THE EFFECT OF COD LIVER OIL AND ORANGE JUICE ON THE GASTRIC ACIDITY OF DOGS MADE ANEMIC BY HEMORRHAGE

by

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A. B., Drury College, 1925

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE AGRICULTURAL COLLEGE

1929

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INTRODUCTION

Anemia presents an unsolved problem with various suggestions as to the prevention and cure of its different types. Since the acidity of the gastric juice is often lowered in anemia, it seems probable that a means of controlling this reaction may prove of value in treating the disease.

The present investigation has been carried on as a continuation of work recently done at Kansas State Agricultural College (1) which indicated that cod liver oil was effective in bringing about a higher gastric acidity in anemic college girls. The purpose of this work has been to determine the relationship of hemorrhagic anemia to the gastric acidity of adult dogs and to ascertain the effect of cod liver oil, and of cod liver oil with orange juice on this condition.

REVIEW OF LITERATURE

One of the most important internal secretions of the body is the gastric juice. Its function is a digestive one and this activity is largely dependent on its acidity which is quite variable.

A study of gastric conditions from a clinical standpoint began about 1844 (2). Formerly the attention of physicians had been given chiefly to the lungs and heart, but

William Brinton who was an English physician of note realized the importance of gastric conditions and founded a system for the study of gastro-intestinal diseases, contributing much of the early knowledge concerning them.

Various methods have been used for obtaining samples of gastric juice. In some cases vomiting was induced and again a sponge, placed in a perforated capsule, was swallowed and recovered by means of a string. Later the gastric pouch or fistula was perfected by Pavlov (3), a Russian scientist. For this the stomach was cut and a portion so arranged that the mucous membrane protruded on the outside. From this fistula the pure secretion could be collected. At the present time this method is often employed with experimental animals.

For clinical examinations and much research the stomach tube is used, and a fractional method of analysis carried out. The Lyon's modification of the Rehfuss tube, made of flexible rubber, is three-sixteenths of an inch in diameter and has a metal end-piece which is perforated to admit the gastric juice and is of sufficient weight to help carry the tube down. Another called the Sawyer tube is made entirely of rubber and is equipped with a wire which is stiff enough to aid in guiding the tube downward. When the tube has reached the stomach the wire is withdrawn, a syringe attached and the contents removed by suction. After a sample of

the fasting juice has been obtained a standard test meal is fed. Then at regular intervals a series of samples are collected. These usually show an ascending and descending curve of acidity and so can be more accurately used for comparison than can any one sample.

The gastric juice may be tested for each of its constituents although its acidity, both free and total, is the property most commonly determined. This may be done colorimetrically by using indicators, the colors of which change at certain pH values. These colors may be matched with a series of standard tubes which possess different pH values and so show a gradation of colors. Since there is a tendency for the colors to fade on standing, and since the end point is not easily determined, this method is open to criticism.

More commonly the gastric juice is titrated with sodium hydroxide. The indicator phenolphthalein is used in determining the total or maximum acidity since it is so delicate that it will detect even the very weak organic acids and those which are temporarily combined with protein. The hydrochloric acid is determined with Topfer's reagent, which is di-methyl-amino-azobenzene, and is not affected by the weaker acids.

The gas-chain method gives a more accurate determination of hydrogen ion concentration but is too complicated

for clinical work. For this process platinum electrodes which are saturated with hydrogen gas are used, one immersed in the solution to be tested and another in a solution of known hydrogen ion concentration. As the solutions become saturated with hydrogen the electrodes become more negative. There is thus set up at each electrode a difference of potential between the electrode and the solution. When this reaches equilibrium it can be measured as voltage and used to compute the hydrogen ion concentration.

Clinically there is a great variation in acidity and the following terms are used to describe these differences. Hypoacidity and anacidity denote a lower, and hyperacidity a higher acidity. Achlorhydria denotes the entire absence of hydrochloric acid and achylia the absence of hydrochloric acid, rennin and pepsin. These terms are further defined by Michaelis and Davidsohn, as quoted by McClendon (4), as hyperacidity meaning a hydrogen ion concentration of from .011 to .088, the average acidity from .028 to .0015, and hypoacidity from .00041 to .0000001. Mathews (5) states the acidity in clinical units and for the normal juice at the end of the digestive period gives the total acid as from 74 to 90 and the free acid as from 40 to 75. Keefer and Bloomfield (6) studied the gastric juice of normal adults and found that, at the peak of digestion after a test meal, it had a pH of approximately 1.2 to 1.4. In studying gastric

anacidity they based the diagnosis on the absence of free acid in the gastric content 45 minutes to one hour after the ingestion of an Ewald meal. Carlson (7) believes that there may be a hypersecretion but that there is no hyperacidity in the sense of a gastric juice of greater than normal acidity. As a result of many normal gastric analyses Rehfuss, Bergeim and Hawk (8) observed three types of acidity curves, varying as to the position of the peak. These were designated as the hyposecretory with a slow ascension and rapid decline, the hypersecretory with a rapid ascension and slow decline, and the isosecretory in which both periods were characterized by equal rapidity. They also found that on different days each person showed the same type of curve but with variations.

The volume of gastric juice secreted during any given period of digestion varies with the quantity and kind of food (9). Pavlov (3) found that on a mixed diet of 50 gm. of meat, 50 gm. of bread and 300 cc. of milk there was a secretion of 42.0 cc. of gastric juice. When the amount of food was doubled there was a secretion of 82.2 cc. Also the digestive power varied with the type of food. When fed bread, dogs secreted a gastric juice of 38 mm. digestive strength, if fed flesh its strength was 16 and if milk it was 10.

Since the gastric juice is variable as to both quantity

and quality, a study of the mechanisms concerned with its secretion becomes a question of importance. That the flow of gastric juice follows the sight, smell and taste of food is a matter of common knowledge. According to Ivy (10), these reactions usually disappear in about 30 minutes and the remainder of digestion results chiefly from mechanical and chemical stimuli. Pain usually inhibits the flow of gastric juice and there is no result from the direct stimulation of the vagi nerves (7).

There have been numerous experiments to determine the cells which secrete hydrochloric acid. The most generally used method has been to test portions of the gastric mucosa with dyes which did not have a toxic effect but which gave an indication of pH value. Using neutral red and cyanamin, Ivy and Dawson (11) found that the canaliculi and cytoplasm of the active parietal cells had a pH of between 3.0 and 6.8, indicating the presence of hydrochloric acid.

Some investigators (3) have suggested that the gastric juice is secreted with a constant acidity and that the differences are due to a secondary neutralization by the mucous membrane. Later evidence points to the theory that the total chlorides of the gastric juice are secreted in constant amounts even though the hydrochloric acid varies. McLean and Griffiths (12,13) believe that the chlorine ion

is brought to the glands by the blood as sodium chloride and is secreted at a definite fixed concentration, part of it unchanged as sodium chloride and part changed into hydrochloric acid. The extent of this change governs the acidity of the secreted juice.

So many factors are concerned with gastric secretion that the real cause of abnormalities is difficult to determine. Cheney (14) states that achlorhydria may follow chronic gastritis and that gastritis may be caused by habitual indiscretion in food habits as to quantity, quality, temperature or regularity, by the chronic swallowing of infectious material or by a stasis in the circulation through the gastric walls. Some investigators suggest that heredity may play a part in gastric conditions. Supporting this is Martinez (15) who has found achlorhydria existing in both parents and children in 20 or 30 cases.

Studies show striking relationships between varying degrees of acidity and other conditions of the body. The presence of achylia with pernicious anemia was first demonstrated about 1870 by Fenwick (16) and this condition has now been evidenced by many authorities. Carlson (7) has concluded that "the anemias, pernicious, secondary and experimental, are practically always associated with gastric hypoacidity and anacidity parallel with the degree of

cachexia". Basu (17) reported a marked deficiency in hydrochloric acid in the gastric juice in the cases of anemia among the troops in Bombay. Cheney (14) and Skoag (18) state that achlorhydria is always found with pernicious anemia and may precede it for years. According to Vanderhoof, as quoted by Larimore (19), there is good reason to believe that every person with true achylia gastrica has a potential case of either pernicious anemia or combined spinal sclerosis.

Gastric acidity would seem to be an important factor in the absorption of iron since mineral salts are more soluble in an acid medium and since the acidity of the small intestine, where absorption takes place, is largely dependent upon the hydrochloric acid of the gastric juice. That gastric digestive ability is a factor in preventing anemia was shown by the fact that after complete gastrectomy dogs lost weight, the hemoglobin decreased and anemia developed (20). Castel (21) showed that the feeding of skeletal muscle which had been previously digested in the human stomach was effective in curing pernicious anemia.

Whether the gastric anacidity is a cause or effect of anemia has not been definitely determined. Carlson (7) believes that hypoacidity is more probably a result than a causative factor of the diseases with which it is associated while Cheney (14) states that both may be the result of the

same cause. There is at least a sure relationship between a lowered gastric acidity and anemia.

Clinical statistics show other conditions also to be associated with varying acidities. In an examination of 35 cases of pellagra,Guthrie (22) found a large percentage of cases with entire absence of hydrochloric acid in the gastric contents. Babbott, Johnson and Haskins (23) reported that rachitic infants developed an infection with fever which in turn gave a diminished gastric acidity. The bacterial flora, which will flourish or die according to the gastric acidity seems to be a factor in the production of pernicious anemia (24). Hyperacidity is found with ulcer of the stomach and a great diminution or complete suppression of free hydrochloric acid with gastric carcinoma (5). Eisen (25) states that the most severe anemia is found associated with cancer of the stomach. Achlorhydria may be the result of long standing or severe diabetes (26) and often occurs in exophthalmic goiter but, according to Moll and Scott (27), is not a predisposing cause.

Keefer and Bloomfield (6) made a study of 500 gastric analyses. Although in the past the condition of gastric anacidity had been associated with many disorders such as gall stones, dental disturbances, asthma, hyperthyroidism, tuberculosis and anemia, these workers found a low degree of

relationship. Their conclusion was that there was more anacidity in older people regardless of other conditions; of the diseases those of the stomach were the only ones which had a sure and definite relationship.

Cheney (14) reported an extensive study of the significance of achlorhydria. Aside from its connection with pernicious anemia he stated that achlorhydria or a hyposecretion seemed to be related to gallbladder disease and was almost always found with sclerosis of the spinal cord. In the diagnosis of disease he considered the discovery of achlorhydria a matter of great importance.

As in the case of anemia, many of the foregoing instances of anacidity indicate that it is cause rather than effect of the accompanying disturbances. For this reason methods are being tried by which the acidity may be controlled. For many years this has been done by the oral administration of an acid in the case of hypoacidity, or an alkali in the case of hyperacidity (27). Hydrochloric acid is given in varying amounts with good results but, according to Kern, Rose and Austin (29) it does not stimulate the spontaneous appearance of free acid in the digestive juice.

Subjecting the stomach to low millamperage with medium voltage (diathermy) immediately stimulated the acidity and when such treatment was continued at frequent intervals over

long periods of time, it tended to increase both the free and total acid (30). The drinking of mineral water containing sodium chloride has been tried with only slight success (31).

If, as Carlson believes, hypoacidity is an effect of all dietary diseases when far enough advanced, it seems probable that an improved diet factor would be of advantage in its prevention and treatment. Some recent experimental evidence (1,20,32) suggests that cod liver oil may be of value in controlling the gastric acidity.

The curative value of cod liver oil has long been recognized. According to historical evidence given by Guy (33) it was first used as a family remedy by fishing peoples and peasantry; later the beneficial effects were noticed and made use of by physicians. It was used as a treatment for rheumatism and was found very effective in curing a disease which would probably now be recognized as osteomalacia. By 1824 there were reports of the use of cod liver oil as a cure for rickets.

Recently the great therapeutic action of cod liver oil has been attributed to its high potency in vitamins A and D (34). Vitamin D has a beneficial effect on calcium and phosphorus metabolism and is used in preventing rickets. The vitamin A content makes it of value in preventing and

curing diseases of the respiratory tract and it is widely used in the treatment of tuberculosis. Koessler, Maurer and Loughlin (35) reported that blood regeneration could not take place without vitamin A and that a definite relationship existed between a state of chronic vitamin deficiency and certain anemias.

Other uses of cod liver oil are being investigated. Ibsen (36) found less mortality from pneumonia and a general improvement in vitality among guinea pigs fed cod liver oil. It also acted as a cure and preventative of snuffles in rabbits. According to Schlingman (37) cod liver oil was found effective in treating dogs convalescing from distemper. In experimental jaundice it aided in the coagulation of blood (38).

In the anemia resulting from gastrectomy of dogs Farrell (20) found that cod liver oil and iron together induced recovery. On cod liver oil alone body weight was maintained although anemia developed, and on iron alone no improvement whatever resulted. Grayzell and Miller (32) demonstrated that dogs on a rickets-producing diet developed a lowered gastro-intestinal acidity which was changed back to its normal range by the daily addition of 10 cc. of cod liver oil to the diet. Colburn's work (1) indicated that cod liver oil was effective in increasing the gastric

acidity of anemic college girls.

A study of the literature indicates a definite relationship between a lowered gastric acidity and anemia, and from what is known of cod liver oil and its action it seems possible that it may in some way stimulate the flow of a more highly acid gastric juice.

EXPERIMENTAL PROCEDURE

Three adult female fox-terrier dogs were used for this study. They were kept in separate metabolism cages in a well-heated and ventilated room.

The standard diet used throughout the experiment was that worked out by Cowgill and his associates (39) and is given in Table I. Table II gives Karr's salt mixture which was recommended by Cowgill (40) and was used in this experiment.

TABLE I

Cowgill's Synthetic Diet For Dogs

Food	Amount	
	gm.	cal.
Casein	6.3	20.6
Sucrose	4.5	18.0
Lard	2.8	25.2
Butter fat	1.1	9.0
Agar-agar	0.4	0.0
Salt mixture	0.2	0.0
Total	15.3	72.8

TABLE II

Karr's Salt Mixture

Salts	Amount
	gm.
Sodium chloride	10
Calcium lactate	4
Magnesium citrate	4
Ferric citrate	1
Lugol's solution	few drops

Vitamin D was supplied by irradiating the casein. It was spread about one-fourth inch thick on paper and exposed to the rays of a Cooper-Hewitt Mercury Vapor Quartz Arc lamp at a distance of 12 inches for five minutes, then stirred and exposed another five minutes.

To stimulate the dogs' appetites vitamin B was provided in the form of Powdered Yeast Foam Tablets prepared by the Northwestern Yeast Company of Chicago. Cowgill recommends 60 mg. of standardized Harris Yeast Vitamine Powder per kilo of body weight per day. Since the dogs weighed approximately seven kilos they were at first given 0.5 gm. yeast. As their appetites lagged this was increased to first 1.0 gm. and then to 1.5 gm. per dog per day. This amount proved sufficient for maintaining the appetite and was given daily throughout the entire experiment.

The amount of food given in Table I furnishes 72.8 calories and is called by Cowgill a kilo unit or the amount

each dog needs per kilo per day to maintain body weight. Calculations of food requirements were also made according to body length (39) and the results of these two methods of determining food needs are given in Table III.

TABLE III

Food Requirement of Dogs

Dog	Weight		Length	Amount of food predicted by		Amount of food given	
	Sept.	May		Weight	Length		
	kg.	kg.	cm.	cal.	cal.	cal.	gm.
A	7.3	6.4	66	531	520	475	100
B	5.7	5.9	64	415	484	475	100
C	7.0	6.8	71	510	627	570	120

Dog A was of short heavy build and a quiet temperament, Dog B was small, thin and very restless and dog C was long and active. During the first days of the preliminary period each dog was fed a quantity of food equivalent to the largest amount predicted by Cowgill's methods of determining food needs and observations were made of food actually eaten. The appetites of the dogs were considered an indication of their food needs and the grams of food fed were soon adjusted to the level fed throughout the experiment (Table III). A quantitative record of food intake was kept during the study.

Enough food was prepared for a week at a time and kept in a cool place. Each morning the dogs were fed and cared

for. The powdered yeast, mixed with a small amount of distilled water was given first; then a weighed portion of food sufficient for the day placed in the cage. Distilled water was furnished ad libitum. During a preparatory period of about two months the dogs became accustomed to this diet and methods of technique were developed.

The experimental work was divided into four periods. The first of these lasted five weeks and during this time the normal gastric acidity was determined. On January 16, 17 and 19 the dogs were bled to approximately one-half the total blood volume. This began the second period and for the following five weeks, while the standard diet was being fed, the effect of this type of anemia on the gastric acidity was studied. Since at the end of this time the blood regeneration was definitely retarded the third period was begun.

The dogs were again bled to approximately the same anemic condition as before and cod liver oil was added to the diet in place of butter fat. The amount of food was lessened by 8 gm. and each dog received 8 cc. of Squibb's cod liver oil* daily. It was measured with a pipette and added to a small amount of food which the dogs

*The cod liver oil used in this experiment was furnished gratis by the E. R. Squibb & Sons Company, New York

ate before the remainder of the day's portion was placed in the cage. When it was discovered that the dogs liked the oil it was poured in a depression made in the center of the food and they ate that which was moistened with cod liver oil first. This period lasted from February 28 to April 8 and when compared with the second gave the effect of cod liver oil on the gastric acidity of the anemic dog.

During the fourth period consisting of another five weeks, each dog received 30 cc. of orange juice daily in addition to the standard diet and cod liver oil. Since the orange juice seemed slightly irritating it was mixed with one tablespoonful of sugar and then, as the dog's head was tipped back, poured down its throat, a little at a time.

Tests were made at weekly intervals by means of the fractional method of gastric analysis. Both the free and total acid determinations were made. Lyon's modification of the Rehfuss stomach tube was used for obtaining samples and each dog was provided with a separate tube which was marked to indicate the distance from the stomach to the mouth. In this way samples were always obtained from the same part of the stomach. To facilitate guiding the tube downward a copper wire was inserted and as the end of the tube neared the stomach the wire was removed an inch or two at a time. After the tube was properly located the

remainder of the wire could easily be withdrawn. The gastric juice, which was then obtained by means of suction, was removed only in amounts necessary for titrations.

The evening before tests were to be made, all food was removed from the cages so that on the following morning the fasting juice could be obtained. In order to introduce the tube into its stomach the dog was placed in a hammock which was made of a rectangular piece of material and was provided with four holes for the legs. By means of ropes this was suspended at a convenient working height. In this position the dog was comfortable and offered no resistance. A wooden block was then put between the teeth and tied in place at the back of the head. This block measured one by three by one and one-eighth inches with a three-fourths inch hole bored in the center for the passage of the tube. After the fasting juice had been aspirated a test meal consisting of 10 gm. of standard food and 50 cc. of distilled water was fed. The time was noted when the dog finished eating and a sample was taken at the end of 15 minutes and from then on at 30 minute intervals. During each interval the dog was removed from the hammock and allowed to rest.

As each sample of gastric juice was obtained it was filtered through a double thickness of cheese cloth into a test tube which was labeled and stoppered until titrations

could be made. For this, one cc. of the juice was diluted with 25 cc. of distilled water and titrated with 0.01 N NaOH. Phenolphthalein was used as the indicator for total acid and Topfer's reagent for free acid. A bureau of Standards calibrated pipette and burette were used in making all measurements. In most cases duplicate titrations were made with each indicator and the average of these results used. When the amount obtained was small, both the free and total determinations were made on the same sample. The NaOH was standardized with 0.1 N HCl each day that tests were made. The results of the gastric titrations were recorded in cc. of NaOH, corrected for normality and then converted into clinical units. A clinical unit is defined as the number of cc. of 0.1 N NaOH required to neutralize 100 cc. of gastric juice.

DISCUSSION OF RESULTS

The following tables show the results of the experiment. Tables IV, VI and VIII give the total acid determinations made on the gastric juice of Dogs A, B and C respectively and Tables V, VII and IX give the free acid determinations of the same dogs. In each case Period 1 deals with the normal condition. Period 2 when compared with this gives the effect of hemorrhagic anemia and

TABLE IV

Total Acid Determinations Made on the Gastric Juice of Dog A

Experimental conditions	Date	Time of obtaining sample: Fasting	15 min.	45 min.	1 hr. 15 min.	1 hr. 45 min.	2 hr. 15 min.
		units	units	units	units	units	units
Period 1							
Normal dog.	Dec.12	*	17.9	95.1	*	*	31.1
Fed the standard diet.	Dec.17	62.9	27.8	71.6	89.4	*	*
	Jan. 9	*	13.6	37.9	101.0	*	*
	Jan.11	60.1	26.2	*	94.0	81.3	*
	average	61.5	21.4	68.2	94.8	81.3	31.1
	range	60.1-62.9	13.6-27.8	37.9-95.1	89.4-101.0		
Period 2							
Dog made anemic by	Jan.21	27.2	23.4	32.5	99.0	81.5	*
bleeding to approxi-	Jan.26	50.0	19.8	36.1	65.8	55.5	*
mately one-half hemo-	Feb. 2	7.6	17.5	31.8	108.1	35.5"	*
globin level.	Feb. 9	35.9	27.0	35.0	121.5	61.6	*
Bled on Jan.16,17,19.	Feb.16	59.8	25.5	43.1	104.3	60.4	*
Fed the standard diet.	Feb.22	57.0	31.2	56.5	121.2	38.4	54.1
	range	7.6-59.8	17.5-31.2	31.8-56.5	65.8-121.2	35.5-81.5	
Period 3							
Dog made anemic by	Mar. 2	42.9	37.3	68.7	68.2	52.2	70.3
bleeding to approxi-	Mar. 9	40.9	26.7	66.4	56.3"	70.6	*
mately same hemoglob-	Mar.23	48.5	22.6	60.6	99.9	57.7	*
in level as before.	Mar.29	30.8	20.2	72.7	94.2	56.9	*
Bled on Feb.26,28.	Apr. 6	45.1	16.6	25.8	84.6	46.3	*
Fed cod liver oil	range	30.8-48.5	16.6-37.3	25.8-72.7	56.3-99.9	46.3-70.6	
diet.							
Period 4							
Orange juice added							
to cod liver oil							
diet.	Apr.13	36.6	27.6	77.0	54.9	*	*
	Apr.20	*	20.2	57.7	80.0	53.0	38.6
	Apr.27	30.9	17.8	34.9	93.4	*	*
	May 4	29.4	15.4	38.2	69.4	*	*
	May 11	46.0	24.5	44.0	67.9	94.4	*
	range	29.4-46.0	15.4-27.6	34.9-77.0	54.9-93.4	53.0-94.4	

* No sample obtained
" Bile present

TABLE V

Free Acid Determinations Made on the Gastric Juice of Dog A

Experimental conditions	Date	Time of obtaining sample					
		Fasting	15 min.	45 min.	1 hr. 15 min.	1 hr. 45 min.	2 hr. 15 min.
		units	units	units	units	units	units
Period 1							
Normal dog.	Dec.12	*	3.1	71.5	*	*	12.4
Fed the standard diet.	Dec.17	39.9	12.7	49.5	70.1	*	*
	Jan. 9	*	1.9	20.3	74.7	*	*
	Jan.11	41.7	12.2	*	65.3	62.8	*
	average	40.8	7.5	47.1	70.0	62.8	12.4
	range	39.9-41.7	1.9-12.7	20.3-71.5	65.3-74.7		
Period 2							
Dog made anemic by	Jan,21	15.3	4.6	14.6	70.4	68.3	*
bleeding to approxi-	Jan.26	39.5	5.8	18.2	47.0	43.2"	*
mately one-half hemo-	Feb. 2	1.2	0.0	13.5	79.4	25.8"	*
globin level.	Feb. 9	25.4	6.0	14.2	88.8	46.5	*
Bled on Jan. 16,17,19.	Feb.16	43.0	5.5	19.5	78.9	44.0	*
Fed the standard diet.	Feb.22	46.3	19.8	33.0	98.5	24.8	43.0
	range	1.2-46.3	0.0-19.8	13.5-33.0	47.0-98.5	24.8-68.3	
Period 3							
Dog made anemic by	Mar. 2	13.6	6.8	33.4	29.5	16.7	45.8
bleeding to approxima-	Mar. 9	25.1	7.8	38.4	37.9"	54.7	*
tely same hemoglobin	Mar.23	35.9	10.8	42.6	79.0	43.1	*
level as before.	Mar.29	19.3	0.0	48.4	70.8	37.8	*
Bled on Feb. 26, 28.	Apr. 6	33.0	4.9	9.5	55.2	32.3	*
Fed cod liver oil diet.	range	13.6-35.9	0.0-10.8	9.5-48.4	29.5-79.0	16.7-54.7	
Period 4							
Orange juice added	Apr.13	22.6	12.1	48.4	40.5	*	*
to cod liver oil	Apr.20	*	8.6	41.8	54.7	35.7	28.4
diet.	Apr.27	17.1	7.3	12.5	71.8	*	*
	May 4	16.5	3.3	21.9	47.3	*	*
	May 11	32.3	12.9	23.5	44.1	69.2	*
	range	16.5-32.3	3.3-12.9	12.5-48.4	40.5-71.8	35.7-69.2	

* No sample obtained
" Bile present

TABLE VI

Total Acid Determinations Made on the Gastric Juice of Dog B

Experimental conditions	Date	Time of obtaining sample					
		Fasting	15 min.	45 min.	1 hr. 15 min.	1 hr. 45 min.	2 hr. 15 min.
		units	units	units	units	units	units
Period 1							
Normal dog.	Dec.14	*	26.3	41.9	55.2	81.2	46.5
Fed the standard diet.	Dec.17	46.0	15.1	31.6	71.4	51.8"	*
	Dec.19	*	27.6	49.7	63.5	89.4	*
	Jan. 9	65.5	18.3	*	104.6	*	*
	average	55.7	21.8	41.1	73.7	74.1	46.5
	range	46.0-65.5	15.1-27.6	31.6-49.7	55.2-104.6	51.8-89.4	
Period 2							
Dog made anemic by	Jan.21	15.5	24.3	31.8	69.9	50.1"	36.9
bleeding to approxima-	Jan.26	*	22.1	26.5	85.3	39.6"	*
tely one-half hemo-	Feb. 2	*	22.9	56.4	*	83.1	69.2
globin level.	Feb. 9	*	32.6	37.8	88.0	60.1	45.7
Bled on Jan. 16,17,19.	Feb.16	*	31.9	26.0	67.9	57.4	39.3
Fed the standard diet.	Feb.22	*	19.8	48.3	82.8	59.9	28.0"
	range		19.8-32.6	26.0-56.4	67.9-88.0	39.6-60.1	28.0-69.2
Period 3							
Dog made anemic by	Mar. 2	*	39.2	47.9	64.8	87.0	21.4
bleeding to approxima-	Mar. 9	18.9"	23.8	77.9	75.4	44.6"	*
tely same hemoglobin	Mar.23	81.0	28.8	45.0	71.8	61.1	26.4
level as before.	Mar.29	41.8	26.0	45.4	64.1	43.0	*
Bled on Feb. 26, 28.	range	18.9-81.0	23.8-39.2	45.0-77.9	64.1-75.4	43.0-87.0	
Fed cod liver oil diet.							
Period 4							
Orange juice added	Apr.13	*	12.9	36.3	87.2	34.8	*
to cod liver oil	Apr.20	*	18.3	21.4	58.0	71.0	50.2
diet.	Apr.27	19.3"	24.2	57.5	82.4	57.0	"
	May 4	42.6	21.6	38.4	77.9	51.0	35.8
	May 11	*	27.7	32.4	56.0	79.4	*
	range	19.3-42.6	12.9-27.7	21.4-57.5	56.0-87.2	34.8-79.4	358-50.2

* No sample obtained
" Bile present

TABLE VII

Free Acid Determinations Made on the Gastric Juice of Dog B

Experimental conditions	Date	Time of obtaining sample: Fasting	15 min.	45 min.	1 hr. 15 min.	1 hr. 45 min.	2 hr. 15 min.
		units	units	units	units	units	units
Period 1							
Normal dog.	Dec.14	*	13.0	27.4	28.3	53.2	30.8
Fed the standard diet.	Dec.17	33.8	5.1	14.5	47.7	40.0"	*
	Dec.19	*	11.4	15.7	37.6	57.1	*
	Jan. 9	41.8	3.1	*	77.2	*	*
	average	37.8	8.1	19.2	47.7	50.1	30.8
	range	33.8-41.8	3.1-13.0	14.5-27.4	28.3-77.2	40.0-53.2	
Period 2							
Dog made anemic by	Jan.21	7.6	4.6	13.4	47.6	31.6"	18.8"
bleeding to approxima-	Jan.26	*	11.2	9.9	58.3	23.5"	*
tely one-half hemo-	Feb. 2	*	9.7	3.3	*	50.8	50.6
globin level.	Feb. 9	*	13.7	16.7	58.6	42.0	30.0
Bled on Jan. 16,17,19.	Feb.16	*	13.0	9.5	42.9	41.5	21.5
Fed the standard diet.	Feb.22	*	3.2	50.0	61.2	46.0	8.0
	range	*	3.2-13.7	9.5-50.0	42.9-61.2	23.5-50.8	8.0-50.6
Period 3							
Dog made anemic by	Mar. 2	*	9.1	26.1	46.7	74.7	21.9
bleeding to approxima-	Mar. 9	21.2"	2.5	54.5	57.8	27.5"	*
tely same hemoglobin	Mar.23	74.6	10.3	22.5	46.9	41.2	12.5
level as before.	Mar.29	31.9	4.6	17.5	38.5	20.3	*
Bled on Feb. 26, 28.	range	21.2-74.6	2.5-10.3	17.5-54.5	38.5-57.8	20.3-74.7	12.5-21.9
Fed cod liver oil diet.							
Period 4							
Orange juice added	Apr. 13	*	1.0	16.9	35.0	18.5	*
to cod liver oil	Apr. 20	*	7.8	8.4	36.9	47.9	34.8"
diet.	Apr. 27	12.7"	9.6	37.9	55.1	34.5	*
	May 4	32.9	9.2	18.7	51.1	33.0	23.6
	May 11	*	14.3	17.2	31.9	50.5	*
	range	12.7-32.9	1.0-14.3	8.4-37.9	35.0-55.1	18.5-50.5	23.6-34.8

* No sample obtained
" Bile present

TABLE VIII

Total Acid Determinations Made on the Gastric Juice of Dog C

Experimental conditions	Date	Time of obtaining sample					
		Fasting	15 min.	45 min.	1 hr. 15 min.	1 hr. 45 min.	2 hr. 15 min.
		units	units	units	units	units	units
Period 1							
Normal dog.	Dec.12	17.1	*	52.0	55.0	56.3	*
Fed the standard diet.	Dec.14	25.2	*	38.9	72.3"	30.0"	*
	Jan. 9	49.0	17.3	58.4	107.6	51.0	30.0"
	Jan.11	43.1	44.2	62.4	86.0	70.6	59.9
	Jan.19	*	18.9	47.7	79.6	80.8	*
	average	33.6	25.1	51.9	80.1	57.7	44.9
	range	17.1-49.0	17.3-44.2	38.9-62.4	55.0-107.6	30.0-80.8	30.0-59.9
Period 2							
Dog made anemic by	Jan.21	21.8	14.7	50.9	89.3	105.05	102.1
bleeding to approxi-	Jan.26	16.6"	29.0	79.2	80.3	92.8	64.2"
mately one-half hemo-	Feb. 2	*	25.5	41.0	84.6	66.3	*
globin level.	Feb. 9	26.5	46.4	40.4	108.1	78.2	*
Bled on Jan. 16,17,19.	Feb.16	89.7	40.6	74.7	109.5	122.3	122.0
Fed the standard diet.	Feb.26	48.8	22.8	74.3	91.8	55.6	59.9
	range	16.6-89.7	14.7-46.4	40.4-79.2	80.3-109.5	55.6-122.35	59.9-122.0
Period 3							
Dog made anemic by	Mar. 2	*	74.6	55.6	86.1	98.5	79.1
bleeding to approxi-	Mar. 9	21.1	22.8	83.1	87.8	67.9	54.8
mately same hemo-	Mar.23	25.9	24.0	62.7	64.1	84.8	*
globin level as be-	Mar.29	20.4	23.1	64.9	60.3	56.4	*
fore.	Apr. 6	13.6	16.2	25.0	38.5	31.7	*
Bled on Feb. 26,28.	range	13.6-25.9	16.2-74.6	25.0-83.1	38.5-87.8	31.7-98.5	*
Fed cod liver oil diet.							
Period 4							
Orange juice added	Apr.13	27.7	27.5	58.6	60.6	18.8	23.3
to cod liver oil	Apr.20	11.1"	10.3	56.9	*	*	*
diet.	Apr.27	6.8	32.8	90.8	99.5	62.3	*
	May 4	27.4"	20.3	33.4	54.9	65.4	*
	May 11	46.6	14.0	46.0	81.5	*	*
	range	6.8-46.6	10.3-32.8	33.4-90.8	54.9-99.5	18.8-65.4	

* No sample obtained
" Bile present

TABLE IX

Free Acid Determinations Made on the Gastric Juice of Dog C

Experimental conditions	Date	Time of obtaining sample: Fasting	15 min.	45 min.	1 hr. 15 min.	1 hr. 45 min.	2 hr. 15 min.
		units	units	units	units	units	units
Period 1							
Normal dog.	Dec.12	0.9	*	30.7	36.8	45.3	*
Fed the standard diet.	Dec.14	14.2	*	22.8	56.2"	17.7"	*
	Jan. 9	45.7	75.9	40.1	88.5	38.2	16.7
	Jan.11	18.0	24.4	36.5	56.0	48.0	48.2
	Jan.19	*	3.4	24.6	50.7	50.2	*
	average	19.7	27.3	30.9	57.6	39.9	32.4
	range	0.9-45.7	3.4-75.9	22.8-40.1	36.8-88.5	17.7-50.2	16.7-48.2
Period 2							
Dog made anemic by	Jan.21	3.8	0.0	19.7	44.6	71.0	66.0
bleeding to approxi-	Jan.26	0.0"	14.7	50.6	59.4	77.2	51.0"
mately one-half hemo-	Feb. 2	*	9.8	26.9	61.2	48.8	*
globin level.	Feb. 9	18.3	27.9	20.3	84.5	65.7	*
Bled on Jan. 16,17,19.	Feb.16	82.8	22.0	48.7	82.2	97.7	100.2
Fed the standard diet.	Feb.26	39.2	10.9	52.5	79.6	46.1	50.0
	range	0.0-82.8	0.0-27.9	19.7-52.5	44.6-84.5	46.1-84.5	50.0-100.2
Period 3							
Dog made anemic by	Mar. 2	*	36.1	20.8	52.2	67.1	60.6
bleeding to approxima-	Mar. 9	13.6	2.2	57.2	66.8	54.9	42.2
tely same hemoglobin	Mar.23	16.6	6.7	43.8	47.7	75.3	*
level as before.	Mar.29	10.2	2.5	35.5	36.1	35.6	*
Bled on Feb. 26, 28.	Apr. 6	6.0	3.6	7.5	31.2	23.2	*
Fed cod liver oil diet.	range	60- 16.6	2.2-36.1	7.5-57.2	31.2-66.8	23.2-75.3	
Period 4							
Orange juice added	Apr.13	14.4	10.8	40.7	46.2	1.0	8.2
to cod liver oil	Apr.20	0.0"	11.2	46.6	*	*	*
diet.	Apr.27	5.7	15.2	68.9	78.6	47.4	*
	May 4	13.7"	8.6	19.2	38.7	49.0	*
	May 11	32.5	1.0	25.5	54.1	*	*
	range	0.0-32.5	1.0-15.2	19.2-68.9	38.7-78.6	1.0-49.0	

* No sample obtained
" Bile present

regeneration of blood on gastric acidity; the diet was the same as that of the first period. During Period 3 conditions were the same except that cod liver oil was substituted for butter fat and the effect of this change is determined by comparing Periods 3 and 2. Period 4 considered in relation to the second and third gives the additional effect of orange juice.

The determinations for total and free acid were studied separately throughout the experiment. For the normal period the figures were averaged, but for Periods 2, 3 and 4 it seemed more desirable to study each test in relation to those preceding or following it. The peak of each curve was determined and used in making comparisons. The range of the acidity of the fasting juice was considered significant.

A comparison of the figures in any one period shows a wide variation in acidity which may be considered normal for a dog.

Dog A. The average of four gastric analyses may be considered the normal figure for this dog. The peak of acidity which was reached about one hour and 15 minutes after the test meal was fed had an average total and free acidity of 101.0 and 74.7 units respectively. The average fasting juice had a total acidity of 61.5 units and a free acidity of 40.8 units.

Hemorrhage did not have an immediate lowering effect

upon the peak; the figures were 99.0 units for total and 70.4 for free acid. One week after bleeding the curves were at their lowest, with total and free peaks of 65.8 and 47.0 units. Then as blood was regenerated from 8.00 to 12.42 gm. hemoglobin the acidity gradually increased. At the end of this period the peaks were higher than any of Period 1, being 121.0 units for total and 98.5 for free.

The ascent of the curves was more gradual than normal indicating a hyposecretion. The fasting juice had a lowered acidity throughout much of this period, at one time being as low as 7.6 for total and 1.2 for free. Most of the samples taken 15 minutes after and all those taken 45 minutes after the test meal were lower in free acid than the normal figure at that point in the curve.

After the second hemorrhage, when cod liver oil was added to the diet, the peaks of the total and free acid were 68.7 and 33.4 units. This was definitely lower than those in Periods 2 and 1. The acidity then increased for two weeks but in no case reached the high point of the previous period. By the last of this period the acidity had dropped to 84.6 and 55.2 units which was definitely lower than all but one in the period before. The fasting juice was also below the normal range. This retarded condition of gastric acidity was accompanied by a much slower

hemoglobin regeneration of only 1.79 gm. during the five weeks.

The first test after orange juice had been added was lower than the preceding one but the acidity gradually increased, as did the hemoglobin regeneration. The highest acidity came in the last week of the period and was 94.4 units for total and 69.2 for free. This was within the normal range. The fasting juice was again lower than normal.

In many cases the differences between periods were more evident in the free acid than in the total.

Dog B. The normal gastric acidity for this dog was determined from the results of four analyses. The peak was reached from one hour and 15 minutes to one hour and 45 minutes after the test meal was fed and had an average total acidity of 86.6 units and free acidity of 58.8 units. The fasting juice averaged 55.7 units for total and 37.8 for free acid.

Immediately after hemorrhage the peak of the total acidity was 69.9 units and the free, 47.6. This was slightly lower than the normal average but not below the normal range. During this period the acidity of the peaks was variable. The last one was 61.2 units for free which was the highest of the period and for total it was 82.8 units. This was definitely higher than the normal average.

After the second hemorrhage the peaks were higher than normal but the addition of cod liver oil to the diet had no beneficial effect and they steadily decreased each week. The last test showed a total acidity of 64.1 and a free acidity of 38.5 units which was lower than any in the preceding periods. There was no hyposecretion but the fasting juice was lower than normal. This lowered gastric acidity was accompanied by a blood regeneration of 2.62 gm. hemoglobin as compared to the period before when regeneration was 2.76 gm.

Upon the addition of orange juice the downward trend in acidity was checked. The peak for total acid was 87.2 and for free 53.0 units. The following tests did not vary greatly. All peaks were within the normal range and in no case reached the lowest of the preceding period. Again the acidity of the fasting juice was below normal.

Dog C. The normal gastric acidity for this dog was determined from the results of five analyses. The peak which was reached from one hour and 15 minutes to one hour and 45 minutes after the test meal had been fed had an average total and free acidity of 80.6 and 59.3 units respectively. The fasting juice averaged 33.6 units for total and 19.7 for free acid.

Immediately after hemorrhage the peaks were 105.0 and 71.0 units but by the second week had dropped to 84.6 and

61.2 units for total and free acid. The highest point came toward the end of the period and was 122.3 units for total and 100.2 for free. Both were definitely higher than the normal range. During this period the blood regenerated 3.03 gm. hemoglobin.

There was a tendency toward hyposecretion. The fasting juice was lowered after hemorrhage and the sample obtained in the second week contained no hydrochloric acid. Most of the 15 minute samples were of lower acidity than normal, there being no free acid at that point in the curve the first week.

After the second hemorrhage the peaks were lower than after the first - 98.5 units for total and 67.1 for free. During this period cod liver oil was fed and blood regeneration was 2.89 gm. hemoglobin. The range of the fasting juice was below normal and no acidity peaks reached the highest of the periods before. The last week gave the lowest figures - a total and free acidity of 38.5 and 31.2 units respectively. There was no tendency toward hyposecretion. Many of the samples taken 45 minutes after the test meal were of a higher acidity than the normal for that time.

Upon the addition of orange juice the downward trend was immediately checked. The highest peak for the period was 99.5 and 78.6 units for total and free. Each peak of this period was within the normal range. At the beginning

of this period the acidity of the fasting juice was lower than normal but the last test showed a figure higher than the average for the normal dog.

SUMMARY

In the total and free acid determinations made on the gastric juice of three dogs it was found that:

1. The normal gastric juice was quite variable from day to day although the general curve was the same. The peak of acidity was reached from one hour and 15 minutes to one hour and 45 minutes after the test meal was given.

2. In five out of six cases the peak of the acidity curve was not lowered as an immediate result of hemorrhage.

3. However during the first week or two of anemia a definite drop in the peaks of the curves occurred.

4. Throughout the entire period of anemia the fasting juice was lower than normal.

5. During the first anemic period there was a tendency toward hyposecretion.

6. As blood regeneration progressed the gastric acidity increased and at the end of this period both the total and free acid were higher than in the normal dog.

7. Throughout the period during which cod liver oil was fed the acidity was lower than normal. The peaks

dropped from week to week in two dogs and in the third, although the acid was variable in amount, the last peak was definitely lowered.

8. No hyposecretion was observed when cod liver oil was included in the diet.

9. The addition of orange juice checked the downward trend in acidity noticed during the third period.

10. A more rapid blood regeneration was accompanied by a higher acidity and a slower regeneration by a lower acidity. This was shown in each of the anemic periods.

11. These results were more evident in the free acid than in the total.

ACKNOWLEDGMENTS

Grateful acknowledgment is given to Dr. Margaret Chaney, Professor in Food Economics and Nutrition, under whose guidance this study was planned and who so willingly gave her advice and assistance in all matters pertaining to it. Gratitude is also due Dr. E. R. Frank, Assistant Professor in Surgery and Medicine for his assistance in bleeding the dogs.

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