THE DIAGNOSTIC WORTH OF THE GLYCYLTRYPTOPHAN AND THE TRYPTOPHAN TESTS IN DISEASES OF THE STOMACH *

A REPORT OF 1,175 CASES STUDIED BY A UNIFORM METHOD

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The surgeon and the pathologist have shown that when cancer of the stomach is diagnosed early that affection is as amenable to treatment as is cancer in other parts of the gastro-intestinal tract. At present, it would seem that early diagnosis rests largely on microscopic examination of sections of extirpated tissue. Inasmuch as prognosis is directly dependent on the process at diagnosis, it would appear desirable to elaborate certain diagnostic procedures that might anticipate laparotomy findings.

Recently the physiologic chemist has undertaken the investigation of biologic problems bearing on clinical medicine. Various workers, notably Müller,[1] Fischer,[2] and Abderhalden[3] have reported that malignant neoplasmata contain certain peptidolytic enzymes. This discovery appeared to have clinical value when Neubauer and Fischer[4] announced that simple peptids, particularly the dipeptid, glycyltryptophan, were hydrolyzed by cancerous ferments. In the case of glycyltryptophan, the amino-acid, tryptophan, which is liberated by this cleavage, can be recognized readily in acid solution by the rose-pink color occurring on the addition of bromin. This reaction forms the basis of the "glycyltryptophan test" for cancer of the stomach, advanced by Neubauer and Fischer.

Clinicians generally have disagreed widely on the actual value of the test. The reaction's sponsors, together with Lyle and Kober[5] and Weinstein[6] early reported enthusiastically on the procedure. Later

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1. Müller: Ztschr. f. klin. Med., 1889, xvi, 496.
2. Fischer: Deutsch. Arch. f. klin. Med., 1902, lxxii, 415.
3. Abderhalden: Ztschr. f. physiol. Chem., 1909, lxii, 136.
4. Neubauer and Fischer: Deutsch. Arch. f. klin. Med., 1909, xciii, 499.
5. Lyle and Kober: New York Med. Jour., 1910, xci, 1151.
6. Weinstein: Jour. Am. Med. Assn., 1910, lv, 1085.

observers, especially Warfield,[7] Oppenheim,[8] Kohlenberger[9] and, most recently, Sanford and Rosenbloom,[10] declare that the test is of dubious value. They admit that while certain cases of cancer of the stomach undoubtedly give the reaction, many non-malignant gastric disturbances give similar tests. Factors claimed to influence the reliability of the reaction are swallowed saliva, bacteria, bile or blood in the gastric extracts, low or absent free hydrochloric acid and regurgitated duodenal contents.

In October, 1911, Weinstein[11] announced that he had improved on the Neubauer and Fischer test. He stated that in extracts from cases of carcinoma ventriculi there exist free amino-acids, notably tryptophan, and that the latter can be tested for directly with bromin. This procedure appeared to render unnecessary the addition of glycyltryptophan to such gastric contents, with search for its cleavage products subsequently. This so-called "tryptophan test" was claimed as a reaction pathognomonic of cancer of the stomach. Weinstein did not, however, go so far as to state just how early in the progress of the disease this test could be regarded as pathognomonic. Certainly, in the clinical cases which he briefly quoted, when the tryptophan test was positive, other evidences of cancer were not lacking. Recently Hall and Williamson[12] and Sanford and Rosenbloom[10] have recorded observations which appear to indicate that Weinstein's test has even less value than, in their experience, had the glycyltryptophan test.

The great difference of opinion regarding the practical worth of the two tests mentioned led us to make the observations herewith submitted.

AUTHOR'S STUDY

From October 1, 1911 to May 15, 1912, the Ewald test breakfast was administered to 1,626 different individuals at St. Mary's Hospital (Mayo Clinic). On the gastric extracts from more than 1,400 of these patients, glycyltryptophan and tryptophan tests were made. On 1,175 different individuals, the gastric extracts were tested according to the modification of the glycyltryptophan and the tryptophan tests recently

7. Warfield: Bull. Johns Hopkins Hosp., May, 1911, 150.
8. Oppenheim: Deutsch. Arch. f. klin. Med., 1910-11, ci, 293.
9. Kohlenberger: Deutsch. Arch. f. klin. Med., 1910, xcix, 148.
10. Sanford and Rosenbloom: THE ARCH. INT. MED., 1912, ix, 445.
11. Weinstein: Jour. Am. Med. Assn., 1911, lvii, 1420.
12. Hall and Williamson: Lancet, London, 1911, clxxxi, 731.

suggested by me.[13] This modification appears to have the advantages of requiring less of the test ingredients than the Neubauer and Fischer method, of being a controlled procedure, and one in which the end-reaction may be easily determined. It is the purpose of this communication to report our experience with the cases tested by this uniform method.

Certain precautions taken in the manipulation of the reaction might be mentioned briefly. All glassware was boiled in distilled water and dried before using. The solution of glycyltryptophan employed was obtained, in bulk and unopened, direct from the makers. To guard against its tendency to crystallize out, in cold solution, the preparation was kept in a water-bath at 37 C. until used. All gastric extracts were carefully filtered before testing, and the tests were set up within two hours, at the outside, from the time the contents were taken from the

13. The test is set up as follows:

1. Test-tubes of 10 c.c. capacity are employed. These should be carefully cleaned with boiling water and dried inside. They are numerically marked for identification with a wax pencil. Into each test-tube is carefully measured, by means of a sterile graduated pipet, 0.5 c.c. of the glycyltryptophan solution. Five c.c. of the recently secured filtered gastric extract are then measured by a clean, graduated pipet and poured into the correspondingly numbered test-tubes to which glycyltrytophan solution has been already added. Two control tubes are used. In one is placed 0.5 c.c. of glycyltryptophan solution and 5 c.c. of normal salt solution, and into the other is placed 5 c.c. of normal salt solution, without added glycyltryptophan solution. In the entire series, each tube next receives 0.5 c.c. of toluol (Toluene, Merck). The contents of the tubes are then mixed by inverting several times. The tubes are next placed in a water-bath (an incubator may be used) at 37 C. for twenty-four hours.

2. At the expiration of the incubation period, the test-tubes are removed from the water-bath. Clean test-tubes of 10 c.c. capacity and numbered to correspond with the gastric extracts tested, as well as the controls, are set in racks. Into each of these tubes is measured by means of a graduated pipet, 2 c.c. of the glycyltryptophan-gastric-extract mixture lying below the toluol in the recently incubated tubes. To each tube are then added three drops of a 3 per cent. glacial acetic acid in distilled water solution. The tubes are well shaken. Bromin vapor is allowed to flow into each tube until it appears amber yellow above the contained fluid. The tubes are again shaken. Examination by daylight (preferred) or by white, artificial light is now made for evidences of the characteristic rose-pink reaction between the amino-acid (tryptophan) and the bromin.

Tryptophan Test: As suggested by Weinstein, this is made, as routine, on the fresh gastric extracts, inasmuch as, occasionally, swallowed saliva, amino-acids, regurgitated duodenal contents and the like may give the bromin vapor reaction, before incubation or without the addition of a dipeptid such as glycyltryptophan. Five c.c. of each fresh, filtered gastric extract are poured into test-tubes of 10 c.c. capacity, acidulated with the 3 per cent. acetic acid solution and treated with bromin vapor as above. If no characteristic rose-pink color results, the tubes are incubated with the corresponding specimens that have been mixed with glycyltryptophan solution. For accurate work, it has seemed best to us to cover these "tryptophan test" contents with a layer of toluol. At the end of twelve, twenty-four and forty-eight hours, note is made of changes in color, and these results are compared with those obtained with the preparations in the first series. *Jour. Am. Med. Assn.*, 1912, lvii, 1008.

patients. In testing for tryptophan, before or after incubation, bromin vapor was preferred over bromin water. It is more readily controlled quantitatively and permits of better color determination. All end reactions were read by daylight.

TYPICAL REACTIONS

When bromin vapor is used for the detection of amino-acid (tryptophan), its presence is indicated, even in small amounts, by lilac-violet to rose-pink shades. The color is usually a lively one, and appears quickly. Admixtures of much blood and bile produce, respectively, dirty, brownish-yellow and muddy-green to drab. In such, gradations in shade are impossible. High organic acidity often gives rich purple or magenta hues. When the color change is opalescent, with bluish or delicate lilac cast, the results may be classed safely as negative.

RESULTS

The gross results of our observations are as follows: Of 1,175 gastric extracts from individuals with gastric symptoms, clinically, 110, or 9.36 per cent., were glycyltryptophan positive. In the same cases, 24, or 2.04 per cent., were tryptophan positive, either before or after incubation. Tables 1 and 2 show, respectively, the number of positives with each test, associated with different diseases. Tables 3 and 4 respectively consider the clinical and laboratory data.

TABLE 1.—SUMMARY OF CASES GIVING POSITIVE GLYCYLTRYPTOPHAN TEST

Diagnosis	No. of Cases	Diagnosis	No. of Cases
Carcinoma ventriculi	31	Achylia gastrica	6
Ulcus ventriculi	9	Appendicitis	8
Carcinoma of the liver	3	Primary anemia	3
Ulcer of duodenum	3	Syphilis—stomach	1
Non-malignant pyloric obstruction	1	Various (gastritis, gastric neurosis, chronic diarrhea, epilepsy)	10
Cholecystitis	11		
Gall-stones	6		
Hypochlorhydria	7	Total	110
Achlorhydria	11		

TABLE 2.—SUMMARY OF CASES GIVING POSITIVE TRYPTOPHAN TEST

Diagnosis	No. of Cases	Diagnosis	No. of Cases
Carcinoma ventriculi	7	Appendix lesions	1
Ulcus ventriculi	3	Various (neuroses, achlorhydria, arteriosclerosis)	6
Ulcer duodenum	3		
Carcinoma of the liver	1		
Gall-stones	3	Total	24

It will be noted that one of the valuable features of the tables is the fact that the majority of the cases exhibiting positive reactions were treated surgically; hence, the conclusions derived from consideration of the figures returned have a fairly definite pathologic basis.

TABLE 3.—CLINICAL AND LABORATORY DATA OF THE CASES RETURNING POSITIVE GLYCYLTRYPTOPHAN TEST

Number and Name	Diagnosis	Total Acidity	Free HCl	Blood	Bile	Lactic Acid	Degree of Reaction*
61563—Leslie	Gall bladder infect.	46	36	+	0	0	+
9532—Graham	Anemia (post. mort.)	0	0	+	0	0	+
61743—Belanger	Duodenal ulcer—opr.	86	80	0	++	0	+
61000—Smith	Gall bladder	46	40	0	0	0	+
61795—Hillis	Gastritis chr.	14	6	+	0	0	+
61857—Kise	Carcinoma stom.—opr.	0	0	++	0	0	++
61802—Fuller	Gastritis; chr. append.	4	0	+	+	?	+
38406—Pew	Gall bladder infect.	6	0	0	0	0	++
53228—Reede	Gastric neurosis	18	18	+	0	0	++
61852—Koss	Carcinoma stomach	4	0	+	0	0	++
61910—Graybill	Carcinoma stomach—opr.	0	0	++	0	++	+
61812—Letoman	Epilepsy	10	10	+	+	0	++
61940—Beck	Gastritis—chr.	24	24	0	0	0	+
61862—Huseby	Gastric neurosis	40	30	0	+++	0	+
61974—Longtime	Gastritis—alcoholic	0	0	+	+	+	+++
62100—Schaffer	Carcinoma stomach—opr.	20	0	+	0	+	+
62171—Plan	Carcinoma stom. opr.	48	0	+	0	++	+
62086—Haley	Gastritis—chr.	10	0	0	++	0	+
62089—Moldenhauer	Gastric ulcer	40	40	0	+	0	+
62219—Biehl	Carcinoma stom.—opr.	24	12	+	0	0	+
62154—Ahlborn	Carcinoma stom. opr.	8	0	0	+	0	++
62233—Flick	Carcinoma liver—expl.	24	24	+	+	0	++
62260—Chapman	Pyloric obstr. non-malignant.	40	32	++	+	0	+
62399—Baum	Carcinoma stom.—opr.	0	0	+	0	0	+
53032—Kopplow	Duodenal ulcer—opr.	50	50	Tr.	+	0	+
62562—Dunn	Carcino. Gall Bl. opr.	6	0	+	+	0	+
62665—Warner	Gastric ulcer	28	28	Tr.	+	0	++
37124—Nelson	Carcin. stom. recur.	8	0	0	+	0	+
61072—Zielsdorf	Gall stones—opr.	56	40	0	Tr.	0	+
62876—Johnson	Cholecystitis	10	0	Tr.	++	0	+++
62912—Erickson	Chr. Ap. opr.	22	18	Tr.	+	0	+
62971—Maxwell	Chr. Ap. opr.	22	0	0	+	0	+
62977—Hanson	Ulcer stomach	48	40	0	0	0	+
63026—Brooks	Chr. Appendicitis	32	32	+	+	0	+
63051—Marian	Neg. Stom. Ap.	36	32	0	+	0	+
63093—Taylor	Chr. Diarrh. stom. neg.	4	0	0	0	0	+
63129—Erickson	Stom. ulcer and G. B. opr.	32	30	+	0	0	0
63130—Weech	Chr. App. opr.	0	0	0	0	0	+
62699—Wright	Cholangitis	0	0	Tr.	0	0	+
63030—Gladens	Carcinoma stomach	8	0	0	0	0	+
63241—Berend	Carcinoma stomach opr.	56	18	+	0	0	+
63292—Glaesner	Gastric ulcer	46	46	+	+	0	+
63197—Smith	Multiple sclerosis	20	6	0	+	0	+
63335—Given	Duod. ulcer; cholecystitis	48	40	0	+	0	+
52034—Rice	Recurrent Ca. stomach	10	0	+	+	0	+
62876—Johnston	Syph. stom.	0	0	0	0	0	+
63506—Bragen	Gastritis	38	22	0	+	0	+
63547—Hanson	Resect. stom. ca.	0	0	+	0	?	+
63562—Arms	Arteriosclerosis	0	0	0	0	0	+
63600—Lutke	Appendicitis—neg.	44	40	0	+	0	+
63616—Rarity	Appendix and G. B. opr.	12	0	0	+	0	+
63653—Allen	Ulcer stom. opr.	32	28	Tr.	+	0	+
63636—Carlson	Gall stones—opr.	22	0	0	0	0	+
63778—Thompson	Carc. stom.	14	0	+	+	+	+
36634—McEvan	Ulcer stom.	40	30	+	0	0	+

TABLE 3.—CLINICAL AND LABORATORY DATA OF THE CASES RETURNING POSITIVE GLYCYLTRYPTOPHAN TEST (*Continued*)

Number and Name	Diagnosis	Total Acidity	Free HCl	Blood	Bile	Lactic Acid	Degree of Reaction*
63883—Bond	Gall stones—opr.	6	6	0	0	0	+
64057—Atol	Hypochlorhydia	8	0	+	++	0	++
64039—Olson	Achlorhydria	0	0	0	0	0	+
61915—Even	Hypochlorhydria	4	4	0	0	0	+
64270—Gregg	Ulcer stom. opr.	50	46	+	0	0	+
64330—Schuler	Carc. stomach	14	0	+	0	+	+
64360—Berg	Neurosis	54	50	0	+	0	+
64482—Smeath	Carc. stom. resect	8	0	+	0	0	+
64455—Norres	Hypochlorhydria	4	0	+	0	0	+
64281—Seedhug	Achylia gastr.	0	0	+	0	0	+
34078—Johnston	Neuroses	42	40	0	0	0	+
64877—Hutchinson	Cholecystitis	4	0	0	0	0	+
65179—Otter	Appendicitis	36	26	0	Tr.	0	+
65229—Arnold	Ca. stom. and liver	0	0	+	0	0	+
65293—Aklund	Achlorhydria	8	0	0	+	0	+
65337—Verner	Cholecystitis, appendix	16	0	0	Tr.	0	+
37889—Gehrke	Appendicitis opr.	12	12	Tr.	0	0	+
65693—Rule	Gall stones and appendix opr.	38	14	24	+	0	+
22528—Shaffer	Duod. ulc. opr.	8	0	0	0	0	+
65703—Gaskill	Degen. gast. ulc.	8	0	0	0	0	+
65835—Ellwell	Gen. carc. prim. stom.	0	0	0	0	0	+
65901—Hovelsrud	Carc. stom.	6	0	+	0	0	+
5231—Graff	Achlorhydria	8	0	0	+	0	+
65953—Eaton	Achlorhydria	12	0	0	+	0	+
66017—Richie	Carc. stom.	30	0	+	0	0	+
66108—Graham	Gall stones opr.	8	0	0	+	0	+
66225—Bryant	Pernicious anemia	40	0	+	0	0	+
64942—Hester	Second. anemia	10	0	0	0	0	+
66314—Spellman	Cholecystitis	30	30	0	+	0	+
66333—Wickman	Gall stones opr.	10	0	+	0	0	+
66409—Bronson	Achlorhydria	20	0	+	0	0	+
66462—Bram	Gastric ulcer	26	26	+	0	0	+
56586—Carr	Cancer stom. recur.	48	48	0	0	0	+
66466—Stevens	Achlorhydria	48	0	0	0	0	+
63547—Harrison	Cancer stom. resect.	12	0	Tr.	0	0	+
66511—Lane	Achlorhydria	4	0	0	0	0	+
66583—Miller	Cholecystitis	50	50	0	0	0	+
66644—Andrew	Carc. stom.	12	0	0	0	0	+
66787—Leutke	Achlorhydria	20	0	Tr.	0	0	+
66904—Boe	Achlorhydria	34	0	Tr.	0	0	+
66855—Fozenden	Carc. stom. opr.	26	26	+	0	0	+
66864—Hennessy	Hypochlorhydria	12	12	+	0	0	+
67000—Stanley	Deg. gast. ulc.	26	26	+	0	0	+
45833—Thompson	Hypochlorhydria	8	8	+	0	0	+
67110—McKay	Cancer stomach	20	0	+	0	+	+
67112—Rasmussen	Gastric neurosis	38	24	0	0	0	+
67077—Snyder	Carc. liver and spleen	80	80	0	0	0	+
67206—Allen	Carc. stom.	14	0	0	+	0	+
67295—VanHook	Hypochlorhydria	18	8	Tr.	+	0	+
67368—Tucker	Carc. stom.	50	50	+	0	0	+
67690—O'Rourke	Pernicious anemia	4	0	0	+	0	+
67928—Hayes	Ca. stom. P. A. (?)	14	0	+	0	0	+
67562—Wessling	Gall stone opr.	12	0	0	0	0	+
67537—Owmen	Expl. cancer stom.	20	0	0	0	0	+
67644—Haggen	Achlorhydria	4	0	0	0	0	+

*Degree of Reaction: Lilac=+; Rose-pink=++; Rose-purple=+++.

Cancer: The total number of proven cases of cancer of the stomach, primary or secondary, in this series is eighty-seven. Of this number, thirty-one, or 35.6 per cent., gave positive glycyltryptophan tests, while seven, or 8.04 per cent., were tryptophan positive. Of the thirty-one cases of cancer in which the glycyltryptophan test was positive, the tryptophan test was positive but four times. In three cases in which the tryptophan test was positive, the glycyltryptophan test was negative.

TABLE 4.—CLINICAL AND LABORATORY DATA OF THE CASES RETURNING POSITIVE TRYPTOPHAN TEST

Number and Name	Diagnosis	Total Acidity	Free HCl	Blood	Bile	Lactic Acid	Degree of Reaction*
61508—Theen	Stom. neg	36	36	0	+	0	+
61496—Fitzsimmons ..	Duod. ulcer opr.	56	38	0	0	0	+
61567—Crane	Gastric ulcer	42	20	0	+	0	+
61552—Eldred	Carcinoma stom. inop.	38	18	+	0	+	++
62233—Flick	Carcinoma liver and G. B...	24	24	+	+	0	++
62784—Drechsler	Gastric ulcer clin.	12	12	+	0	0	+
62876—Johnson	Achlorhydria and G. B.	10	0	+	++	0	+
62865—Zenk	Appendix chronic	24	18	Tr.	+	0	+
63051—Marion.....	Gastric neurosis	36	32	0	+	0	+
63230—Gladens	Carcinoma stom. (mass) ...	8	0	0	0	0	+
63241—Burend	Gastric carcinoma, resect. ..	58	18	+	0	0	+
63221—Quinn	Gall-stone empyema G. B. ..	20	20	0	Tr.	0	+
63414—Hanson	Duodenal ulcer opr.	80	80	0	+	0	+
63408—Lynch	Carcinoma stom. opr.	66	60	0	0	0	+
63653—Allen	Gastric ulcer clin.	32	28	Tr.	0	0	+
63354—Kissman	Tabes—crises	4	0	+	0	0	+
63563—Arms	Arteriosclerosis Gen.	0	0	0	0	0	+
63536—Carlson	Gall stone opr.	22	0	0	0	0	+
64394—Davis	Duodenal ulcer opr.	66	60	0	0	0	+
64294—Thoet	Carcinoma stom. opr.	14	4	+	0	+	+
65693—Rule	Gall stones and append. opr.	38	14	+	0	0	++
5231—Graf	Gastric ulcer degen. post opr.	8	0	0	+	0	++
56586—Carr	Carcinoma stom. recur.	48	0	0	0	0	+
67112—Rasmussen ...	Gastric neurosis	38	24	0	+	0	++

*Degree of Reaction: Lilac=+; Rose-pink=++; Rose-purple=+++.

Of nine gastric ulcers with fair evidence of carcinomatous degeneration (of the type described by MacCarty[14]), two, or 22.2 per cent., gave the glycyltryptophan reaction. In these same cases there was no positive tryptophan test. If we combine the returns from these cases with those from the specimens of advanced carcinoma, we note that the glycyltryptophan test is positive in 35.4 per cent. and the tryptophan in 7.28 per cent., or the glycyltryptophan test is positive approximately five times as frequently as is the tryptophan test.

14. MacCarty: Surg., Gyn. and Obst., 1910, x, 449.

Gastric Ulcer: In none of thirty-five operated gastric ulcers (microscopically carcinoma-free) was the glycyltryptophan test positive. The tryptophan reaction was obtained once.

Thirty-nine cases were clinically diagnosed as gastric ulcer. Three of these (7.4 per cent.) were glycyltryptophan-positive, and two (5.2 per cent.) were tryptophan-positive.

Duodenal Ulcer: Operations were performed on seventy-eight patients with duodenal ulcers. Of this number, three (2.6 per cent.) gave glycyltryptophan and tryptophan tests. They were not identical cases and the reactions were not always associated with low acidity.

Fifty-seven individuals had duodenal ulcer, clinically. One (1.7 per cent.) was glycyltryptophan-positive. None gave the tryptophan test.

TABLE 5.—THE RELATION OF GLYCYLTRYPTOPHAN TEST TO ACIDITY

Group	No. of Positives	No. of Negatives	Group	No. of Positives	No. of Negatives
Extracts having no acidity	14	20	Extracts having decreased T. A.	88	515
Extracts having no free HCl	52	31	Extracts having normal T. A.	17	213
Extracts having diminished HCl	15	214	Extracts having increased T. A.	5	337
Extracts having normal HCl	22	369	Totals	110	1,065
Extracts having increased HCl	7	431	Extracts having lactic acid	11	33
Totals	110	1,065			

Other Gastric Conditions: It has been advanced by Weinstein, Warfield, and Sanford and Rosenbloom that positive glycyltryptophan reactions are usually obtained in gastric extracts exhibiting achylia or low hydrochloric acid. These reactions are claimed to result from the presence of a peptid-splitting enzyme (Warfield) existing in saliva. Gies[15] thinks that mouth-bacteria may be capable of splitting simple peptids under these conditions. In order to determine the results in our cases from the view point of acidity, we have compiled Tables 5 and 6. It will be seen that about 60 per cent. of the positive glycyltryptophan tests were obtained from extracts showing no free hydrochloric acid, while in an additional 13.6 per cent., the free hydrochloric acid was low. In other words, nearly three-fourths of the positives occurred in gastric extracts showing diminished acidity. Table 5 also brings out the interesting fact that approximately 80 per cent. of the

15. Gies: Quoted by Weinstein, Jour. Am. Med. Assn., 1911, lvii, 1420.

glycyltryptophan reactions were returned by contents in which the total acidity was low.

The support which these figures apparently give to Warfield's saliva ferment-action on peptids is qualified when one considers the negative glycyltryptophan tests in Table 5. Fifty-one of these extracts showed no free hydrochloric acid. In 214 extracts the free hydrochloric content was diminished. The combination of these results demonstrates that about one-fourth (24.8 per cent.) of the negatives was associated with low free hydrochloric acid. It could scarcely be maintained that all these extracts were saliva-free. Table 3 shows that some of the extracts were from cancerous patients. Approximately one-half (48.3 per cent.) of the negative glycyltryptophan tests were on extracts with diminished total acidity.

TABLE 6.—THE RELATION OF TRYPTOPHAN TEST TO ACIDITY

Group	No. of Positives	No. of Negatives	Group	No. of Positives	No. of Negatives
Extracts having no acidity	1	33	Extracts having decreased T. A.	17	586
Extracts having no free HCl	6	77	Extracts having normal T. A.	2	228
Extracts having diminished HCl	10	219	Extracts having increased T. A.	5	337
Extracts having normal HCl	4	387	Totals	24	1,151
Extracts having increased HCl	3	435	Extracts having lactic acid	2	42
Totals	24	1,151			

A consideration of the relation of the tryptophan test to acidity is of interest. Of the positives seven, or 28.9 per cent., of the contents contained no free hydrochloric acid. In seventeen (75 per cent.) of the positives the free hydrochloric acid was diminished or absent. This combined figure is practically identical with that returned by the glycyltryptophan positives, although the percentage of extracts containing no free acid is much lower. In the tryptophan positives it will be seen that 75 per cent. showed diminished total acidity as against 80 per cent. in the case of glycyltryptophan positives.

Studying the negative tryptophan reactions, we note that in 329 instances (28.6 per cent.) there was absent or diminished free acid, while in 586 cases (50.8 per cent.) the total acidity was low. These figures closely approximate those shown by the tabulations from the negative glycyltryptophan reactions.

It would appear that Weinstein's contention that his tryptophan test removes the consideration of contaminating saliva as a source of

error is not borne out by our study. Further, the presence of negative glycyltryptophan reaction, in so large a percentage of extracts with low acidity, leads one to the opinion that the significance of the peptidase, said to exist in saliva, as a factor in hydrolyzing glycyltryptophan added to gastric extracts, is quite questionable. This opinion is substantiated by work we have done on saliva, soon to be reported.

Organic Acid: Ten per cent. of the positive glycyltryptophan tests were associated with the presence of lactic acid. With the exception of one, the cases were carcinoma. Thirty-three negative reactions (3.9 per cent.) were in contents containing lactic acid. Eight and one-third per cent. of the positive tryptophan tests were present in lactic-acid-containing extracts, while forty-two (3.6 per cent.) negative tryptophan contents contained lactic acid. It would seem that organic acids have little bearing on the relative variation of the two tests.

TABLE 7

(A) THE RELATION OF BILE TO GLYCYL-TRYPTOPHAN TEST

Groups	Bile present	Bile absent
Glycyltryptophan positive.	39	71
Glycyltryptophan negative	320	945
Totals	359	816

(B) THE RELATION OF BILE TO TRYPTOPHAN TEST

Groups	Bile present	Bile absent
Tryptophan positive.....	10	14
Tryptophan negative.....	349	802
Totals	359	816

Of the entire number of gastric extracts (1,175) analyzed in this series, forty-four, or 3.7 per cent., contained lactic acid by the controlled Uffelmann test. Of the cases proved to be carcinoma ventriculi, lactic acid was present in twenty-five (28.7 per cent.). As we have shown in these cases, the glycyltryptophan reaction was positive in thirty-one (35.6 per cent.) and the tryptophan test in seven (8.04 per cent.). The relatively low percentage of extracts containing lactic acid may be explained on the basis of early diagnosis, many cases being operated on before marked obstruction and retention had developed. Emerson[16] states that in his series of cases of carcinoma ventriculi, lactic acid was present in approximately 90 per cent. From our experience, it would appear that the great majority of his cases were far advanced and exhibited marked retention. High mixed organic acidity frequently gives confusing Uffelmann reactions.

It has been held that the *chyle* in gastric extracts vitiates the glycyltryptophan test, but need not be considered when making the tryptophan test. The presence of bile or evidencess of tryptic digestion has been used as proof that duodenal contents have been mixed with gastric juice.

16. Emerson: "Clinical Diagnosis," 1906.

The significance of this supposition is shown by analysis of Table 7. The gastric extracts were judged macroscopically as to the presence of bile and were also tested by means of the Pettinkofer or the fuming nitric acid reaction. It will be seen (a) that of 110 positive glycyltryptophan reactions, thirty-nine (35.4 per cent.) contained bile; of 1,065 negative reactions, 320 (20.4 per cent.) showed bile; of the twenty-four positive tryptophan tests (b), ten (41.6 per cent.) were in bile-containing extracts, while 349 (30.4 per cent.) negative tryptophan tests were bile-positive. These figures do not demonstrate that the tryptophan test is uninfluenced by bile in the extracts. It is worthy of note that a relatively high number of both glycyltryptophan and tryptophan reactions are found in bile-containing chyme.

TABLE 8

(A) THE RELATION OF BLOOD TO GLYCYLTRYPTOPHAN TEST

Groups	Bile present	Bile absent
Glycyltryptophan positive.	56	54
Glycyltryptophan negative	236	829
Totals	292	883

(B) THE RELATION OF BLOOD TO TRYPTOPHAN TEST

Groups	Bile present	Bile absent
Tryptophan positive.....	10	14
Tryptophan negative.....	282	869
Totals	292	883

The effect of blood, traumatic or "occult," in gastric extracts has at least two points worthy of consideration with regard to the glycyltryptophan and tryptophan tests. Traumatic blood of itself gives a tan or definitely red cast to filtrates. A color reaction such as we are discussing is readily effected by such shades. The second point of note is the possibility of tryptophan resulting from split digestion products of the blood itself, particularly in those cases in which there is marked gastric retention with much flora. Table 8 furnishes interesting data on the above points. In fifty-six (50.9 per cent.) of the glycyltryptophan positive extracts, blood, traumatic or altered, (benzidin test) was present. Of the glycyltryptophan negative extracts, in 236 (22 per cent.) blood was demonstrated. Of the tryptophan positive extracts, ten (41.6 per cent.) contained blood. In 282 (24.6 per cent.) tryptophan negatives, blood was proved. These figures for both tests so closely approximate that it does not seem possible to state that advantage lies with either. The relatively high percentage of positives in extracts containing blood should, however, be borne in mind.

SUMMARY

The work submitted makes apparent the following:

1. In our series, more than one-third of the proved cases of cancer of the stomach gave positive glycyltryptophan reactions; more than one-fourth were lactic-acid-positive and about one-thirteenth of the number

exhibited the tryptophan test. Diagnosis of malignant disease of the stomach was in each case quite possible, independent of the above chemical reactions. As a test associated with cancer of the stomach, it will be seen that in our series the glycyltryptophan reaction proved more consistent than tests for common organic or existing free amino-acid (tryptophan).

2. While gastric conditions other than cancer exhibit positive glycyltryphan reactions, in no single class of disease of the stomach is this test obtained so frequently as in cancer. This fact is of considerable significance chemically and, perhaps, etiologically. While cancer of the stomach can doubtless be diagnosticated clinically without the glycyltryptophan test, one cannot state that the study of this and allied reactions will prove valueless.

3. Our work does not show that the tryptophan test is, as has been advanced, pathognomonic of cancer.

4. Low free hydrochloric or total acidity is frequently determined in gastric contents exhibiting positive glycyltrophan, lactic acid and tryptophan reactions. One cannot state positively that this diminished acidity is causative. Many cases of low acidity were negative to the above tests.

5. Approximately one-half of the positive glycyltryptophan and tryptophan reactions were in gastric extracts containing bile and blood elements. Approximately one-fourth of the negative extracts contained blood and bile elements.