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School of Biological Sciences

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To the Committee on Graduate Study:

I submit herewith a thesis by Mr. C. F. Hoffman, Jr.,
"The Substance in Yeast Which Stimulates Gastric Secretion," and
recommend that it be accepted for eighteen quarter hours credit in
fulfillment of the requirements for the degree of Master of Science,
with a major in Chemistry.

E. F. Williams

Major Professor

At the request of the
Committee on Graduate
Study, I have read this
thesis and recommend its
acceptance.

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February, 1942
Memphis, Tennessee
Accepted by the Committee

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UNIVERSITY OF TENNESSEE

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INTRODUCTION

A number of substances are known which stimulate gastric secretion either when introduced into the lumen of the stomach or intestine, or when injected intravenously or intramuscularly. Among substances that have been tested experimentally by direct introduction into the stomach and have been found active as stimulating agents are beta-alanine, histamine, adrenaline, and peptone (1).

Substances which affect gastric secretion following their intramuscular or intravenous injection act through a humoral mechanism. Among compounds which have been tested for such effect are:

Histidine. This has no effect on gastric secretion (2).

Choline. Intravenously it weakly stimulates volume and acid (3).

Lecithin. Its action is similar to that of choline (3).

Acetyl-beta-methyl-choline. This was found to be inactive (4).

Bromcholine. Following subcutaneous injection gastric stimulation occurred, but the flow of juice was slower and contained less acid than following a comparable injection of histamine (5).

Cystamine. It produced an acid gastric secretion when injected intramuscularly (6).

Pilocarpine. Intravenous injection of the substance stimulated gastric secretion, and its action was inhibited by atropine (7).

"Acaprine." Subcutaneously injected it caused an increase in the flow of gastric juice (8).

Histamine. When injected intramuscularly this is a very effective gastric secretagogue (9).

"Gastrin." Intramuscular injection of an aqueous acid extract of

gastric mucosa causes the secretion of a greater volume of gastric juice with a higher concentration of acid than before injection (10).

Extracts of three natural vegetable materials have been shown to have a stimulating effect on gastric secretion. Using spinach, Bickel (11) prepared an active extract which was stable to dry heat up to about 140° C. and also to boiling with concentrated hydrochloric acid. Gleichmann (12) found filtered tomato juice to be active when injected intramuscularly. Williams, Cox, and Nash (9) prepared a yeast extract which stimulated the previously resting stomachs of dogs.

Since the last mentioned report showed that the active substance could be concentrated from a hot acid-aqueous extract of yeast, it was the purpose of the studies reported in this thesis to purify the extract further and to isolate and identify the active principle if possible.

The primary approach to the problem was to devise a more efficient and facile method of extraction and this was found in the acid-alcohol extraction of dry yeast. Since this gave a reliably active extract, the procedure of differential solubilities in the major organic solvents was followed as a possible means of purification and identification.

A still further approach was made by testing various organic compounds for their activity on gastric secretion, the hope being that the active substance in yeast might be identical with one of them. The compounds were selected on the basis of their known presence in yeast or their suspected presence as indicated by the results of extraction and separation procedure.

METHODS

Chapter 1.

Preparation of Extracts from Yeast

Extracts of yeast were first made using commercial foil-wrapped cake yeast exactly after the method of Keeton and Koch (10) for the extraction of "gastrin" from animal tissues. This method soon proved to be much too long and tedious. It had the further disadvantage that the extract was not sufficiently concentrated. This made necessary the injection of large amounts of the extract to elicit a response.

Acid alcohol was then used since it evaporated easily and reduced the bulk of the extracted material. This method did not decrease the potency of the extract but still had one disadvantage. The difficulty was removing the hydrochloric acid from the concentrate. This was accomplished only after repeated additions and subsequent evaporations of absolute ethyl alcohol.

The use of sulphuric acid in conjunction with alcohol as an extracting agent was undertaken since this acid might be removed by precipitation, and the insoluble sulphate filtered off. The procedure was not successful partly due to the difficulty of removing all of the sulphate. There was also a marked decrease in the activity of the extract.

In order to prove that the active material was preformed in the yeast and not produced by the action of heat and strong acid, several procedures were carried out. Acid alcohol was used as an extracting agent in the absence of heat. The yeast was treated with ethyl acetate, to lyse the cell membrane, with subsequent acid alcohol extraction. A portion of the acid alcohol extract was neutralized with sodium hydroxide to eliminate the action of the concentrated acid as the volume of the extract became more concentrated.

Formic acid, a weak acid, was used in place of the strong hydrochloric acid in acid-alcohol mixture. For the results of these experiments consult pages 19-20, 22-23, 25.

A purer form of wet yeast was extracted with the acid alcohol mixture in the hope that extraneous material would be still further reduced. The results are shown on page 27.

AQUEOUS HYDROCHLORIC ACID EXTRACTION OF COMPRESSED YEAST

Fresh yeast was mixed with 5 times its weight of 0.4 per cent hydrochloric acid. The thoroughly mixed material was then heated to 90° C. in the hot water bath and set aside to cool at room temperature overnight. On the following day it was again heated to 90° and after cooling and again standing overnight was filtered.

Filtration was slow, and the filtrate was cloudy when made through paper alone. In order to avoid these difficulties a filter of diatomaceous earth on paper in a Buchner funnel was used with suction. The clear filtrate was of a light brown color with slight bluish fluorescence. The volume of the filtrate, which contained some of the water content of the yeast in addition to that of the acid solution, was reduced by concentration in vacuo at a temperature of 70° or less to 1/18 to 1/10 of the original volume. To this concentrated filtrate was added 6 volumes of 95 per cent alcohol. After standing 1 to 3 days this mixture was filtered to remove some gummy solid matter which had settled out. Under diminished pressure in a hot (but not over 70°) water bath the alcoholic filtrate was evaporated to dryness.

Much of the hydrochloric acid was removed at this point by repeated treatments with absolute alcohol followed each time by evaporation under reduced pressure.

When the now dry residue was treated with distilled water, a gummy suspension ensued. The brown gummy matter was flocculated upon adding sufficient distilled water, and the diluted mixture was centrifuged at high speed. Most of the insoluble material floated on the liquid which could be poured off easily, the remainder of the insoluble matter being removed by filtration, frequently through diatomaceous earth.

Finally the clear water solution was concentrated in vacuo as before to such a volume that 1 cc. would be equivalent to 30 gm. of original yeast, or even to a smaller volume if the solution remained suitable for intramuscular injection. It was found that even a water bath temperature of 70° led to some charring of the extract, presumably by the hydrochloric acid, and therefore in later preparations the water bath temperature was reduced to 60°. Even at this lower temperature the extract had a dark brown color, and the final concentrated product was very viscid and had an unpleasant odor resembling that of mixed organic acids.

ALCOHOLIC HYDROCHLORIC ACID EXTRACTION OF DRY YEAST

In order to avoid the long and tedious evaporations and filtrations of the foregoing procedure, search was made for a solvent to replace water. The active principle of yeast was found to be soluble in 95 per cent alcohol, and a new procedure was devised to make use of this solvent as the extracting agent. Also, dry yeast was used instead of compressed yeast since the water content of the latter would dilute the alcohol appreciably.

The dry brewer's yeast when suspended in water had a pH of about 5.5. We have found it necessary to reduce the pH considerably below this level for efficient extraction of the active principle. This acidification was brought about by the addition of 37 cc. of concentrated (12 N) hydrochloric

acid to each mixture of 450 gm. of yeast with 1500 cc. of 95 per cent alcohol. The calculated concentration of hydrochloric acid in the final mixture was about 0.3 N. While being continuously stirred the acid mixture was heated at 70° in the water bath for 2 hours.

After settling and cooling overnight, the clear supernatant fluid was decanted through a suction filter of the diatomaceous earth. When it had passed through, the remaining soft yeast mixture was poured on the same filter. The nearly dry cake was washed with a volume of hot 95 per cent alcohol, approximately a third that of the original alcohol used. After standing 5 minutes the washing alcohol was drawn through the cake. This washing treatment was repeated twice using fresh washing alcohol each time. The combined washings and filtrate were evaporated to a thick syrup under diminished pressure in the hot water bath at a temperature of 60° to 70°. A portion of the hydrochloric acid escaped with the distillate.

When the thick syrupy residue was treated with distilled water, a large amount of the material dissolved imparting to the solution a brown color. The insoluble portion was removed by centrifugation and filtration. Approximately two-fifths by volume of the original syrupy residue was removed by this procedure. The clear water solution was evaporated to dryness in vacuo at 60° to 70° on the water bath. Absolute ethyl alcohol was added and subsequently evaporated several times removing portions of the hydrochloric acid yet remaining. Finally an absolute ethyl alcohol solution was made of the residue, only a small portion being insoluble. This insoluble material was light, fluffy, and brown in color and was easily removed by simple filtration.

After the clear absolute alcohol solution was evaporated to dryness, the dry residue then dissolved in such a volume of water that 1 cc. of the

resulting solution would be equivalent to 30 to 80 gm. of dry yeast.

The above procedure has been standard for most of the extracts made during the past year.

ALCOHOLIC SULFURIC ACID EXTRACTION

To a mixture of 1500 cc. of 95 per cent alcohol and 50 cc. of concentrated sulfuric acid was added 450 gm. of dry yeast. This was heated on the water bath at 70° for 2 hours. The filtrate resulting from this mixture was treated in the cold with barium hydroxide or solid barium carbonate until the solution became only slightly acid. Centrifugation and filtration removed solid matter, and the filtrate was evaporated to dryness on the water bath at 60°. The water-insoluble fraction was then removed by centrifugation and filtration, and the remaining water-soluble fraction concentrated to dose level volume. Solid sodium bicarbonate was used to neutralize any small excess of sulfuric acid that remained before injection into the animal.

COLD ALCOHOLIC HYDROCHLORIC ACID EXTRACTION

An extract was made in the proportions of 450 gm. of dry yeast with 1500 cc. of 95 per cent ethyl alcohol and 30 cc. of 12 N hydrochloric acid at room temperature. The mixture was stirred with a motor stirrer for two hours without heating and then allowed to stand overnight. Subsequent processing of the preparation was guided by the standard alcoholic hydrochloric acid extraction procedure.

EXTRACTION AFTER TREATMENT WITH ETHYL ACETATE

Dry yeast was added to an equal weight of ethyl acetate. The mixture had the appearance of a suspension, but upon the addition of a weight of distilled water equal to the original weight of yeast the mixture assumed a doughy appearance with very little free liquid observable. Frequent stirring by hand to assure thorough mixing of the pasty mass was kept up

for 4 hours.

(a) To a portion of this mass was added with thorough stirring 1500 cc. of 95 per cent alcohol per pound of dry yeast, and the resulting mixture allowed to stand 2 hours before filtering through diatomaceous earth.

The clear light brown filtrate was termed "neutral alcohol extract."

(b) Another portion of the yeast-acetate-water mass was mixed with 1500 cc. of 95 per cent alcohol (plus 35 cc. of concentrated hydrochloric acid) per pound of dry yeast. This was allowed to stand for two hours and then filtered through diatomaceous earth. The brown filtrate was termed "acid-alcohol extract."

(c) A part of the "acid-alcohol extract" having been immediately neutralized with 4 normal sodium hydroxide to a pH of about 5, the remaining portion was allowed to stand in the strongly acid solution. At the higher pH a brown, fluffy material separated and was removed by centrifuging. This separated solid material was found to be soluble neither in 95 per cent alcohol for the most part nor in distilled water. The clear solution was called "neutralized acid-alcohol extract."

(d) Each of these three extracts (a,b,c) was then concentrated to dryness and taken up in approximately 150 cc. of absolute ethyl alcohol, the insoluble residue being centrifuged off. After the absolute alcohol had been evaporated away under reduced pressure at 60° and the dry residue taken up in water, the water-insoluble portion was removed by centrifugation. The now aqueous extracts were evaporated separately to dose level volume for injection.

ALCOHOLIC FORMIC ACID EXTRACTION

One pound of dry yeast was mixed with 1500 cc. of 95 per cent alcohol and 50 cc. of formic acid, heated at 60° for one hour, allowed to stand overnight, and then filtered. The filtrate was evaporated to a thick

syrup in vacuo at 60° and then taken up in about 60 cc. of 95 per cent alcohol. This was evaporated to dryness in vacuo and the residue treated with about 60 cc. of absolute alcohol which was evaporated off also. The absolute alcohol treatment was repeated twice more. Approximately 300 cc. of water were added to the dry residue and the resulting water solution filtered. The filtrate was evaporated to a thick syrup, to which water was added to make a concentration suitable for injection. Finally the solution was sealed in ampules and sterilized in boiling water.

ALCOHOLIC HYDROCHLORIC ACID EXTRACTION OF WET COMPRESSED STARCH-FREE YEAST

Two pounds of wet compressed yeast (specially prepared free of starch) were treated with 95 per cent alcohol and concentrated hydrochloric acid in the usual manner, except that the amount of acid was reduced to 30 cc. per pound of yeast. The only difference noted between this extraction and those made similarly from other types of yeast was that the color of the filtrate was somewhat lighter, and that upon a subsequent concentration on the water bath this paleness of color was maintained. When the final residue had been treated with distilled water and reconcentrated, it was taken up in distilled water to a volume suitable for injection.

Chapter 2

Treatment of Concentrated Extracts from Yeast

Having successfully prepared an extract that was dependable in its effect when injected, further purification procedures were employed. The concentrated extract was treated with phosphotungstic acid which almost universally precipitates organic bases. Decolorizing charcoal (Nuchar) was used on the extract which also removed in addition to the color a large amount of solid material.

Various organic solvents, such as absolute methyl or ethyl alcohols and acetone, were used to separate the active material from the bulk of the extract.

Finally the extract was treated with nitrous acid since it was known from experiments of others that this treatment destroyed the activity of primary amines such as histamine. By this means the active material was shown not to be histamine or a primary amine, thus deciding a controversial point.

PHOSPHOTUNGSTIC ACID PRECIPITATION

The precipitating agent was 20 per cent phosphotungstic acid (in 5 per cent sulfuric acid). Approximately 400 cc. of this reagent were found necessary for complete precipitation of the aqueous solution of the usual alcoholic hydrochloric acid extract, equivalent to 900 gm. of dry yeast. The heavy yellowish precipitate carried down with it most of the color of the solution. With a slight excess of phosphotungstic acid present, the mixture was covered and allowed to stand at room temperature for 24 hours. On the following day the supernatant was tested again with a few drops of the phosphotungstic acid reagent and if no further precipitation occurred, the heavy precipitate was removed by centrifuging. The precipitate was washed in the centrifuge cup several times with dilute sulfuric acid.

In order to release the organic bases from the precipitate, saturated baryta water was added with stirring until the mixture became and remained just slightly alkaline.

Residues of barium phosphotungstate and sulfate were removed by centrifuging and then washed with distilled water, the washings being added to the slightly alkaline supernatant. The removal of these barium precipitates was difficult and in some cases the mixture was allowed to stand overnight in order that the crystals might grow by accretion and

then was heated and filtered.

The slightly alkaline supernatant was treated with carbon dioxide under pressure until saturated and then was heated on the water bath at the boiling temperature for 15 to 30 minutes to convert soluble barium bicarbonate into insoluble normal carbonate, which was then filtered off. After the filtrate had been evaporated under reduced pressure at 60° to a very small volume, any residual barium was removed by adding dilute sulfuric acid. This solution was concentrated to dose level volume in the usual manner and adjusted to pH of about 5 before injection.

The filtrate from the original phosphotungstic acid precipitation, including the washings, was treated in a similar manner, the barium being completely removed before injection. This material was termed the "non-precipitated" fraction.

DECOLORIZATION BY CHARCOAL

A concentrated aqueous solution of an alcoholic hydrochloric acid extract was treated with decolorizing charcoal (Nuchar) at almost 100° for 3 to 5 minutes. When the charcoal had been filtered off, the filtrate was found to be clear and colorless in contrast with the original dark brown sticky solution. The charcoal was washed with warm water, the filtrate and washings then being concentrated to dryness in vacuo. Fine white needles crystallized out, but on injection were essentially inactive in stimulating gastric secretion. An attempt was made to elute active material from the charcoal by dilute sulfuric acid, but without success.

ABSOLUTE ETHYL ALCOHOL SOLUBLE FRACTION

Treatment of a concentrated active extract of dry yeast with a relatively large volume of absolute ethyl alcohol (as routinely done in the alcoholic hydrochloric acid method of extraction) caused the separation of a fluffy, flocculent precipitate which could be centrifuged off easily.

The absolute alcohol soluble fraction was then evaporated to dryness under diminished pressure at 60° . Distilled water was added to the solid residue until a volume suitable for injection was reached. Any excess hydrochloric acid still remaining was neutralized with solid sodium bicarbonate before injection.

ABSOLUTE METHYL ALCOHOL SOLUBLE FRACTION

When the usual alcoholic hydrochloric acid extract was evaporated almost to dryness, fine white crystals were seen to form. These crystals were found to be fairly insoluble ⁱⁿ methyl alcohol and were removed by the use of this solvent which apparently dissolved the remainder of the syrupy mass. On filtering, the colored material remained in solution while the white crystals collected on the filter paper. Upon reconcentration of the filtrate at 60° under diminished pressure, more crystals similar in appearance to the first crop appeared. These were removed in the same way, and then the alcohol was evaporated off completely from this second filtrate. The residue from this filtrate was dissolved in water to make a volume suitable for injection.

ACETONE EXTRACTION

A concentrated water solution of the usual alcoholic hydrochloric acid extract of a pound or two of yeast was stirred with successive 50 cc. portions of redistilled acetone. A bulky, light brown material was left behind after the acetone soluble fraction had been poured off. As the acetone treatments were repeated, this material became more and more viscid until on stirring it "balled" in the vessel. Further addition of acetone beyond this point was found to extract no more soluble matter. The combined acetone extracts were evaporated to dryness at 60° under diminished pressure. At this point the dry residue was found to contain a small amount of light

brown crystals. These crystals were readily water soluble imparting a light yellow or brownish color to the solution. Due to the slight acidity of the aqueous solution, it was neutralized with sodium bicarbonate before injection.

NITROUS ACID TREATMENT

To 2 cc. (equivalent to about 60 gm. dry yeast) of an aqueous solution (of pH between 2 and 3) of active alcoholic hydrochloric acid extract were added 50 mg. of sodium nitrite. The mixture was then heated on the water bath at 60° for 3 minutes.

Upon the addition of 25 mg. of urea, vigorous effervescence resulted indicating an excess of nitrite. The mixture was again heated on the water bath at 60 for 3 minutes. Finally the solution was neutralized with solid sodium bicarbonate to pH of about 6 just before injecting into the animals.

Chapter 3

Operative Technique for Gastro-Jejunal Fistula

The animals after starving 24 hours were given a subcutaneous injection of 1/4 gr. morphine and 1/120 gr. atropine pre-operatively. This usually caused emesis insuring a minimum amount of residue in the stomach, and also assisted the anesthesia produced by ether.

The anterior abdominal wall was prepared using iodine-alcohol antiseptics. A lateral rectus incision was made for a distance of about 10 cm. On opening the peritoneal cavity, the jejunum was located from the duodeno-jejunal junction which is indicated by a ligament corresponding to the ligament of Treitz in the human.

A section of jejunum approximately 8 cm. in length with a good fan-shaped blood supply was selected, and after the direction of peristalsis

had been noted, it was clamped using the least amount of pressure possible. The blood supply was double ligated at each end, and the section was cut through and placed in sponges soaked in warm 0.9 per cent sodium chloride.

The jejunum was anastomosed using the end-to-end technique. Single black silk No.1 or Deknatel No.1 was used, and a through-and-through inverting suture of the Connell or Lembert type was used and tied on itself. Special care must be taken at the separation of the two layers of mesentery where it meets the bowel, and often an extra suture was used at this location.

The jejunal segment was reversed and the distal end sutured into a stab wound made into the greater curvature of the stomach using the same sutures and technique. The proximal end was brought to the exterior through a left rectus stab wound and fixed with one or two skin sutures. The wound was closed by a single suture of white silk No. 1 or Deknatel No. 1; the peritoneum was sutured serosa to serosa, and then the abdominal parietes in layers, a continuous running suture of white silk No. 1 or Deknatel No. 1 being used for aponeuroses, muscle, and skin, although interrupted sutures and occasionally Halsted's subcuticular suture were sometimes used in the skin.

Chapter 4

Assay of Extract in Dogs

ANIMALS

The data represent ten dogs weighing from 9 to 15 kilograms. All of the dogs with the exception of one (No. 41) which had a Spivack gastric fistula, were of the gastric intestinal fistula type prepared according to the usual procedure (See operative technique). In two cases (No. 63 and No. 64) the isolated loop was of very distal jejunum or of proximal ileum, it being almost impossible to say definitely in which area the resection

was made.

The dogs gave negative tests for free hydrochloric acid in 78 per cent of the control gastric samples for the 30 minute period preceding injection. In the 22 per cent giving a positive test for free acid, the highest value was only 0.7 of a millequivalent of free hydrochloric acid. Of the remaining values 63 per cent or more were within the range of 0.2 or less of a millequivalent of free hydrochloric acid, and this value is not considered statistically significant.

Five additional dogs were used for observation, but because of the fact that these dogs would spontaneously secrete free hydrochloric acid in the control periods, their results are not included in the data. There is, however, less tendency apparently for gastric intestinal fistula dogs to secrete free acid in control periods than for dogs with the Spivack fistula. Apparently this latter preparation developed either an internal or external gastric ulcer with subsequent spontaneous or continuous acid secretion. On the other hand, there is no basis for concluding that the gastric intestinal fistula may not develop internal gastric ulcers, although observations have not shown the presence of any. Of necessity, however, they do not develop gastric ulcers of the external type, and there is no apparent ulceration of the intestinal segment.

EXPERIMENTAL

The animals were fed daily with "Purina dog chow" in biscuit form to which some sodium chloride was occasionally added, experiments with histamine on dogs by Goebel and Bartosieroiz (13) having shown that alkalosis results from excessive chloride removal. For this reason, as well as general nutritional considerations, the dogs were not used on consecutive days.

The animals in all of the experiments received no food for 20 to 30 hours before injections. Gastric contents were collected by a 20 cc. syringe from a No. 20 rubber catheter inserted through the fistula. It was necessary that the stomach contain no food residue, since this would bring about the secretion of free hydrochloric acid. Also, the particles of food would occlude the opening of the catheter and syringe and thus prevent complete emptying of the stomach. The technique was attempted of washing with water the stomach in which a bit of food residue remained, but this procedure was found to be unsatisfactory. At the beginning of the experiment the dogs' stomachs were completely emptied and the material discarded. In later experiments if this material showed any signs of free hydrochloric acid, the animal was not used. For the next two hours, the samples were collected at 15 minute intervals, the entire contents being withdrawn. The two samples collected during each 30 minute period were combined for analysis, making a total of 4 samples for each animal. At the end of the first 30 minute interval the material to be tested was injected intramuscularly into either the gluteal or thigh region. In no case was more than 1 to $1\frac{1}{2}$ cc. given in any one location. If the extract was not sufficiently concentrated so that this amount contained the necessary yeast equivalent, the dose was divided and given into both sides. In no case was more than 4 cc. used, and in the majority of cases the dose injected was less than 3 cc., particularly as the extract became more purified.

The animals were kept as quiet as possible to prevent any spontaneous gastric secretion. In some particularly refractive cases intravenous injections of Nembutal were used, the initial dose being approximately $1/2$ gr. per kilo of body weight; this was shown not to have any effect on the gas-

tric response to histamine or to yeast extract.

ANALYTICAL METHODS

For free hydrochloric acid 2 to 5 cc. of the gastric sample were titrated to pH 2.4 with 0.1 N sodium hydroxide using thymol blue as an indicator and a phthalate buffer solution with the same indicator as a color standard. Total acid in the same sample was measured by further titration with 0.1 N sodium hydroxide to the second color change of thymol blue at a pH of 8.0 using a phosphate buffer solution as a color standard (14).

Since the values for pepsin had been shown previously (9) not to be essential to the assay of the active substance, these were not routinely done.

Chapter 5

Probability of Significance in a Single Set of Data

The results of experiments were analysed statistically according to the formula of Mainland (15) for the determination of t. This value for t was then compared with Fisher's (16) table to determine the probability of the results being due to chance. A value that corresponded to 0.05 in the table was taken to be significant. This represents a probability that the increases were due to chance only one in twenty times.

The values for the 30 minute interval before injection represent the normal resting secretion of the stomach. These values were consistently observed in experiments in which 0.9 per cent sodium chloride or inactive material was injected. All calculations were based on values obtained in the intervals following the injection.

EXPERIMENTAL RESULTS

Chapter 1

Simple Extracts

Experiment I

ALCOHOLIC HYDROCHLORIC ACID EXTRAOT OF DRY YEAST

In Table I are shown the results of 4 experiments in which 1 to 2 cc. of an alcohol hydrochloric acid extract (equivalent to 30 to 45 gm. of yeast) were injected intramuscularly. In the same Table, the probabilities of a chance increase in free hydrochloric acid, total acid, and volumes are shown as compared with the first 30 minute (or control) period preceding injection.

As compared with the control averages there is an apparent increase in all three of the factors recorded. However, this increase is not statistically significant. This equivocal result may be due to the small number of observations recorded and to the relatively small dose. The dose here employed falls into the category of liminal dosage. Later it was found that a dose equivalent to 60 to 90 gm. of yeast does produce a significant response. Large values for the control period in the case of two of the dogs also affected the statistical significance. While these results, therefore, fall into the classification of orientation experiments, further work has indicated that the apparent increases are real. (See Table IX).

Experiment II

COLD ALCOHOLIC HYDROCHLORIC ACID EXTRACT

In Table II are shown the results of the injection of 3 cc. of an extract prepared from dry yeast using hydrochloric acid with 95 per cent

TABLE I

Effects of injection of alcoholic hydrochloric acid extract in doses equivalent to 30 to 40 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	12.5	0.5	0.8
2.	22.0	0.1	0.2
3.	6.5	0.7	1.0
4.	11.5	0.0	0.3
First 30 minute period after injection			
1.	36.0	1.0	1.5
2.	15.0	1.3	1.7
3.	2.5	0.0	0.0
4.	31.0	2.0	2.2
P*	0.4	0.1	0.2
Second 30 minute period after injection			
1.	21.0	0.6	1.1
2.	13.0	0.7	0.9
3.	23.5	0.9	1.2
4.	14.0	1.0	1.4
P*	0.3	0.4	0.2
Third 30 minute period after injection			
1.	14.5	1.2	1.6
2.	18.0	1.3	1.6
3.	11.0	0.0	0.1
4.	14.5	0.5	1.1
P*	0.7	0.2	0.2

*P = probability of chance increase.

TABLE II

Effect of injection of alcoholic hydrochloric acid extract (prepared in the cold) in doses equivalent to 60 to 100 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	5.0	0.2	0.3
2.	2.0	0.0	0.0
3.	16.0	0.2	0.5
First 30 minute period after injection			
1.	17.0	0.6	1.5
2.	14.0	0.8	1.4
3.	43.5	1.9	3.6
P*	0.1	0.1	0.08
Second 30 minute period after injection			
1.	8.0	0.1	0.3
2.	6.0	0.3	0.6
3.	24.0	0.8	1.2
P*	0.5	0.2	0.2
Third 30 minute period after injection			
1.	11.0	0.1	0.3
2.	1.5	0.0	0.0
3.	9.5	0.3	0.5
P*	1.0	1.0	1.0

*P = Probability of chance increase

alcohol in the cold. The dry yeast equivalent is 60 to 100 gm. per dose.

Although the results are statistically insignificant in all cases, they do indicate that some fraction of the active material may have been extracted. If so, this fraction must represent a very minute amount as compared with extraction at the higher temperature.

Experiment III

ALCOHOLIC SULFURIC ACID EXTRACT

In Table III are shown the results of the injection of 1.5 to 3 cc. of the alcoholic sulfuric acid extract representing a dry yeast equivalent of from 45 to 55 gm. or a wet compressed starch-free yeast equivalent of 60 gm.

The results are essentially negative indicating failure of this method to extract active principle from the quantities of yeast tried.

Experiment IV

EXTRACTION WITH ETHYL ACETATE FOLLOWED BY ALCOHOLIC HYDROCHLORIC ACID

In Table IV are shown the results of the injection of 1.3 to 3 cc. of extract representing a dry yeast equivalent of 52 to 60 gm. The results are very definitely negative, except that dog No. 43 showed a slight increase in secretion. No statistical significance can be attached to this exception, however.

Experiment V

ALCOHOLIC FORMIC ACID EXTRACT OF DRY YEAST

The results of the injection of 1.5 to 2 cc. of the absolute alcohol soluble fraction of the alcoholic formic acid extract are shown in Table V. The extract, which was similar in appearance to the hydrochloric acid extract, was neutralized to a pH of approximately 6 just before injection.

Increases in volume, free hydrochloric acid, and total acid are shown, and they are significant statistically.

TABLE III

Effects of injection of alcoholic sulphuric acid extract in doses equivalent to 45 to 60 grams of wet or dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc	meq.	meq.
1.	1.0	0.0	0.0
2.	0.5	0.0	0.0
3.	8.0	0.0	0.0
4.	6.0	0.2	0.5
5.	1.0	0.0	0.0
6.	17.5	0.7	1.2
First 30 minute period after injection			
1.	3.0	0.0	0.0
2.	2.0	0.0	0.0
3.	63.0	0.0	0.0
4.	11.-	0.5	0.9
5.	15.0	0.0	0.0
6.	14.0	1.2	1.5
P*	0.2	0.6	0.7
Second 30 minute period after injection			
1.	10.0	0.0	0.0
2.	3.0	0.0	0.2
3.	31.0	0.0	0.0
4.	14.0	0.0	0.5
5.	5.5	0.0	0.0
6.	9.0	0.1	0.3
P*	0.2	0.4	1.0
Third 30 minute period after injection			
1.	13.0	0.0	0.0
2.	3.5	0.9	1.7
3.	24.0	0.0	0.0
4.	7.0	0.0	0.3
5.	2.0	0.0	0.0
6.	8.5	0.2	0.4
P*	0.2	0.9	0.7

*P = Probability of chance increase.

Effects of injection of ethyl acetate alcoholic hydrochloric acid extract in doses equivalent to 45 to 60 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	2.5	0.0	0.0
2.	0.5	0.0	0.0
3.	0.5	0.0	0.0
4.	2.5	0.0	0.0
5.	4.0	0.0	0.0
6.	0.0	0.0	0.0
7.	3.0	0.0	0.0
8.	2.5	0.0	0.0
First 30 minute period after injection			
1.	0.0	0.0	0.0
2.	4.0	0.0	0.0
3.	19.0	1.3	2.0
4.	0.0	0.0	0.0
5.	2.0	0.0	0.0
6.	2.0	0.0	0.0
7.	1.0	0.0	0.0
8.	15.0	0.7	1.2
P*	0.2	0.4	0.2
Second 30 minute period after injection			
1.	1.0	0.0	0.0
2.	2.0	0.0	0.0
3.	14.5	0.9	1.4
4.	0.5	0.0	0.0
5.	0.5	0.0	0.0
6.	0.0	0.0	0.0
7.	0.5	0.0	0.0
8.	7.0	0.4	0.6
P*	0.5	1.0	0.2

(Cont.)

Third 30 minute period after injection

	cc.	meq.	meq.
1.	2.0	0.0	0.0
2.	1.0	0.0	0.0
3.	2.5	0.0	1.1
4.	6.0	0.0	0.0
5.	0.5	0.0	0.0
6.	0.5	0.0	0.0
7.	2.0	0.0	0.0
8.	6.0	0.4	0.6
P*	0.5	1.0	0.1

*P = Probability of chance increase.

1.	13.3	1.3	1.4
2.	2.3	0.0	2.3
3.	22.0	2.1	2.8
4.	16.0	1.1	1.6
5.	14.5	0.0	7.0
6.	15.0	6.0	2.9
P*	0.05	0.1	0.05

Second 30 minute period after injection

1.	18.0	1.5	2.1
2.	17.0	1.0	1.4
3.	34.0	2.4	3.6
4.	12.0	0.4	0.4
5.	2.0	0.0	0.1
6.	15.5	1.4	1.9
P*	0.01	0.02	0.02

First 30 minute period after injection

1.	16.0	1.4	1.9
2.	16.0	1.0	1.2
3.	5.0	0.3	0.5
4.	13.0	0.3	0.5
5.	11.0	0.3	0.4
6.	4.0	0.0	0.1
P*	0.01	0.1	0.2

*P = Probability of chance increase.

** = Not used in calculations for volume.

TABLE V

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Effects of injection of absolute ethyl alcohol soluble fraction of alcoholic formic acid extract in doses equivalent to 60 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	6.0--	0.4	0.6
2.	1.2	0.0	0.0
3.	3.5	0.0	0.0
4.	2.0	0.0	0.1
5.	**45.0	0.0	0.6
6.	3.0	0.0	0.0
First 30 minute period after injection			
1.	13.0	1.1	1.4
2.	2.5	0.0	0.0
3.	52.0	3.1	3.8
4.	19.0	1.1	1.6
5.	14.5	0.0	0.0
6.	13.0	0.5	0.9
	0.05	0.1	0.05
Second 30 minute period after injection			
1.	18.0	1.5	2.1
2.	17.0	1.0	1.4
3.	36.0	2.6	3.8
4.	12.0	0.4	0.8
5.	9.0	0.0	0.1
6.	15.5	1.4	1.9
	0.01	0.02	0.02
Third 30 minute period after injection			
1.	16.0	1.4	1.9
2.	13.0	1.0	1.2
3.	5.0	0.1	0.3
4.	13.0	0.3	0.6
5.	12.0	0.2	0.4
6.	2.0	0.0	0.1
P*	0.01	0.1	0.1

* Probability of chance increase.

** Not used in calculations for volume.

It is interesting to note that the greatest increase in the secretion is to be found in the second 30 minute period following injection rather than in the first 30 minute period as had been seen previously with the injection of the alcoholic hydrochloric acid extract.

Experiment VI

ALCOHOLIC HYDROCHLORIC ACID EXTRACT OF WET COMPRESSED STARCH-FREE YEAST

In Table VI are shown the results of the injection of 1.5 to 2 cc. of an acid alcohol extract of wet compressed starch-free yeast, each dose representing 60 to 80 gm. of the yeast. In this extraction the amount of concentrated hydrochloric acid was reduced from the usual 37.5 cc. to only 30 cc. per 450 gm. of yeast. This fact and the high water content of the wet yeast, which reduces the yeast equivalent as compared with the dry yeast may account for the statistically insignificant results which were observed.

Chapter 2

Extracts Subjected to Further Treatment

Experiment VII

PHOSPHOTUNGSTIC ACID TREATMENT OF ALCOHOLIC HYDROCHLORIC ACID EXTRACT OF YEAST

In Table VII are shown the results of the injection of 1 to 2 cc. of the fraction of the yeast extract precipitated by phosphotungstic acid, each dose representing an equivalent of 45 to 70 gm. of dry yeast. The results are negative, although some slight increase is apparent in total acid in the second 30 minute period; this, however, is statistically insignificant.

Effects of injection of alcoholic hydrochloric acid extract of wet compressed starch-free yeast in doses equivalent to 60 to 80 grams of the yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	16.0	0.4	0.7
2.	8.0	0.0	0.0
3.	5.0	0.0	0.0
4.	2.5	0.0	0.0
First 30 minute period after injection			
1.	13.0	0.7	1.1
2.	16.0	0.0	0.0
3.	15.0	1.0	1.4
4.	8.5	0.2	0.5
P*	0.2	0.2	0.1
Second 30 minute period after injection			
1.	4.0	0.0	0.1
2.	8.0	0.1	0.2
3.	5.5	0.1	0.2
4.	2.5	0.0	0.1
P*	0.4	0.7	0.9
Third 30 minute period after injection			
1.	0.0	0.0	0.0
2.	8.0	0.0	0.0
3.	4.5	0.0	0.0
4.	0.0	0.0	0.0
P*	1.0	1.0	1.0

*P = Probability of chance increase.

Effects of injection of phosphotungstic acid precipitable fraction of an active extract in doses equivalent to 45 to 70 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	1.5	0.0	0.0
2.	38.0	0.0	0.0
3.	11.0	0.0	0.0
4.	8.0	0.0	0.0
5.	4.0	0.0	0.0
First 30 minute period after injection			
1.	2.0	0.0	0.0
2.	16.0	0.0	0.0
3.	29.0	0.0	0.0
4.	9.5	0.0	0.0
5.	42.0	0.0	0.0
P*	0.5	1.0	1.0
Second 30 minute period after injection			
1.	1.5	0.0	0.0
2.	12.0	0.0	0.0
3.	30.0	0.0	0.0
4.	21.0	0.2	0.5
5.	9.0	0.0	0.4
P*	0.8	0.3	0.1
Third 30 minute period after injection			
1.	4.0	0.0	0.0
2.	15.0	0.0	0.0
3.	14.0	0.0	0.0
4.	8.0	0.0	0.2
5.	2.0	0.0	0.0
P*	1.0	1.0	0.3
*P = Probability of chance increase			

ALCOHOLIC HYDROCHLORIC ACID EXTRACT DECOLORIZED WITH CHARCOAL

In Table VIII are shown the results of the injection of 2 to 5 cc. of a decolorized acid alcohol extract of dry yeast, each dose representing 22.5 to 135 gm. of the yeast. The results are negative, indicating that the charcoal removed the greater part of the activity. However, it may be seen from the Table that the material injected was not entirely inactive.

Experiment IX

ABSOLUTE ALCOHOL SOLUBLE FRACTION OF ALCOHOLIC
HYDROCHLORIC ACID EXTRACT OF YEAST

Further tests of the absolute alcohol fraction of the alcoholic hydrochloric acid extract of yeast are shown in Table IX. The dosage was increased so that the dry yeast equivalent amounted to 60 to 67 gm., 2 to 4 cc. of the final solution being injected.

The results of 8 tests show increases in free hydrochloric and total acid as well as in volume, which are statistically significant, being far out of the range of chance in the first and second periods following injection. In the third period after injection the results do not show a any significant increase, presumably due to the wearing off of the effect of the active agent and the return of the secretion of the stomach to its normal level.

In 6 out of the 8 cases the animals were anesthetized with Nembutal in order to prevent them from becoming excited as well as to avoid excessive swallowing of saliva, which was allowed to drain from the mouth in the anesthetized animals. This latter arrangement obviates the possibility that the volume of gastric secretion might be augmented by swallowed saliva and also that the acidity might be more or less neutralized by the alkaline

Effects of injection of an active extract decolorized with charcoal in doses equivalent to 22.5 to 135 grams of dry yeast.

Experiment No.	**Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
-	cc.	meq.	meq.
1.	3.0	0.0	0.0
2.	35.0	0.0	0.0
3.	10.0	0.0	0.0
4.	27.0	0.2	0.9
5.	1.0	0.0	0.0
6.	16.0	0.2	0.5
First 30 minute period after injection			
1.	32.0	0.3	0.8
2.	41.0	0.1	0.2
3.	29.0	1.4	2.0
4.	27.0	0.0	0.0
5.	24.0	0.0	0.2
6.	27.0	1.1	1.6
P*	0.02	0.2	0.2
Second 30 minute period after injection			
1.	33.0	0.2	0.5
2.	20.0	0.6	0.8
3.	16.0	0.5	0.8
4.	70.0	0.0	0.0
5.	19.5	1.1	1.5
6.	13.5	0.0	0.3
P*	0.2	0.2	0.2
Third 30 minute period after injection			
1.	37.5	0.0	0.5
2.	7.0	0.0	0.0
3.	20.0	0.6	0.1
4.	60.0	0.0	0.0
5.	8.0	0.4	0.6
6.	18.0	0.8	1.1
P*	0.3	0.2	0.5

*P = Probability of chance increase.

** = Large volume increases due to saliva.

Effects of injection of absolute ethyl alcohol soluble fraction of alcoholic hydrochloric acid extract in doses equivalent to 60 to 67 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	4.0	0.0	0.0
2.	21.0	0.2	0.5
3.	7.5	0.0	0.0
4.	0.0	0.0	0.0
5.	0.0	0.0	0.0
6.	0.0	0.0	0.0
7.	1.0	0.0	0.0
8.	7.0	0.0	0.0
First 30 minute period after injection			
1.	46.0	4.4	5.8
2.	67.0	2.0	3.7
3.	45.0	1.3	2.0
4.	52.0	4.8	6.4
5.	29.0	1.8	2.5
6.	31.0	2.9	3.8
7.	10.0	0.7	1.0
8.	30.0	2.9	3.5
P*	0.001	0.001	0.001
Second 30 minute period after injection			
1.	36.0	3.2	4.3
2.	35.0	0.5	1.3
3.	21.0	0.2	0.5
4.	14.0	0.8	1.3
5.	34.5	2.9	3.6
6.	37.0	3.6	4.6
7.	10.0	0.7	1.0
8.	37.0	3.8	4.5
P*	0.001	0.001	0.001

(Cont'd)

Third 30 minute period after injection

	cc.	meq.	meq.
1.	37.0	3.3	4.6
2.	20.0	0.3	0.8
3.	8.0	0.1	0.2
4.	4.0	0.0	0.0
5.	6.0	0.5	0.6
6.	12.5	0.4	0.8
7.	6.5	0.3	0.5
8.	15.0	1.3	1.7
P*	0.1	0.05	0.1

*P = Probability of chance increase.

saliva, which I have found in dogs may run as high as pH of 9. In the two remaining cases the animals were not anesthetized, since their behavior was of such nature as to make anesthesia unnecessary.

Experiment X .

ABSOLUTE METHYL ALCOHOL SOLUBLE FRACTION OF ALCOHOLIC HYDROCHLORIC ACID EXTRACT OF YEAST

In Table X are shown the results of the injection of 3 cc. of the absolute methyl alcohol soluble fraction of an alcoholic hydrochloric acid extract of dry yeast, this dose being equivalent to 100 gm. of dry yeast.

The results are statistically significant but to a lesser degree than might be expected from such a yeast equivalent.

Experiment XI

ACETONE SOLUBLE FRACTION OF ALCOHOLIC HYDROCHLORIC OR FORMIC ACID EXTRACT OF YEAST

In Table XI are the results of the injection of 1 to 1.5 cc. of the acetone soluble fraction of either hydrochloric or formic acid alcoholic extraction, each dose representing an equivalent of 60 to 200 gm. of dry yeast.

The results were not significant statistically at any point, the values being essentially those that might be observed following the injection of isotonic sodium chloride solution.

Experiment XII

THE NON-PRECIPIATED FRACTION OF YEAST EXTRACT WITH PHOSPHOTUNGSTIC ACID

In Table XII are shown the results of the injection of 1.25 to 1.5 cc. (equivalent to 45 to 100 gm. dry yeast) of the non-precipitated fraction of yeast extract which had been treated with phosphotungstic

TABLE X

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Effects of injection of the absolute methyl alcohol fraction of an active alcoholic hydrochloric acid extract in doses equivalent to 100 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	8.0	0.2	0.5
2.	0.5	0.0	0.0
3.	7.0	0.3	0.4
4.	0.0	0.0	0.0
First 30 minute period after injection			
1.	26.0	1.4	2.0
2.	9.0	0.5	0.8
3.	50.0	1.3	2.4
4.	30.0	1.7	2.3
P*	0.02	0.01	0.01
Second 30 minute period after injection			
1.	15.5	1.0	1.5
2.	15.5	0.6	1.2
3.	26.0	0.5	1.0
4.	12.5	1.2	1.5
P*	0.01	0.02	0.001
Third 30 minute period after injection			
1.	11.0	0.4	0.9
2.	0.0	0.0	0.0
3.	17.0	0.6	0.9
4.	2.0	0.0	0.0
P*	0.4	0.5	0.5

*P = Probability of chance increase.

TABLE XI

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Effects of injection of acetone soluble fraction of an active extract in doses equivalent to 60 to 200 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	ccs	meq.	meq.
1.	9.0	0.2	0.7
2.	11.0	0.0	0.0
3.	5.5	0.0	0.0
4.	3.5	0.0	0.0
5.	9.0	0.1	0.3
First 30 minute period after injection			
1.	23.0	1.0	1.4
2.	19.0	0.1	0.3
3.	3.5	0.0	0.0
4.	13.0	0.0	0.0
5.	3.5	0.0	0.0
P*	0.3	0.4	0.7
Second 30 minute period after injection			
1.	23.0	0.3	0.7
2.	19.0	0.1	0.4
3.	2.0	0.0	0.0
4.	9.0	0.0	0.0
5.	6.5	0.0	0.0
P*	0.6	0.8	1.0
Third 30 minute period after injection			
1.	11.0	0.1	0.3
2.	16.0	0.0	0.1
3.	3.5	0.0	0.0
4.	2.0	0.0	0.0
5.	7.5	0.0	0.0
P*	0.8	1.0	1.0

*P = Probability of chance increase

Effect of injection of the non-precipitated fraction of yeast extract treated with phosphotungstic acid in doses equivalent to 45 to 100 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	8.0	0.0	0.4
2.	27.0	0.0	0.0
3.	2.5	0.0	0.0
4.	3.0	0.0	0.1
5.	4.0	0.0	0.0
6.	4.0	0.0	0.0
7.	12.0	0.0	0.0
8.	23.0	0.0	0.0
9.	2.5	0.0	0.0
First 30 minute period after injection			
1.	30.0	0.0	0.2
2.	53.0	0.0	0.0
3.	11.0	0.0	0.0
4.	10.0	0.0	0.5
5.	23.0	0.3	0.8
6.	8.0	0.1	0.4
7.	12.0	0.0	0.2
8.	17.0	0.6	0.9
9.	3.5	0.0	0.0
P*	0.02	0.05	0.02
Second 30 minute period after injection			
1.	36.0	0.0	0.1
2.	41.0	0.0	0.0
3.	3.5	0.0	0.0
4.	8.5	0.1	0.4
5.	6.5	0.3	0.6
6.	10.0	0.5	0.9
7.	9.0	0.0	0.1
8.	10.0	0.0	0.1
9.	1.0	0.0	0.0
P*	0.4	0.05	0.07

(Cont'd)

Third 30 minute period after injection

	cc.	meq.	meq.
1.	16.0	0.1	0.7
2.	28.0	0.0	0.0
3.	2.0	0.0	0.0
4.	3.0	0.0	0.2
5.	1.0	0.0	0.0
6.	10.0	0.3	0.8
7.	12.0	0.0	0.0
8.	4.0	0.0	0.0
9.	3.0	0.0	0.0
P*	1.0	0.1	0.2

*P - Probability of chance increase.

acid. The results show an increase which is statistically significant in the first 30 minutes following the injection. The increase is in no case marked, and there is very little evidence of activity in this fraction of the yeast extract.

Chapter 3

Comparison of Active Yeast Extract with Histamine

Experiment XIII

ALCOHOLIC HYDROCHLORIC ACID EXTRACT OF YEAST TREATED WITH NITROUS ACID

In Table XIII the results of the injection of 2 cc. of an active alcoholic hydrochloric acid extract of dry yeast are given. This dose is equivalent to 64 to 67 gm. of the dry yeast.

The results show values that are statistically significant in all cases except two. The values for volume and free HCL in the last 30 minute period are within the range of chance. These results then are such as might have been expected had the extract received no treatment with nitrous acid. The nitrous acid treatment, therefore, had no effect on the activity of the extract.

Experiment XIV

HISTAMINE TREATED WITH NITROUS ACID

In Table XIV are shown the results of the injection of 0.1 milligram of histamine after treatment with nitrous acid. This amount, when untreated, is known to produce a marked increase in gastric secretion. In this case it will be seen that all results are absolutely negative statistically. The stimulating action of the histamine has been completely destroyed by the nitrous acid treatment.

Effects of injection of an active extract treated with nitrous acid in doses equivalent to 64 to 67 grams of dry yeast.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	2.0	0.0	0.0
2.	3.0	0.0	0.0
3.	25.0	0.0	0.2
4.	31.0	0.0	0.0
5.	1.5	0.0	0.0
First 30 minute period after injection			
1.	66.0	1.7	2.8
2.	31.0	1.8	2.3
3.	64.0	2.8	3.7
4.	20.0	0.5	0.9
5.	61.0	1.8	2.8
P*	0.01	0.001	0.001
Second 30 minute period after injection			
1.	17.0	0.6	0.7
2.	24.0	1.4	1.8
3.	59.0	3.8	4.7
4.	17.0	0.2	0.4
5.	34.0	2.7	3.4
P*	0.05	0.02	0.02
Third 30 minute period after injection			
1.	11.0	0.1	0.2
2.	8.0	0.4	0.6
3.	24.0	1.2	1.6
4.	19.0	0.2	0.5
5.	6.0	0.0	0.1
P*	0.8	0.1	0.05

*P = Probability of chance increase

Effects of injection of 0.1 milligram of histamine treated with nitrous acid.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	1.0	0.0	0.0
2.	12.0	0.0	0.0
3.	6.0	0.0	0.0
4.	5.0	0.1	0.3
5.	13.0	0.5	0.8
First 30 minute period after injection			
1.	1.0	0.0	0.0
2.	5.0	0.0	0.0
3.	32.0	0.0	0.0
4.	10.0	0.0	0.2
5.	49.0	0.8	1.1
P*	0.2	0.8	0.9
Second 30 minute period after injection			
1.	4.0	0.0	0.1
2.	7.0	0.0	0.0
3.	9.0	0.0	0.0
4.	8.0	0.0	0.0
5.	32.0	0.4	0.0
P*	0.4	1.0	1.0
Third 30 minute period after injection			
1.	2.5	0.0	0.0
2.	3.0	0.0	0.0
3.	7.0	0.0	0.0
4.	5.0	0.0	0.0
5.	31.0	0.4	0.7
P*	0.7	1.0	1.0

*P = Probability of chance increase

PAULY REACTION

In Figure 1 are shown the spectrophotometric curves for histamine and an active extract after simultaneous treatment with the Pauly reagent. It will be seen that the absorption curves differ markedly in height, the curve for the extract being much lower at all points examined. Further, the peak for the extract is located at a different wave length from that of the histamine.

Chapter 4

Pure Chemical Compounds Known or Suspected to Be Present in Yeast.

Experiment XVI

NEURINE BROMIDE

The effects of the injection of 25 milligrams of neurine bromide in 1.5 cc. of 0.9 per cent sodium chloride are shown in Table XV. There is seen an increase in the gastric secretion which is statistically significant in the first 30 minute period following the injection. The results in the two following 30 minute periods are not statistically significant. These results indicate that the neurine bromide does stimulate gastric secretion, but the effect lasts only for 30 minutes after injection. It may be possible to increase the length of the effect of the neurine bromide by giving larger doses.

Experiment XVII

BETA-ALANINE

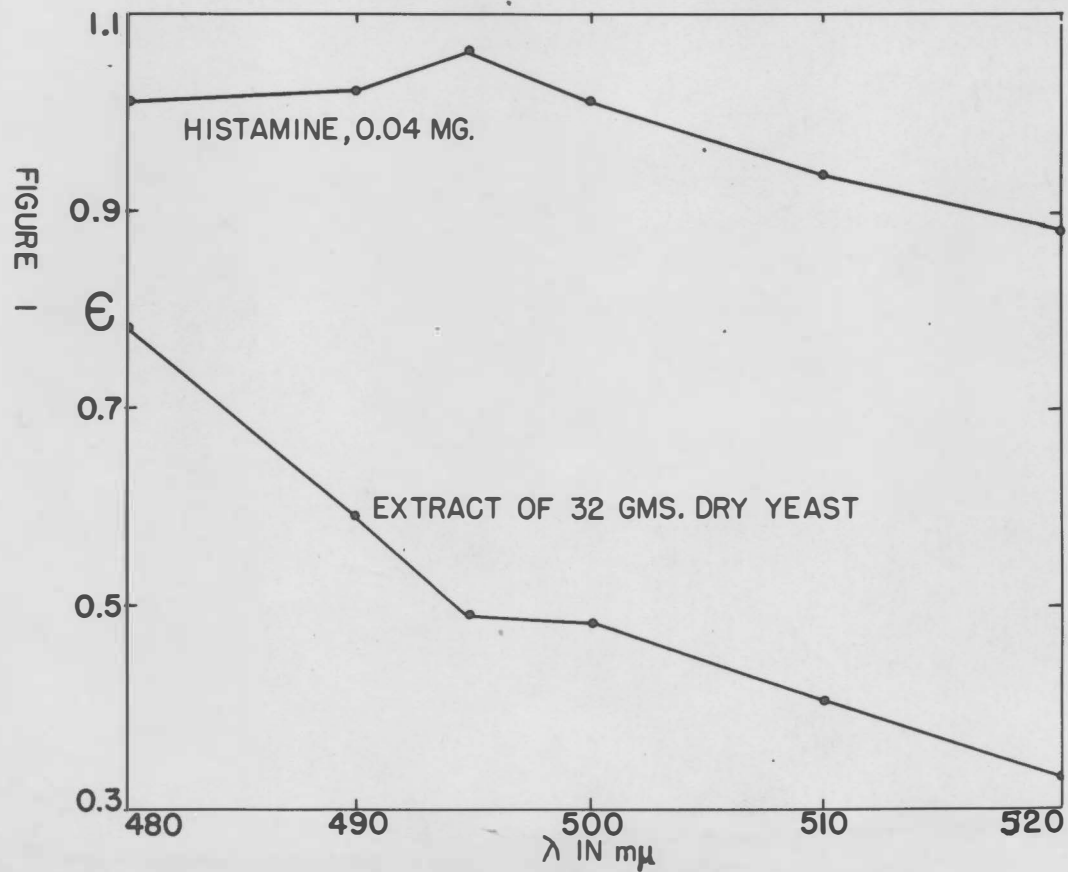
The results of the injection of 5 to 25 milligrams of beta-alanine in 1.5 cc. of 0.9 per cent sodium chloride are shown in Table XVI. The values obtained are statistically negative in all cases.

Experiment XVIII

GLUTATHIONE

The results of the injection of 25 milligrams of glutathione in 1.5 cc. of 0.9 per cent sodium chloride are shown in Table XVII. The values obtained are statistically negative in all cases.

LIGHT ABSORPTION CURVES OF PAULY TESTS



Effects of injection of 25 milligrams of neurine bromide.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	5.0	0.0	0.0
2.	0.0	0.0	0.0
3.	4.0	0.0	0.1
4.	0.0	0.0	0.0
5.	15.0	0.0	0.0
First 30 minute period after injection			
1.	27.0	0.7	1.2
2.	7.5	0.2	0.4
3.	22.0	0.7	1.5
4.	20.0	0.6	1.0
5.	15.0	0.2	0.3
P*	0.002	0.002	0.01
Second 30 minute period after injection			
1.	15.0	0.4	0.6
2.	2.0	0.0	0.0
3.	8.0	0.1	0.3
4.	8.0	0.0	0.0
5.	12.5	0.0	0.1
P*	0.1	0.2	0.1
Third 30 minute period after injection			
1.	12.0	0.1	0.2
2.	3.0	0.0	0.0
3.	6.0	0.1	0.2
4.	4.0	0.0	0.0
5.	5.5	0.0	0.0
P*	0.5	0.1	0.3

*P = Probability of chance increase.

Effects of injection of 5 or 25 mg. of beta-alanine.

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	17.0	0.0	0.0
2.	4.0	0.2	0.3
3.	3.0	0.0	0.1
First 30 minute period after injection			
1.	21.5	0.0	0.0
2.	6.0	0.3	0.5
3.	2.0	0.0	0.1
P*	0.8	0.7	0.7
Second 30 minute period after injection			
1.	15.0	0.0	0.0
2.	10.0	0.3	0.6
3.	0.0	0.0	0.0
P*	1.0	0.7	0.8
Third 30 minute period after injection			
1.	27.0	0.0	0.0
2.	16.0	0.6	1.0
3.	3.0	0.0	0.0
P*	0.4	0.6	0.7

*P = Probability of chance increase.

Effects of injection of 25 mg. of glutathione (Pfanstiehl).

Experiment No.	Volume	Free HCl	Total Acid
(Control) 30 minute period preceding injection			
	cc.	meq.	meq.
1.	5.0	0.0	0.2
2.	12.0	0.0	0.0
3.	1.0	0.0	0.0
First 30 minute period after injection			
1.	6.0	0.1	0.3
2.	9.0	0.0	0.0
3.	1.0	0.0	0.0
P*	1.0	0.4	0.7
Second 30 minute period after injection			
1.	3.0	0.0	0.1
2.	12.0	0.0	0.0
3.	1.0	0.0	0.0
P*	1.0	1.0	1.0
Third 30 minute period after injection			
1.	3.0	0.0	0.0
2.	8.0	0.0	0.0
3.	2.0	0.0	0.0
P*	1.0	1.0	1.0

*P = Probability of chance increase

DISCUSSION

Using the method of extracting dry brewer's yeast with 95 per cent alcohol and hydrochloric acid, it was possible to prepare an extract which could be relied upon as having a fairly constant activity. The use of alcohol in the first step eliminated much of the extraneous material (presumably protein or proteose in nature) which had been present in aqueous extracts. The principal difficulty remaining was in getting rid of the excess of hydrochloric acid. After alcohol had been evaporated off, treatment of the residue with absolute alcohol and its subsequent removal at 60° under diminished pressure eliminated a large part of the acid. At this stage it was found that approximately one-fifth of the solid residue was insoluble in absolute alcohol and could be eliminated by exhaustive extraction with hot absolute alcohol. The soluble fraction contained essentially the entire active substance present. With such a purified extract the equivalent of 60 gm. of dry yeast could be concentrated into a volume of 1 to 1.5 cc. By reducing the volume for injection, less discomfort was experienced by the animals. The free acid present, however, still caused some pain, and it was neutralized routinely with solid sodium bicarbonate just before injection. This neutralization had been shown not to affect the activity. It was rather essential that everything possible be done toward the comfort of the animal, since otherwise the animal chewed at the leg ties and swallowed copious quantities of saliva. The latter complication would render inaccurate the observations on volume on the one hand and acidity, which thereby suffers some neutralization, on the other. This was the reason for the use of Nembutal as an anesthetic in Experiment IX. However, as the improved extract became less discomforting on injection, this procedure was deemed unnecessary, and calm

behavior throughout each assay was the rule.

This purified extract on evaporation almost to dryness showed white crystals dispersed throughout the syrupy liquid. These crystals were found to be nearly insoluble in absolute methyl alcohol. They were removed accordingly by dissolving the brown syrupy fluid in the alcohol and filtering. This process was repeated several times with subsequent evaporation almost to dryness each time, since the crystals also were slightly soluble in a great excess of the solvent. When the crystals were tested for gastric activity in doses equivalent to 100 gm. of dry yeast, they were found to be inactive. On the other hand the activity was found to go unchanged into the absolute methyl alcohol soluble fraction.

At this stage then there remains only approximately a fifth of the original solid residue extracted from the yeast by the alcoholic hydrochloric acid treatment, half being insoluble in distilled water, a fifth insoluble in absolute ethyl alcohol and a tenth insoluble in absolute methyl alcohol.

These properties of solubility in the above three solvents may be taken to suggest that the active substance consists of moderately polar organic molecules probably containing only short hydrocarbon chains.

A further question arose as to whether or not the active substance was present in the yeast as such or was produced from some inactive material by the effect of the hydrochloric acid and heat. Therefore, numerous attempts were made to fragment the yeast cells by other means such as the colloid mill, the ball mill (grinding for as long as 60 hours) grinding with sand in a mortar, grinding with carborundum between glass plates, freezing with subsequent quick thawing, and dissolution of the cell membrane by means of ether or ethyl acetate. It was not possible to

demonstrate any considerable degree of fragmentation microscopically or otherwise by any of these physical means. Treatments with ether or ethyl acetate were also unsuccessful in lysing the membrane.

Since the temperature of the evaporation procedure was so low, it was thought that here the heat would not play any appreciable part in the hypothesized production of the active substance. However, in some cases the pH was adjusted with solid potassium bicarbonate or 4-normal sodium hydroxide to pH 5 or higher before evaporation was undertaken. This precaution had little effect on the activity and it is concluded, therefore, that hydrochloric acid acts merely as an agent to release the active material from the yeast cell.

Attempts to prepare an active extract using a weak acid, such as acetic, instead of hydrochloric were unsuccessful. However, an active product was obtained when formic acid was used.

In a further attempt at purification, an active alcoholic hydrochloric acid extract was treated with acetone repeatedly and a very small amount of the material dissolved in the acetone. This soluble fraction would correspond to the one prepared by Abderhalden and Schaumann (17) and said by them to contain "aschamin," an organic base perhaps identical with neurine. However, the soluble fraction proved to be inactive, while the activity remained unchanged in the insoluble fraction.

Treatment of an active extract with nitrous acid, which is well known to convert primary amines into the corresponding alcohols, had absolutely no effect on its activity. On the other hand, the same treatment applied to 0.1 mg. of histamine completely negated its effect on gastric secretion when injected into the animals. This would seem to prove that the active substance in yeast extracts certainly is not

histamine or any primary amine or secondary amine. Thus, any amino nitrogen in the molecule must be at least tertiary if not quaternary. Such an arrangement is found in many heterocyclic ring compounds, in betaines, and in such quaternary bases as choline or neurine.

An unsuccessful attempt was made to concentrate the active substance by precipitation with mercuric sulfate and with mercuric chloride. Further, precipitation of an active extract with phosphotungstic acid, which is considered to be an universal precipitant for organic bases, led to the preparation of a solution from the precipitated fraction which was inactive. These findings indicate that the substance may not be a basic compound after all, in spite of the fact that acid must be used to bring the substance into solution from the yeast originally. On the other hand, it is suggested that treatment with barium hydroxide, necessary at one stage in the phosphotungstic acid separation, may have destroyed the active principle.

Spectrophotometric evidence also indicates the non-identity of the yeast substance with histamine. When histamine and an active extract are treated simultaneously with the Pauly reagent, very different absorption curves result (see Fig. 1). Although the yeast extract was much more effective in stimulating gastric secretion than the quantity of histamine it was compared with, its absorption curve was much lower at all points examined and the peak was not located at the same wave length as in the case of histamine.

Since the active material was found not to be histamine, other substances which might be present in yeast were tested for activity in doses estimated to be far above the content of each substance in 60 gm. of yeast. In this way have been tried neurine bromide, choline chloride, beta-alanine,

and glutathione. With the exception of neurine, none of these showed any response either quantitatively or qualitatively comparable to that following the injection of an active yeast extract.

CONCLUSIONS

1. By alcoholic acid extraction of dry yeast, an extract may be prepared which is an effective gastric stimulant when injected intramuscularly. The effective dose of the extract in dogs weighing approximately 10 kilograms represents 60 gm. of dry yeast.
2. The active material is soluble in absolute methyl alcohol and absolute ethyl alcohol.
3. The active material is not found in the precipitate obtained by treatment of the extract with mercuric sulphate, mercuric chloride, or phosphotungstic acid.
4. The active material is not histamine since it is not affected by treatment with nitrous acid. Treatment with the Pauly reagent gives a color which exhibits a spectrophotometric curve very different from that of histamine.
5. Choline chloride, beta-alanine, and glutathione are ineffective as gastric stimulants and consequently cannot represent the active material in the yeast.
6. Neurine bromide is an effective gastric stimulant when injected intramuscularly in 25 milligram doses.
7. Our work has not definitely decided the question whether the active material exists preformed in yeast or is produced only in the process of extraction. While conditions are relatively mild and not designed to produce hydrolytic or other changes in chemical structure of the yeast compounds, positive evidence may depend upon discovery of a physical means of fragmenting the yeast cells in the absence of heat and acid.

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