0.5	0.5	-	-	-		
	773	0.30	0.56	0.5	-	-	-		
	804	0.30	0.5	0.5	-	-	#	-	-
1418	729	0.30	0.5	0.49	-	-	-		
	774	0.30	0.5	0.5	-	-	#	-	-
1419	808	0.30	0.5	0.5	-	-	-		
	830	0.30	0.5	0.5	-	-	#	-	small
1420	773	0.32	0.47	0.54	-	-	-		
	729	0.30	0.5	0.5	-	-	#	-	small
*1432	733	0.30	0.5	0.5	-	-	#	+	small
*1433	349	0.30	0.5	0.5	-	-	#	-	small

- killed 24 hours after injection for autopsy

* - used 9 days before in control series.

III. Discussion.

We have shown that atropine will completely antagonize the toxic action of both natural and synthetic dacryorrhetin. The way in which it acts is not yet clear. Some experiments, to be reported elsewhere (6), have shown that it does not act directly upon dacryorrhetin. Since we do not know yet how dacryorrhetin produces gastric ulcers or lung infarcts it is impossible to discuss its action in preventing these changes.

The production of milky tears was noted many years ago (3). The milky appearance is due to the presence of fat droplets. The origin of the secretion is not definitely established, but it is probable that it comes from Harder's gland, which is the tear gland associated with the nictitating membrane (1). The gland is composed of two parts, an upper "white" lobe and a lower "red" lobe (2). Microscopically it is similar in structure to the secreting mamma and fat glands. Microscopic studies were made of the gland in normal and dacryorrhetin-injected guinea pigs and rats. Since no pathological changes were evident, it is probable that dacryorrhetin produces milky tears by stimulation of fat secretion of this gland.

The complete innervation of this gland has not been reported. Krause (2), in his work on the rabbit, speaks

of small nerve stems consisting of two or more "double outlined" ("doppelt konturierten") nerve fibers. This probably refers to medullated fibers, since most parasympathetic nerves are medullated while most sympathetic nerves are not. Stimulation of the parasympathetic nerves causes secretion of most glands. Atropine paralyzes the parasympathetic system. Thus, if the tear production is due to parasympathetic stimulation of Harder's gland atropine will inhibit the secretion. Salivation is caused by parasympathetic stimulation and is abolished by paralysis of these nerves through atropine. We believe therefore that the basis of the antagonistic action of atropine to the production of tears and saliva by dacryorrhetin is due to the paralysis of the parasympathetic nerves.

IV. Conclusions.

1. Dacryorrhetin from beef heart muscle produces milky tears and saliva in guinea pigs.
2. Synthetic dacryorrhetin produces milky tears, saliva, gastric ulcers, and death with lung infarcts in guinea pigs.
3. Atropine prevents the toxic actions of both natural and synthetic dacryorrhetin.
4. The source of the milky tears is probably Harder's gland.
5. It is suggested that dacryorrhetin produces milky tears and saliva by parasympathetic stimulation and that atropine antagonizes these actions by paralyzing these nerves.

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