

tion more than fifty Widal's were performed on specimens of blood obtained from all the persons living on the different dairy farms supplying this distributor. Only one of these Widal's was positive. The man from whom this specimen was obtained gave no history of any recent illness. The evidence that he might be the probable source was strengthened by this fact: the milk from this one of the several dairies was the milk bottled for the more particular customers, and on further analysis of the cases it was found that it was among those consuming this bottled milk that the fourteen cases occurred.

It has been remarked that the light cases of typhoid fever, unrecognized as such, and the carriers of the specific organism constitute the most formidable source of infection in a community possessing a pure water supply. Each epidemic leaves its residue of chronic carriers, that are a threat of future epidemics. These carriers should be sought out and prohibited from engaging in such an occupation as cooking, baking, dairy work, etc., which may from the articles handled and consumed without further cooking endanger the lives of others. Milk, from its nature, is an especially fine culture medium; if contaminated with typhoid bacilli, particularly in the warm summer months, it is a most "effective" intermediary agent from the carrier to the consumer.

The recommendation of the committee of the American Public Health Association on typhoid fever that physicians should be impressed with the necessity of reporting this disease promptly is an excellent one. For, "until the physician, who is really the scout of the public health service, reports the location of the enemy, all our paraphernalia with all its elaborate organization is at a standstill."² The truth of this statement is shown by the epidemic reported in this paper, since the Board of Health had no inkling that such an epidemic was about to burst on the community until physicians reported actual cases, when the two employees as secondary sources of further infection were promptly recognized and removed. But these light cases, for which physicians are not called in, and carriers, because they are unrecognized as such, are the real danger of initiating other epidemics.

Typhoid fever has been called a preventable disease, and to consider it as such is certainly the goal which the public health service must strive to attain. That man constantly harbors the specific organism of typhoid fever is generally recognized at present. Precautions are taken to protect the public from typical cases. If the chronic carriers of this disease could be known, and the food and water-supplies, as intermediate bearers, be protected from their contamination, typhoid fever could soon be stamped out. The disease is preventable only by working along this line. Therefore, the new and serious problem that confronts the health service at present is, How is this knowledge of a carrier, which is of such recent recognition, to be gained, before it is bought at the extravagant price of an epidemic? A method of defense for this new problem, that is in the least satisfactory, has not as yet been advised.

The various methods advocated for the discovery of carriers, as general examination of excreta, or even only the examination of the excreta of those persons with a history of a previous typhoid infection, are propositions too large to be practicable. The second suggestion makes no allowance for the fact that in many cases of typhoid

carriers there is a lack of a previous history of typhoid fever, and again that many carriers excrete the bacilli only intermittently.

From the experience of the last two years as set forth in this paper, the suggestion is made that specimens of blood for Widal's be taken generally from persons living on dairy farms or handling foods that are consumed without further cooking, and then that the excreta of those only that give a positive agglutination reaction be examined. The Widal reaction in most cases of typhoid fever rapidly disappears after defervescence. The presence of post-typhoid suppuration somewhat increases its persistence.³ Most carriers possess it. Therefore, if a Widal reaction persists, that person if employed as mentioned above should be looked on with suspicion until proved free from typhoid organisms by repeated examinations of excreta.

This procedure also is an immense proposition, when, as is the case in the city of Worcester with a population of 145,000 for an example, milk is supplied from more than 600 dairies and 900 stores and distributors. These statistics take no account of bakers, cooks and other persons who come in intimate contact with other kinds of food. This simple procedure of a Widal reaction, however, should certainly be carried out on the blood of all persons handling, or living on farms producing, milk which is put on the market as certified.

61 Pearl Street.

THE TRYPTOPHAN TEST FOR CANCER OF THE STOMACH WITH SPECIAL REFER- ENCE TO PEPTIDOLYTIC ENZYME IN THE SALIVA *

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INTRODUCTION

The attention of the medical profession has recently been drawn to new ways of diagnosing cancer of the stomach by means of tests for the simplest products of protein digestion. Emerson,¹ who was among the first investigators to approach this field, found that "cancer tissue contains a ferment which, in the thermostat as well as in the stomach, is able to digest proteins beyond the peptone ('albumose') stage." He also observed that "this ferment is active in the presence of free hydrochloric acid." Emerson's discovery, coupled with the well-known fact that pepsin does not, in the stomach, conduct the cleavage of proteins beyond the stage of peptones, offered a method of determining whether or not the stomach has been invaded by a cancerous growth. A test for cancerous enzyme, if based on Emerson's findings, could not be applied, however, with the ease and directness which is desirable in clinical work.

THE GLYCYLTRYPTOPHAN TEST²

About two years ago Neubauer and Fischer³ published a new test for cancer of the stomach, which was based on

3. Louissou and French. Guy's Hospital Reports, 1907, Ixi, p. 227.

* From the laboratory of biological chemistry of Columbia University, at the College of Physicians and Surgeons, New York, and under the auspices of the George Crocker Special Research Fund.

1. Emerson: Deutsch. Arch. f. klin. Med., 1901-02, lxxii, 415.

2. In this paper the phrase "glycyltryptophan test" is invariably employed to indicate the Neubauer and Fischer process, which includes the use of glycyltryptophan. The term "tryptophan test" will be used to designate the author's method, which does not involve the use of glycyltryptophan. See Weinstein, J. W.: THE JOURNAL A. M. A., Sept. 24, 1910, p. 1085.

3. Neubauer and Fischer: Deutsch. Arch. f. klin. Med., 1909, xcvi, 490.

2. Am. Jour. Pub. Hyg., February, 1910, xx, No. 1 (New Series vi, No. 1), p. 50.

this same principle, but was applied specifically to the detection of an enzyme having the power of digesting a simple peptid. The substrate selected for this test was glycyltryptophan, a synthetic dipeptid containing the molecular radicals of glycine and tryptophan. Glycyltryptophan is readily hydrolyzed into these two amino-acids. Neubauer and Fischer selected glycyltryptophan as the peptid substrate for their test because tryptophan, which can readily be produced from it by enzymolysis, is the amino-acid that may be detected in the easiest and most direct manner.

In the test, as recommended by Neubauer and Fischer, glycyltryptophan is added to a sample of the stomach contents in question and the mixture is incubated for twenty-four hours. The presence or absence of peptidolytic enzyme is then ascertained by determining with the aid of bromin water, whether or not tryptophan has been produced. A positive result which, according to Neubauer and Fischer, could not occur through the action of anything in normal gastric juice or contents, indicates the presence of a peptidolytic enzyme and implies the occurrence of carcinoma of the stomach. whereas, according to the same authors, a negative result indicates the non-occurrence of carcinoma. Neubauer and Fischer supported their conclusions as to the significance of this test with a series of convincing clinical observations. Naturally, this new test for cancer of the stomach promptly received a great deal of attention and interest.

THE TRYPTOPHAN TEST

I recently published² the results of some experiments which showed that the use of glycyltryptophan is superfluous for the detection of tryptophan-producing enzyme in gastric contents. A gastric cancer secretes enzyme which is capable of hydrating proteins into tryptophan and other amino-acids. Gastric contents always contain proteoses and peptones that are readily convertible by cancer enzyme into their corresponding simpler peptids and amino-acids, including tryptophan.⁴ Consequently, it is unnecessary to add either a specific protein, or a simple peptid, or glycyltryptophan, as a tryptophan-yielding substrate for the easy detection of cancer protease in stomach contents.⁴ It was shown by me that plain stomach contents served more satisfactorily for this purpose than stomach contents mixed with glycyltryptophan.

Previous observers, among them Erdman and Winternitz,⁵ Glaesner,⁶ and Volhard,⁷ employed stomach contents directly for a similar purpose, but as incubation was not a part of the process in any of their methods, their observations as well as their findings, have little practical bearing on the method which was recommended in my original paper.

4. It is not very likely that stomach contents ever contain mixtures of proteins which would wholly fail to yield tryptophan among the products of complete proteolysis. Gastric mucus yields tryptophan under such conditions. Casein, egg albumin, gliadin and practically all other ordinary food proteins yield tryptophan in comparatively large proportions. It is probable that the proportion of tryptophan which is produced from the proteins in stomach contents, under favorable conditions for the test, is sufficient for detection in all cases except those characterized by practical absence of peptidolytic enzyme. But in such instances glycyltryptophan would also fail to yield detectable proportions of tryptophan. Whether the tryptophan that arises from proteins in stomach contents (in a quantity through the continued action of the enzyme), to yield a negative result with bromin water cannot be decided at present. Thus far, however, all observations indicate that no uncertainty is introduced into the test in this way.

5. Erdman and Winternitz: München. med. Wehnschr., 1903, 1, 182.

6. Glaesner: Berl. klin. Wehnschr., 1903, xl, 599.

7. Volhard: München. med. Wehnschr., 1903, 1, 2129.

WARFIELD'S STUDY OF THE ACTION OF SALIVA ON GLYCYLTRYPTOPHAN

There has been wide difference of opinion regarding the value of the glycyltryptophan test for gastric carcinoma. A few have commended it highly, but a large majority have pronounced it valueless. The main objection which has been raised against the test is that glycyltryptophan yields tryptophan in a large number of cases in which no cancer exists, as shown at autopsy and by operation. It has been asserted that most achylia gastrica cases, whether due to carcinoma or not, give a positive reaction with glycyltryptophan.

Warfield⁸ has lately made observations which help us to understand the current differences of opinion regarding the value of the glycyltryptophan test.⁹ Warfield has found that the saliva of some people, when not acid (to litmus) to begin with, is capable of decomposing glycyltryptophan into glycine and tryptophan. He concluded that *swallowed alkaline saliva*, when mixed with neutral or faintly acid gastric liquid, imparts to the latter the power of producing tryptophan from glycyltryptophan, thus rendering "the glycyltryptophan test of no value in the diagnosis of cancer of the stomach." Warfield has shown that the peptidolytic enzyme in saliva is destroyed by heat.¹⁰

THE AUTHOR'S CONFIRMATION OF WARFIELD'S RESULTS

I have verified Warfield's findings. Every specimen of saliva, except one, in a total of eight was found to exert marked peptidolytic power on glycyltryptophan within twenty-four hours. The experiments in this connection were performed with glycyltryptophan and saliva directly, as a simple mixture, *and also after their mixture with stomach contents, which had either been neutralized or which contained a slight proportion of acid*. Neutralization in these cases was effected with one of the following alkalis: calcined magnesia, sodium bicarbonate and sodium hydroxid (1 per cent. solution).

No attempt was made to ascertain the limit of acidity within which saliva is able to induce the production of tryptophan from glycyltryptophan. Warfield states that acidity greater than that of a 0.05 per cent. hydrochloric acid solution prevents such action.

THE SPECIAL SIGNIFICANCE OF WARFIELD'S FINDINGS

The important facts which have been disclosed by Warfield explain in part the past difficulties with the glycyltryptophan test. That they render its positive responses particularly unreliable in cancer diagnosis is obvious, for such results may be due to swallowed saliva. On the other hand, that Warfield's findings do not disqualify my tryptophan test for cancer of the stomach is equally clear, since the salivary protease does not produce tryptophan from the proteins in non-cancerous gastric contents.

All investigators of the subject agree on the proposition that cancer secretes enzyme which is capable of converting protein into amino-acids. The tryptophan test has all the advantages and none of the disadvantages of the glycyltryptophan test. Warfield's peptidolytic salivary enzyme is devoid of effect on the tryptophan test. I have repeatedly added saliva to normal stomach

8. Warfield: Bull. Johns Hopkins Hosp., 1911, xxii, 150.

9. In referring to "the stages in the complete digestion of congluable proteins" Warfield states that metaproteins are "commonly called albumoses." It is evident that in this case *albumoses* is a misprint for "albuminates."

10. Dr. Gies, although suggesting the probability of creptile and tryptic excretion by the salivary glands, thinks it possible also that the tryptophan-producing enzyme in mixed saliva is derived in part from bacteria in the mouth, especially from cavities in carious teeth. He has planned to investigate these possibilities.

contents of different degrees of acidity, but have never been able to detect tryptophan, even after prolonged incubation of the mixtures. In one special instance a specimen of stomach contents that contained no free hydrochloric acid, and which had been carefully neutralized with sodium hydroxid, (1 per cent. solution), was mixed with saliva and kept in a thermostat for fourteen days. Not a trace of tryptophan could be detected in the mixture at any time during the period. A few protocols, in this and other connections, are appended:

Mrs. S. N., suffering from chronic gastritis, has been coming to my service at the Vanderbilt Clinic for the past nine years to get hydrochloric acid, which does her good. Examination of stomach contents shows: Free hydrochloric acid, none; combined acid, none; total acidity, 4; reaction to litmus, faintly acid.

GLYCYLTRYPTOPHAN TESTS (I-III)

- I. Stomach contents, 20 c.c.
Saliva, 10 c.c.
Glycyltryptophan, 1 c.c.¹¹
= Marked tryptophan reaction in twenty-four hours.
- II. Stomach contents, 20 c.c.
Saliva (previously boiled), 10 c.c.
Glycyltryptophan, 1 c.c.
= Marked tryptophan reaction within twenty-four hours.¹²
- III. Stomach contents, 20 c.c.
Water, 10 c.c.
Glycyltryptophan, 1 c.c.
= Tryptophan reaction; not so marked, however, as before, in twenty-four hours.¹²

TRYPTOPHAN TEST (IV)

- IV. Stomach contents (neutralized with NaOH), 28 c.c.
Saliva, 20 c.c.
Glycyltryptophan, None.
Toluol, a few drops.
= No tryptophan reaction.¹³

The effect of saliva on Witte peptone has been tried in experiments of a similar nature—such as are outlined below:

TRYPTOPHAN TESTS (A-B)

- A. Witte peptone, 2 gm.
Saliva (Jacob's), 10 c.c.
Water, 20 c.c.
Incubation, 7 days (with toluol).
= No tryptophan reaction.
- B. Witte peptone, 2 gm.
Saliva (Kraus's), 10 c.c.
Water, 20 c.c.
Incubation, 7 days (with toluol).
= No tryptophan reaction.

THE AUTHOR'S FURTHER EXPERIENCE WITH THE TRYPTOPHAN TEST

I have been employing the tryptophan test for over a year and have found it a valuable sign in the diagnosis of cancer of the stomach. The main objection to the Neubauer and Fischer test is the frequent occurrence of positive responses in non-carcinomatous contents. This is not the case with the tryptophan test. On the contrary, in the case of the tryptophan test, negative responses have occurred in some specimens of stomach

contents from true cancerous cases. By means of the tryptophan test I was able recently to distinguish all the malignant from the benign states in a series of pyloric obstruction cases prior to operation. Everybody knows the unusual difficulties that beset the endeavor to make a differential diagnosis of malignant from benign obstruction of the pylorus, since all the clinical features are the same in both conditions.

I am confident that the tryptophan test outranks every other available laboratory means for the diagnosis of cancer of the stomach. Further investigation is necessary, however, before it can be said that one or two positive tryptophan findings in stomach contents absolutely prove the existence of carcinoma. This degree of uncertainty is much less, however, than that connected with any other "sign of cancer." Among the "other signs" are absence of free hydrochloric acid from stomach contents, presence of lactic acid in stomach contents, occurrence of occult blood in stomach contents or feces, stasis of food, and the results of such newer methods as the Solomon, meiotagmin and hemolytic tests. Free hydrochloric acid is absent from stomach contents not only in gastric cancer, but also in chronic gastritis, pernicious anemia, exophthalmic goiter and achylia gastrica. On the other hand cancer of the stomach, chiefly of the pyloric region, may be accompanied by normal and even hyperacid states of the contents. Lactic acid, an important sign, frequently fails to occur in the contents of the stomach in cancerous cases, and it is occasionally present in undoubted cases of chronic gastritis. In general, lactic acid is prone to occur when free hydrochloric acid is absent and especially, also, when the Boas-motor powers of the stomach are impaired. Boas-Oppler bacilli are an excellent diagnostic feature of carcinoma ventriculi, but only when present in large numbers. Unfortunately they appear only when the invasion of the stomach by the carcinoma is extensive. They are never present unless free hydrochloric acid is absent and, in general, are not of very common occurrence. Occult blood in stomach contents and in feces occurs in ulcer of the gastro-intestinal tract, cirrhosis of the liver, and also in cardiac and renal cases that are attended by congestion of the viscera. The Solomon, meiotagmin and hemolytic tests are positive in conditions other than carcinoma.

I have made hundreds of applications of the tryptophan test to stomach contents. I have never obtained a positive response in any undoubted non-cancerous case, except such as resulted from gastro-enterostomy, in which the resultant artificial conditions favored the flow of intestinal contents into the stomach and thus caused the hydrolysis of the proteins into amino-acids by intestinal protease.

Whether the occurrence of tryptophan in the gastric contents affords an early sign of cancer, I cannot say. Thus far I have not diagnosed a case in its early stages by means of the tryptophan test. I have had several cases, however, in which the first sign of cancer of the stomach was a positive response in the tryptophan test—all other signs failing to give a clear indication. I have lately had an elderly patient (Mrs. O. R.), who suffered from digestive disturbances accompanied by low gastric acidity. Her nutrition and general condition were such as to preclude the diagnosis of carcinoma, and I thought I was dealing with a case of chronic gastritis. The tryptophan reaction, however, was repeatedly positive. Four to five weeks later a tumor appeared in the region of the stomach, which grew very rapidly. The case was then diagnosed by every one who saw the

11. This quantity of glycyltryptophan solution is the volume provided by Kalle & Co. (Biebrich am Rhein) for a single test in accord with the directions of Neubauer and Fischer. The volume is usually a little greater, though it is seldom more than 1.5 c.c. The exact concentration of the solution is unknown to me.

12. This positive result was due undoubtedly to the enzyme which had been introduced into the gastric contents in the saliva swallowed by the patient.

13. This mixture was kept in a thermostat for seven days, but no trace of tryptophan could be detected at any time during that period.

patient as an undoubted case of cancer of the stomach. Operation was proposed, but the patient disappeared from observation.

Another experience of mine related to an elderly man (N. F.) who presented himself to my service at the Vanderbilt Clinic with stomach trouble. I obtained several positive tryptophan tests and on the strength of these only, as the other features did not clearly indicate malignancy, I advised an operation. This was refused by the patient, who disappeared from observation. Three months later I traced him to Mount Sinai Hospital. There an exploratory laparotomy was performed and a lymphosarcoma of the middle of the stomach was found and resected.

ANOMALOUS EXPERIENCES WITH THE TRYPTOPHAN TEST

In numerous applications of the tryptophan test I have had anomalous experiences with several cases. The stomach contents of one patient (Case 16 of my original paper), a man of 54 with a suspicious history of carcinoma, gave repeated tryptophan tests in the presence of free hydrochloric acid. The patient felt well afterwards for a couple of months, when I lost track of him for a time. Lately, however, I have had the opportunity of again examining him. He is now in good health and apparently free from cancer of the stomach.

Another anomalous case was that of a middle-aged woman, who had a typical pyloric obstruction, with marked peristaltic movements and gastrectasia. The tryptophan test was repeatedly positive. The patient refused an operation. Four months later she seemed to be somewhat improved, but still had all the stigmata of pyloric obstruction. At that time two tryptophan tests applied to different specimens of gastric contents were negative. This patient is now in the hands of a colleague who thinks that her pyloric obstruction is a benign one, because it shows no signs of further increase. We should not forget, however, that a scirrhus carcinoma is of slow growth and that, with a very small open passage through the pylorus, and lavage, the patient may hold her own for a long time.

Another case of interest in this connection was that of Mrs. L. S., aged 64, who presented a typical history of cancer of the stomach, such as sudden onset of illness of nine months' duration, loss of appetite, considerable loss of flesh and strength, vomiting, and vomiting of coffee grounds. Occult blood was found repeatedly in the stomach contents and feces. An exploratory laparotomy was performed by Dr. A. A. Berg, who failed to find any abdominal lesion. In this case repeated tryptophan tests were negative. Once, however, it was strongly positive, but in that instance the contents had been rendered very faintly alkaline with magnesium oxid, and the resultant proteolysis was undoubtedly due to intestinal protease. It was intended, in this procedure, to enhance, if possible, the action of the cancer enzyme through alkalization, an expedient which, as was found, may possibly lead to error.

Dr. Berg performed a pylorotomy on another patient, Mrs. S., whose history and findings pointed strongly to the diagnosis of cancer of the stomach. Several tryptophan tests were positive. The pathologic report was "inflammatory," and not malignant.

DISADVANTAGES OF THE TRYPTOPHAN TEST

The tryptophan test is inconstant. Tryptophan may appear in the contents from one meal and fail to appear in the contents from the next. This implies in-

stancy in the secretion of the carcinoma enzyme, but it does not indicate variability in the reactive tendencies of the reagent in the test. I saw in consultation about three months ago an old lady who had all the stigmata of cancer of the stomach (no palpable tumor, however), and I failed to get a positive tryptophan test in a specimen of stomach contents. Only one test was tried in this case. As a rule, however, several tests of different specimens of gastric contents yield a positive reaction in each case of cancer.

SOURCES OF ERROR IN APPLYING THE TRYPTOPHAN TEST

There appears to be one important source of error in the tryptophan test, and that is regurgitated intestinal fluid. Boas¹⁴ was the first to obtain duodenal contents from the stomach. He massaged the duodenum toward the stomach and obtained a variable quantity of bile-stained fluid through a stomach tube. Later Boldyreff,¹⁵ at the instigation of Pawlow, introduced olive oil into the stomach through a tube and subsequently extracted the contents. Olive oil has the double effect of checking gastric secretion and stimulating pancreatic secretion. It also acts as a lubricant, and back flow of intestinal contents is facilitated. By such means, duodenal contents may be secured. This line of work was taken up by a good many investigators, such as Faubel,¹⁶ Volhard,¹⁷ Molnar,¹⁸ Ohrl and Schittenhelm¹⁹ and their experiences coincide in the fact that some duodenal secretion may be secured in this manner. The observations of Ohrl and Schittenhelm indicate that under ordinary conditions spontaneous regurgitation of intestinal contents into the stomach takes place. In considering, however, the degree to which the tryptophan test may be vitiated by erepsin or trypsin, or both, we must not forget that these enzymes are destroyed by the acid of gastric contents. Moreover, I have repeatedly kept non-cancerous stomach contents, devoid of free hydrochloric acid, in a thermostat at 40 C. for days and even weeks, without obtaining a positive response in the tryptophan test. It is obviously necessary, however, in every serious application of the tryptophan test, to prevent all possible regurgitation of duodenal contents. To prevent this occurrence the stomach contents should be withdrawn gently, and also without any straining on the part of the patient. For this purpose the aspiration method instead of the expression process should be employed. A tube with thin walls and a bore not more than 10 mm. in diameter (30 French) should be selected for the purpose. The patient should be instructed to breathe continuously during the extraction. The manipulation should be conducted by one who is skilled in the work and not by an untrained assistant. By disregarding these rules, by letting the patient press forcibly on the tube until he gets blue in the face, some intestinal regurgitation will probably result and may vitiate the test. Vomitus is not reliable for obvious reasons. If, in spite of all these precautions, one obtains a specimen of stomach contents with a greenish color, and which gives the chemical tests for bile, the specimen should be discarded and another one secured.

Various authors have stated from time to time that occult blood vitiates the glycol-tryptophan test. They

14. Boas: *Zentralbl. f. klin. Med.*, 1889, x, 97.

15. Boldyreff: *Zentralbl. f. Physiol.*, 1904, xviii, 457; also, *Arch. f. d. ges. Physiol.*, 1908, cxxi, 13.

16. Faubel: *Hofmeister's Beitr. z. Chem. Phys. u. Path.*, 1907, x, 35.

17. Volhard: *München. med. Wchnschr.*, 1907, liv, 403.

18. Molnar: *Ztschr. f. klin. Med.*, 1909, lxxvii, 188.

19. Ohrl and Schittenhelm: *Zentralbl. f. ges. Phys. u. Path. d. Stoffwechs.*, 1910, v, 881.

might also presume that it vitiates the tryptophan test. In my original paper I discussed these improbabilities. I have not ignored the fact that blood contains proteases. I am certain, however, that blood, even when present (in the tryptophan test) in a quantity sufficient to impart a distinct red tinge to the contents, is not able to produce tryptophan in detectable proportion. I have repeatedly studied this matter, from various points of view, and have always obtained negative tests for tryptophan in contents to which moderate amounts of blood had been added—in one case even after incubation of the mixture for seven days. I have also obtained negative tryptophan results in such mixtures of glycyl-tryptophan and blood, even after incubation for the same length of time. Of course, it is likely that the addition of large quantities of blood would effect hydrolysis of associated protein, but in such cases the tryptophan test could not be carried out, for it would be impossible to discern the characteristic red color resulting from the addition of the bromin.

The presence of *free* hydrochloric acid does not necessarily interfere with the test. I have found tryptophan in several instances of carcinoma with high gastric acidities.

TECHNIC OF THE TRYPTOPHAN TEST

The Test Meal.—The ordinary Ewald test breakfast is not suited to the test. This has been shown repeatedly. A simple and effective test meal is a glass of water, hot or cold and very sweet, with some white bread, or toast or biscuits. Milk may also be added. The contents are extracted in about one hour. A regular dinner with extraction after two to four hours, also serves the purpose. Any substance that is likely to impart color to the contents, such as strong tea, coffee, strawberries, tomatoes, etc., should be excluded from the test meal. After their extraction a portion of the contents should be *filtered* and tested directly for tryptophan. If the test is positive, no further treatment is necessary. If the direct test is negative, however, the contents, filtered or unfiltered and without the addition of toluol or other preservative, should be kept in a thermostat for from twenty-four to forty-eight hours, and then tested again for tryptophan. The addition of a preservative appears to be superfluous, for I have shown repeatedly that the bacteria which develop do not produce tryptophan in a detectable proportion.²⁰ The tryptophan test may often be obtained in positive cases without incubation, by merely keeping the contents at room temperature for from twenty-four to forty-eight hours. Incubation, however, is preferable.

The Test for Tryptophan with Bromin Water.—In completing the tryptophan test, *filtered* stomach contents should be employed. A volume equal to 6 or 7 c.c. is satisfactory for the purpose. This volume in a test-tube is treated with a few drops of a 3 per cent. acetic acid solution and then saturated aqueous solution of bromin is added, drop by drop, from a pipette. The appearance of a reddish-violet or rose-red color shows the presence of tryptophan. If, after the addition of about 4 drops of bromin water, the expected color does not appear, the mixture should be allowed to stand for about fifteen or twenty minutes, when the characteristic color may develop. If, by the end of that time, the rose color fails to show, then more bromin water should be slowly added, drop by drop, until the mixture becomes

yellow or until a rose-red color is imparted. If a reddish color is produced, the mixture should be allowed to stand again, when the tinge may grow deeper. Considerable practice is required for the accurate performance of the test in the presence of very minute proportions of tryptophan, because a slight excess of bromin may make the characteristic color indistinguishable. Excess of bromin imparts a lemon-yellow color to the mixture. Whenever the reddish color merges into a yellow we know positively that there is an excess of bromin in the mixture. The opening at the tip of the pipette from which the bromin solution is dropped should be a very small one. The bromin water should invariably be added drop by drop, and the mixture should be well shaken after each addition.

Bromin itself should be handled with great care, for it is extremely irritating to the respiratory mucous membrane. Under no circumstance should plain liquid bromin be mixed with the gastric contents to be tested. An aqueous bromin solution may be kept saturated by retaining in it a slight excess of the heavy liquid bromin.

Acetic acid is added to the mixture to be tested, because the reaction appears at its best in an acid medium. Alkali, by combining with the bromin, prevents the reaction with tryptophan. Almost all stomach contents are acid and in testing them for tryptophan the addition of acetic acid may not be necessary. In mixtures of very low acidity, however, the addition of acetic acid is especially desirable.

I wish, in conclusion, to express my indebtedness to Prof. William J. Gies for the use of his laboratories and suggestions in my work.²¹

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THE TREATMENT OF HEMOPTYSIS IN PULMONARY TUBERCULOSIS *

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Two months ago I read a paper on the treatment of hemoptysis in tuberculosis before the meeting of the Medical Society of the State of California, at Santa Barbara. When I returned from the meeting, I was notified on my arrival in San Francisco that a dozen of my patients were having hemorrhages. The peculiar feature of this fact was that they all started to bleed at about the same time, which can be explained only by changes in atmospheric pressure. A number of these patients bled for only one or two days, and showed no alarming symptoms, but two of them continued to bleed severely, and in spite of all the usually employed means and manipulations the hemorrhages did not stop. I shall give in detail the history of one of these patients, because this one case will be sufficient to explain the soundness of the method I have used for the

21. The following, together with references numbered 2, 3, 8 and 19, constitute a complete bibliography of the glycyltryptophan and tryptophan tests to the time of writing this paper.
Abderhalden: *Ztschr. f. physiol. Chem.*, 1909, lxi, 130.
Kohlenberger: *Deutsch. Arch. f. klin. Med.*, 1910, xcix, 148.
Lyle and Kober: *New York Med. Jour.*, 1910, xci, 1151.
Kuttner and Pulvermacher: *Berl. klin. Wechnschr.*, 1910, xiv, 2057.

Ley: *Berl. klin. Wechnschr.*, 1911, xlviii, 119.
Oppenheim: *Deutsch. Arch. f. klin. Med.*, 1910-11, ci, 293.
Pechstein: *Berl. klin. Wechnschr.*, 1911, xlviii, 375.
Hall and Williamson: *Lancet*, London, 1911, cxxx, 731.
Neubauer and Fischer: *München. med. Wechnschr.*, 1911, lviii, 674.
Ehrenberg: *Berl. klin. Wechnschr.*, 1911, xlviii, 704.
* Read in the Section on Pharmacology and Therapeutics of the American Medical Association, at the Sixty-Second Annual Session, held at Los Angeles, June, 1911.

20. I cannot say that this would always be the case, for some bacteria and fungi that I have not thus far encountered may be able to produce detectable proportions of tryptophan under the conditions of the test.