VOLUME XXX • NUMBER 12 •

UNIVERSITY OF MINNESOTA

Medical Bulletin

OFFICIAL PUBLICATION OF THE
UNIVERSITY OF MINNESOTA HOSPITALS
THE MINNESOTA MEDICAL FOUNDATION
AND THE MINNESOTA MEDICAL ALUMNI
ASSOCIATION

IN THIS ISSUE:

University of Minnesota Medical Bulletin

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VOLUME XXX April 15, 1959 NUMBER 12

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Published semi-monthly from October 15 to June 15 at Minneapolis, Minnesota

Staff Meeting Report

Gastric Cancer Follow-up Studies at the University of Minnesota Hospitals, 1950-1953*

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In a previous paper[1] we reported definite improvement in survival rates following the surgical treatment of cancer of the stomach at the University of Minnesota Hospitals during the period 1936 to 1949. The increase in the percentage of five-year survivors was considered a direct result of the surgeon's more aggressive extirpation of lymph nodes in approaching this problem. Information gained from the study of this disease during that period has provided us with a better knowledge of the routes of lymphatic spread. This has enabled us to concentrate our surgical attack on the additional lymph-node-bearing areas[2] to which this cancer advances. We have attempted in this paper to assess our accomplishments by this approach and to evaluate the prognostic factors bearing on the outcome of this disease from January 1, 1950, to December 31, 1953. The 327 outpatients and inpatients with cancer of the stomach seen at University of Minnesota Hospitals during this time represented: 10 cases of lymphosarcoma, 1 case of Hodgkin's disease of the stomach, and 316 cases diagnosed as carcinoma. The diagnosis was established by microscopic verification or at operation in 304 of the 327 cases (93.0%).

Sex and Age

The sex and age distribution, presented in Figure 1, shows that 230 (70%) of the 327 patients were men, and 97 (30%) were women. This closely parallels the sex incidence of patients with gastric cancer seen at this hospital during the period 1936 to 1949,[1] as well as that reported by other investigators.[3,4,5]

The average age of all patients was 64.9 years—an increase of two years over the group seen here during the previous 14-year period.

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on April 3, 1959.

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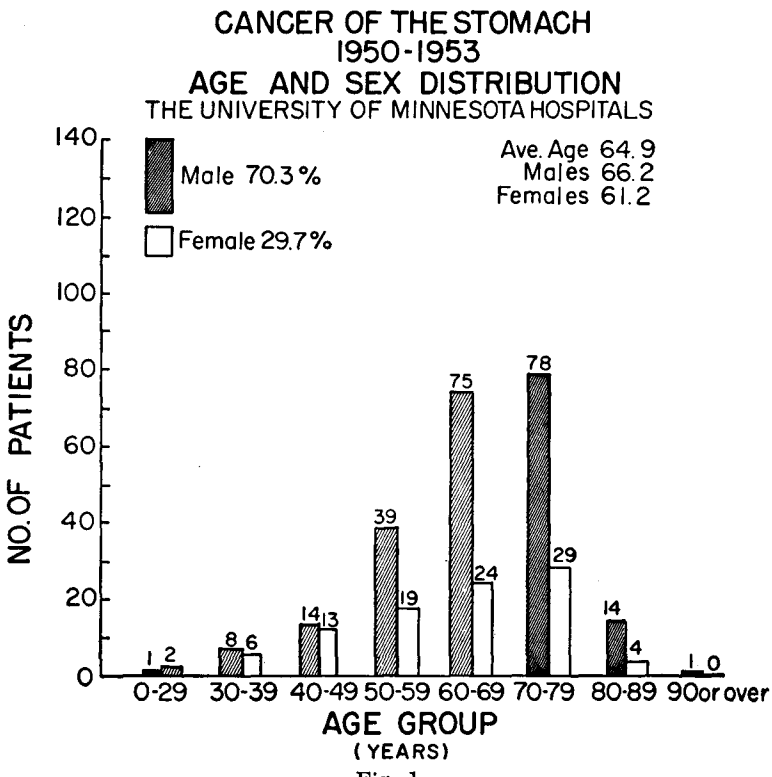

Fig. 1

The average age of men was 66.2 years and of women, 61.2 years. The youngest patient was a girl 18 years old; the oldest, a man 90 years old. Sixty-eight per cent of the patients were 60 years of age or older, and 38.5 per cent were 70 years or older. The average age of patients with cancer of the stomach seen at this hospital is approximately five to ten years higher than that reported by other investigators.[3,6,7,8]

Diagnosis

The great need for early diagnosis continues to be evident. The mean duration of delay in therapy (1) from the onset of symptoms to the time the patient first saw his family doctor was 6.6 months, and (2) from that time until the family doctor referred the patient to the surgeon for definitive therapy was 5.7 months.

An insignificant difference in delay in therapy was noted between the nonsurviors and the patients who survived five years or more. The average overall delay of 10 months from the onset of symptoms to the time the patient was seen by the surgeon represents a decided improvement over the average delay of approximately 18.4 months for all patients seen during the period 1936 to 1949. Evidence suggests that in many instances gastric cancer is a relatively slow-growing disease with a long "silent interval." This was noted by Friesen[9] in cases where carcinoma was left at the line of resection; an average period of 20 months elapsed before symptoms recurred.

The statistical incidence of symptoms is generally known and has been reported in various other publications.[6,10,11,12] An attempt has been made to include in this paper certain laboratory data that may assist the examiner in the early diagnosis of gastric cancer. The findings of occult blood in the stool in 72.1 per cent of patients and of achlorhydria in 68.2 per cent of patients strongly indicates the necessity for using at least these two simple determinations in the diagnosis of gastric cancer. The incidence of anemia in slightly less than 30 per cent of patients is lower than our previously reported figure of 45.1 per cent for patients examined during 1936-1949. (Patients were considered anemic when their hemoglobin was below 11.0 gm/100 ml.)

Roentgenographic examination at this hospital during the period 1939-1951 revealed an accuracy of 91.6 per cent in diagnosing gastric cancer, and recent studies have demonstrated a continued high diagnostic accuracy.

Operability and Resectability

The operability and resectability rates are presented in Table 1 in conjunction with the hospital mortality for the various procedures employed. Table 1 reveals an operability rate of 89.1 per cent and a resectability rate of 73.4 per cent. Curative resections were attempted in 48 per cent of all patients seen. The use of bypass procedures, such as gastrostomy, gastrojejunostomy, or jejunostomy, is fraught with a high mortality rate (15.0 per cent) when compared to exploratory laparotomy alone. The hospital mortality rate of 19.6 per cent for *all* resections is higher than has been noted in the past. This may result from performing resections on patients who are poor risks or whose disease is too far advanced. In addition, the mortality rate of 31.3 per cent for all *palliative* resections during the later period implies that advanced cases of cancer of the stomach were referred to this hospital in greater numbers than before. Perhaps the increased

TABLE 1

CANCER OF THE STOMACH

UNIVERSITY OF MINNESOTA HOSPITALS, 1950–1953

Operability and Resectability Rates

Procedure	No. Patients	Per cent	Hospital Mortality No. Cases	Per cent
No surgery	33	10.1	10	30.3
Operations	294	89.1	52	17.7
Exploration only	34	10.1	2	5.9
Bypass	20	6.1	3	15.0
Resections	240	73.4	47	19.6
All curative	157	48.0	21	13.4
All palliative	83	25.4	26	31.3

hospital mortality rate for resections may be due to the fact that since 1950, gastrectomy for cancer of the stomach has been performed by 34 surgeons, with a great majority of the resections being performed today by the resident house staff. The increase in patients of advanced age, as well as those with lymph node metastases and distant organ metastases, tends to corroborate the assumption that the University Hospitals has been assuming more and more the role of a "court of final appeal."

HOSPITAL MORTALITY RATE

The mortality rates following gastric resection are presented in Table 2. If additional organs were resected at the time of gastrectomy, regardless of whether the gastrectomy was total or partial, operative mortality increased sharply. When gastrectomy was performed without resection of other organs, the operative mortality was only 8.8 per cent for total gastrectomy and 5.2 per cent for partial gastrectomy. Resection of other organs in addition to the stomach caused a fourfold increase in mortality rate for both partial and total gastrectomy. With the increase in the incidence of extended lymph-node dissections associated with curative gastric resections, an increase in operative mortality was anticipated as a possible result of extending this operation to include the hepatic artery and porta hepatis as well as the retroduodenal and paraduodenal nodes. Surprisingly, in those curative resections *with* extensive lymph-node dissection, hospital mortality was only 3.9 per cent. This was less than that following curative resection *without* extensive lymph-node dissection. This may be due to the fact that the extensive lymph-node dissection was employed in

TABLE 2

CANCER OF THE STOMACH

UNIVERSITY OF MINNESOTA HOSPITALS, 1950–1953

Mortality Rates of Gastric Resection

Type of Operation	Curative Resection			Palliative Resection			All Resections		
	No. Cases	Operative Mortality		No. Cases	Operative Mortality		No. Cases	Operative Mortality	
		No.	Per cent		No.	Per cent		No.	Per cent
Partial Gastrectomy	99	9	9.1	53	13	24.5	15.2	22	14.5
Excluding cases with resection of other organs	77	4	5.2						
Cases with resection of other organs only	22	5	22.7						
Total Gastrectomy	58	12	20.7	30	13	43.3	88	25	28.4
Excluding cases with resection of other organs	34	3	8.8						
Cases with resection of other organs only	24	8	33.3						

younger patients and perhaps in those representing better risks. Of patients 60 years of age or less who had extended lymph-node dissection, only 1 in 19 (5.3 per cent) died in the hospital following this procedure. Of those patients who were more than 60 years of age, only 1 out of 33 (3.0 per cent) died as a result of surgery following extensive lymph-node dissection. This suggests that extensive lymph-node dissection, whether performed in patients under or over 60 years of age, still offers a fairly low hospital mortality. Although the hospital mortality for resections has increased over that during the period 1946-49 when the mortality was 8.8 per cent, this increase in mortality cannot be attributed to the performance of extended lymph-node dissections; it appears rather to result from an increased attempt at palliative resection as well as more frequent excision of additional organs, whether for curative or palliative purposes.

Five-Year Survival

We evaluated the influence of certain factors on the mortality rates and length of survival of patients with cancer of the stomach; these factors include the location, gross appearance, and size of the lesion, as well as the incidence of its extension through the gastric wall, and the effect of lymph-node involvement on five-year survival.

Location of Cancer in the Stomach

Figure 2 demonstrates the incidence of gastric cancer by location in the stomach. The majority of cases appeared to originate in the body and antrum. Approximately 9 per cent of cases occurred in the cardia and fundus. Three times as many cancers were noted on the lesser curvature (39.6 per cent) as on the greater curvature (12.9 per cent). The lesions appeared to be equally distributed on the anterior and posterior walls. Only 6.3 per cent of patients were found to have involvement of the entire stomach, and an additional 1.5 per cent had involvement of *almost* the entire stomach. In 13 (28.3 per cent) of 46 patients who had a curative resection, cancer was found in other organs either by direct extension or lymphatic metastases. There were no five-year survivors among the patients with lymphatic metastases to other organs, but three patients with direct extension of the cancer to adjacent organs (such as transverse colon, liver, or pancreas) survived five years or longer.

Table 3 shows the influence of the cancer's location in the stomach on lymph-node extension. The greatest incidence of lymph-node negative cases was noted in those lesions confined to the body or antrum

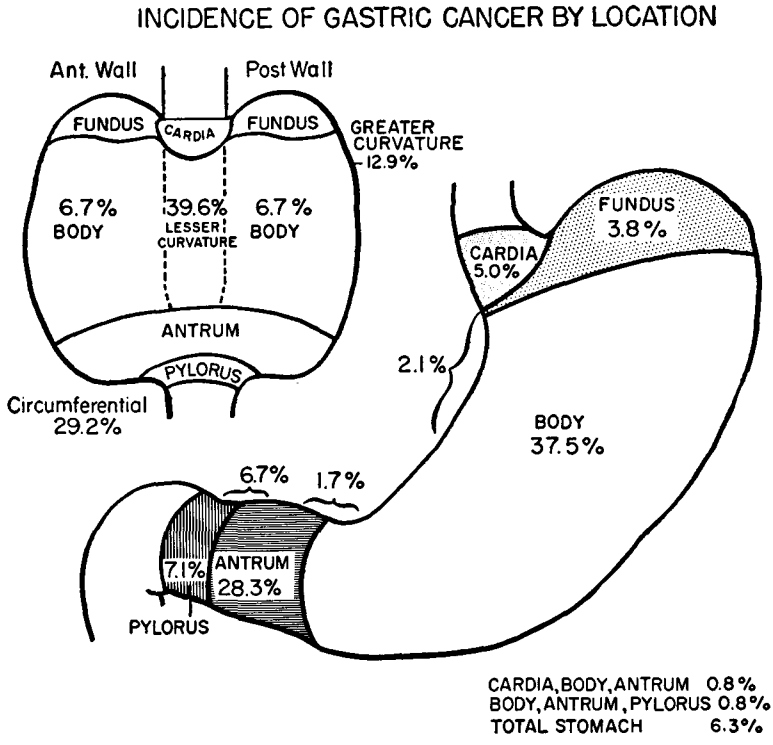

Fig. 2

of the stomach. The incidence of lymph-node negative cases in pyloric lesions was low, and only 5.9 per cent of lesions in this area had extended to other organs at the time of surgery. It appears that a great percentage (94.1 per cent) of pyloric lesions are still in the curable stage. Surprisingly, 26.7 per cent of patients with involvement of the entire stomach undergoing resections (both palliative and curative) had extension to other organs. More than 70 per cent of all patients undergoing resection had lymph-node involvement, and approximately 24 per cent had lesions that had extended to other organs.

Table 4 reveals the influence of the location of the lesion in the stomach on five-year survival. The low percentage of lymph-node negative cases in lesions of the cardia and fundus may account for the very few five-year survivors among patients with cancers in these sites.

TABLE 3

CANCER OF THE STOMACH

UNIVERSITY OF MINNESOTA HOSPITALS, 1950–1953

Location of Cancer Correlated With Lymph Node Extension

Location	No. Cases	Lymph Node Negative	Involvement of Regional Nodes	Distant Nodes	Other Organs
Cardia	12	8.3%	58.3%	16.7%	16.7%
Fundus	9	11.1	55.6	22.2	11.1
Body	90	35.6	21.1	12.2	31.1
Antrum	68	36.8	23.5	17.6	22.1
Pylorus	17	17.6	41.2	25.3	5.9
Pylorus and antrum	16	12.5	37.5	25.0	25.0
Antrum and body	4	25.0	50.0	0.0	25.0
Body and fundus	5	20.0	20.0	60.0	0.0
Pylorus, antrum and body	2	50.0	0.0	0.0	50.0
Antrum, body and fundus	2	0.0	0.0	100.0	0.0
Total stomach	15	13.3	20.0	40.0	26.7
Totals	240	28.8%	27.5%	20.0%	23.9%

TABLE 4

CANCER OF THE STOMACH

UNIVERSITY OF MINNESOTA HOSPITALS, 1950–1953

Influence of Location of Lesion on 5 Year Survival

	No. of Cases	5 Year Survivors All Cases No.	Per Cent	Excluding Hosp. Mort. Per Cent
Cardia	12	1	8.3	12.5
Fundus	9	1	11.1	12.5
Body	90	22	24.4	31.4
Antrum	68	17	25.0	29.3
Pylorus	17	5	29.4	35.7
Fundus and body	5	0	0.0	0.0
Body and antrum	4	0	0.0	0.0
Antrum and pylorus	16	2	12.5	16.7
Cardia, body and antrum	2	0	0.0	0.0
Body, antrum and pylorus	2	0	0.0	0.0
Total stomach	15	0	0.0	0.0

Only two five-year survivors were noted in those cases where the tumor was so large as to involve more than one portion of the stomach.

Gross Classification of Cancer

We have classified our carcinomas of the stomach into the following four groups: ulcerating adenocarcinoma, polypoid adenocarci-

TABLE 5

CANCER OF THE STOMACH

UNIVERSITY OF MINNESOTA HOSPITALS, 1950–1953

Five Year Survival Correlated With Type of Lesion

	All Resections No.	Curative only No.	5 Year Survival			
			No.	% all Resection	% Curative Resection	% Curative Resection Excluding Hosp. Mort.
Ulcerating adenocarcinoma	150	94	29	19.3	30.1	36.3
Polypoid adenocarcinoma	30	28	14	46.7	50.0	56.0
Scirrhous carcinoma	49	25	1	2.0	4.0	4.8
Superficial noninfiltrating	3	3	0	0.0	0.0	0.0
Malignant lymphoma	8	7	4	50.0	57.1	57.1
Total	240	157	48	20.0	30.6	35.3

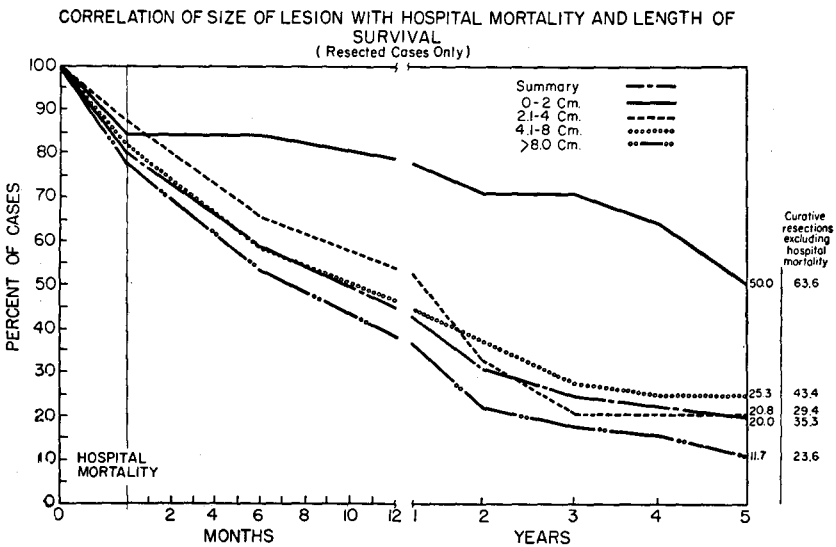

Fig. 3

noma, scirrhous carcinoma, and superficial noninfiltrating carcinoma.[13] The influence of the type of lesion on five-year survival is demonstrated in Table 5. Most of the resected specimens were ulcerating adenocarcinomas. We have surmised that the majority of patients with carcinoma of the stomach seen at this hospital have far-advanced cancers. This assumption is verified by the fact that only three patients were found to have superficial noninfiltrating carcinomas. The best prognosis in the carcinoma group appears to be in the patients with polypoid lesions in whom the five-year survival rate is 56.0 per cent. Although one would expect the best survival rates to occur among patients with superficial noninfiltrating lesions, this was not our experience; the three patients with this type of lesion all died less than five years after gastric resection. More than 75 per cent of patients with malignant lymphhomata survived five years or more, the prognosis being much better in this group than in patients with carcinoma of the stomach. Only one of 49 patients (2.0 per cent) with scirrhous carcinoma survived five years or more.

Size of Lesion

The influence of the size of the lesion on hospital mortality and on length of survival is demonstrated in Figure 3. Of those patients

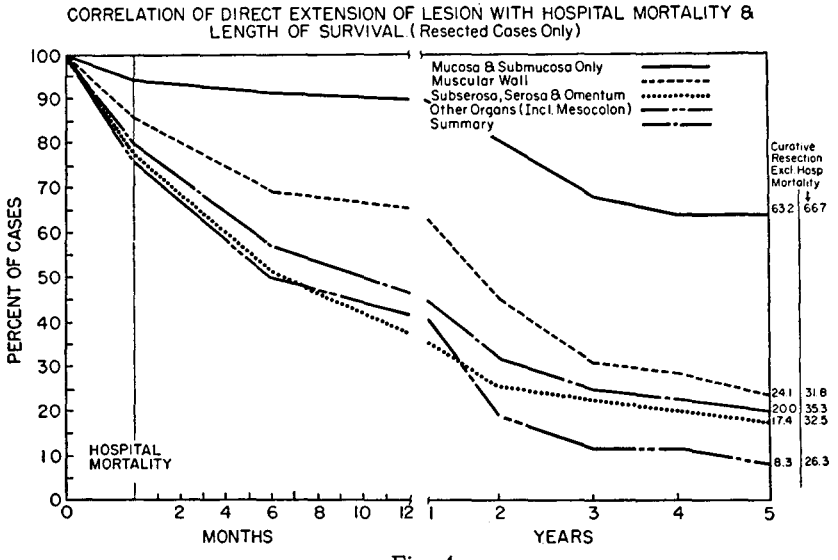

Fig. 4

who underwent curative resections for lesions of 2 cm. in diameter or less, excluding hospital mortality, 63.6 per cent survived five years or more. The five-year survival rate for *all* patients undergoing resection for lesions of less than 2 cm. was 50 per cent. In patients with lesions measuring more than 8 cm. in diameter, less than one-fourth of those who underwent curative resections and left the hospital alive survived five years or more. Figure 3 indicates that the size of the lesion appears to influence to some extent the length of survival. The hospital mortality of the various groups classified by size of lesion is demonstrated on the left side of the graph.

Extension of Lesion Through Gastric Wall

An attempt to determine the influence on survival of the extension of the cancer through the wall of the stomach is presented in Figure 4. This factor appears to have a slightly greater influence on five-year survival than does the size of the lesion. If the cancer has not extended beyond the mucosa or submucosa, the five-year survival rate in patients who underwent curative resections and survived the operation was 66.7 per cent. Once the cancer has extended beyond the submucosa the five-year survival rate drops sharply, to less than half the above figure (31.8 per cent). Little change is noted from the

muscularis-involved group when the cancer extends to serosa or to involve other organs by direct extension, if one views only those patients who underwent curative resection and excludes the hospital mortality. In the entire group of patients with resections, however, only 8.3 per cent of those who had involvement of other organs—including the transverse mesocolon—survived five years or more.

Lymph-Node Metastases

Lymph-node involvement appears to have the greatest influence on five-year survival of any of the factors conisdered. From the data presented in Figure 5, 44.9 per cent of all patients in whom the lesion had not extended to the lymph nodes survived five years or more after either palliative or curative resection. If we exclude hospital mortality and limit this group to the patients who survived the curative resection, we see that 52.5 per cent of those whose lymph-nodes were negative survived five years or more, as contrasted to only 22.1 per cent of those whose lymph-nodes were positive. The five-year survival rate for *all* patients who underwent curative gastrectomy was 45.6 per cent for those without lymph-node involvement, as compared to 19.1 per cent for those with the lymph-node involvement. The distance of involved nodes from the stomach also appears to have some

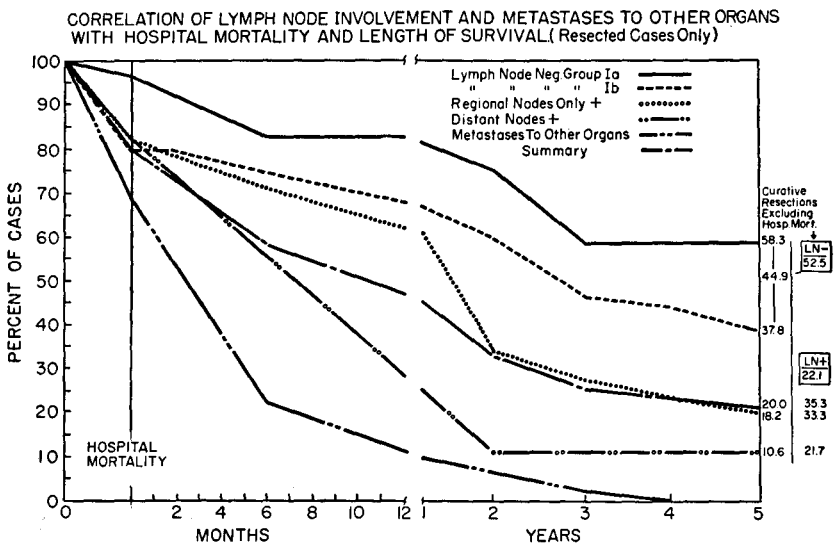

Fig. 5

bearing on the overall five-year survival rate. Where regional nodes only were involved, the overall five-year survival rate was 18.2 per cent; and for curative resections excluding the hospital mortality, 33.3 per cent, while among patients in whom the distant nodes were involved, the comparable figures were 10.6 per cent and 21.7 per cent respectively. By "distant" we mean those nodes beyond the nodes of the lesser and greater curvature. This includes patients with extension to the lymph nodes of the hepatic artery, the porta hepatis and/or paraduodenal or retroduodenal areas. All five patients in this group who survived five years or more had involvement of the porta hepatis nodes only. There were no five-year survivors in the group with lymphatic metastases to other organs, the longest survival in this group being four years, with more than 85 per cent of these patients having died by the end of one year.

The influence of the extent of the extirpative surgery (i.e., whether total or subtotal gastrectomy) on length of survival is demonstrated in Table 6. The overall cure rate for all patients seen at the University of Minnesota Hospitals from the period January 1, 1950, to December 31, 1953, was 14.7 per cent (48 of 327 patients). Excluding the hospital mortality, that figure increases to 15.2 per cent. Of patients undergoing a curative resection, among those whose lymph nodes were found to be negative, 45.6 per cent survived five years or more, as compared to only 19.1 per cent of patients whose lymph nodes had been invaded by cancer. The increase in the five-year survival of lymph-node positive patients is a direct result of the increase in the extent of the extirpative procedure, including dissections of the lymph nodes of the porta hepatis, hepatic artery, paraduodenal and retroduodenal areas.

In the period 1946 to 1949, a greater percentage—57.1 per cent —of lymph-node negative patients survived five years or more. This may be due to the fact that the increased operative mortality during 1950-1953 caused fewer patients with negative lymph-nodes to survive the surgery itself, and thus reduced the number of patients who had an opportunity to survive five years or longer. If, for example, all the nine lymph-node negative patients who died as a result of curative surgery had lived, the statistical expectation is that four of them would have survived five years or more. This would have increased the five-year survival rate for all patients to 16 per cent and that for lymph-node negative patients to 62.2 per cent.

An attempt to correlate the extent of the operation with: (1) hospital mortality and (2) length of survival following partial gastrec-

TABLE 6

CANCER OF THE STOMACH

UNIVERSITY OF MINNESOTA HOSPITALS, 1950–1953

Results of Extirpative Surgery

	Curative Resections			5-Year Survivors					
		Curative Resections		Lymph Node Positive		Lymph Node Negative		All Cases	
	No. Cases	Lymph Node Positive	Lymph Node Negative	No.	%	No.	%	No.	%
Partial gastrectomy	99	54	45	12	22.2	24	53.3	36	36.4
Total gastrectomy	58	35	23	5	14.3	7	30.4	12	20.7
Total	157	89 (56.7%)	68 (43.3%)	17	19.1	31	45.6	48	30.6
All patients seen	327		68 (20.8%)					48	14.7
Excluding hospital mortality	316	77	59	17	22.1	31	52.5	48	15.2

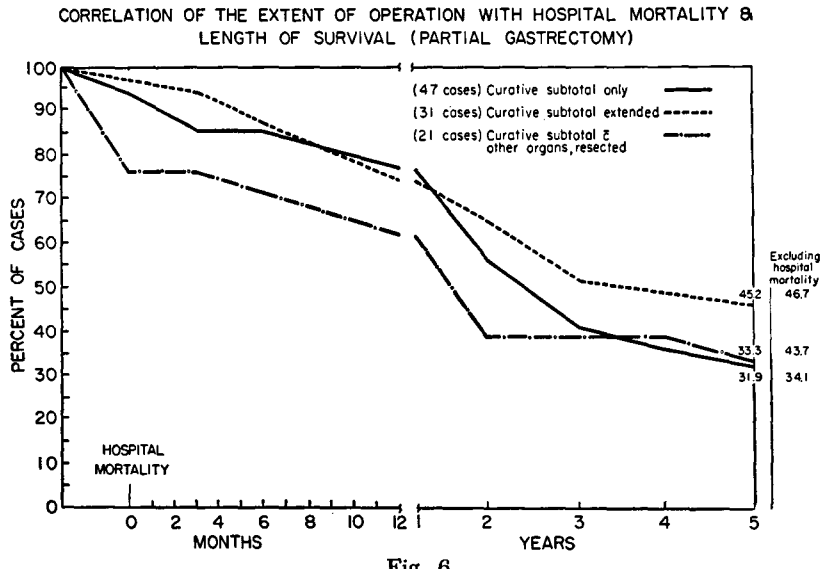
CORRELATION OF THE EXTENT OF OPERATION WITH HOSPITAL MORTALITY & LENGTH OF SURVIVAL (PARTIAL GASTRECTOMY)
(47 cases) Curative subtotal only
(31 cases) Curative subtotal extended
(21 cases) Curative subtotal & other organs, resected
PERCENT OF CASES
HOSPITAL MORTALITY
MONTHS
YEARS
Excluding hospital mortality
45.2
46.7
33.3
43.7
31.9
34.1

Fig. 6

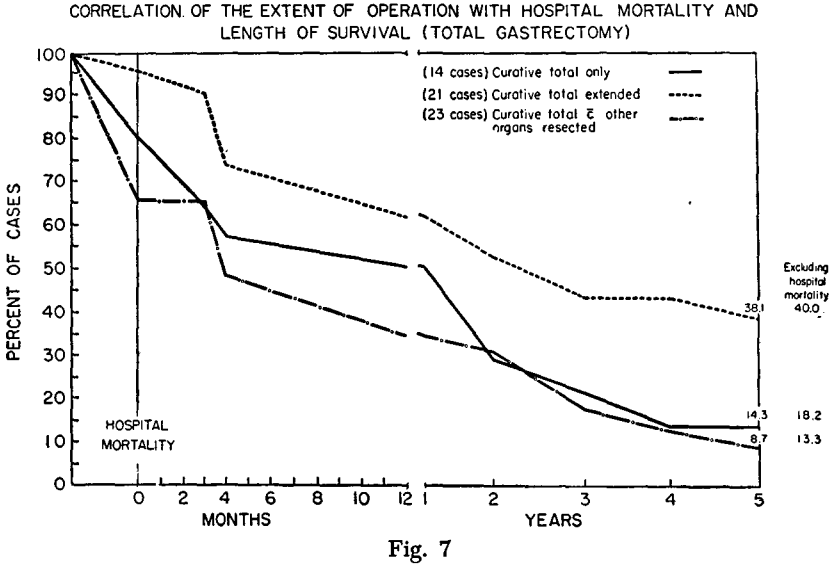
CORRELATION OF THE EXTENT OF OPERATION WITH HOSPITAL MORTALITY AND LENGTH OF SURVIVAL (TOTAL GASTRECTOMY)
(14 cases) Curative total only
(21 cases) Curative total extended
(23 cases) Curative total & other organs resected
PERCENT OF CASES
HOSPITAL MORTALITY
MONTHS
YEARS
Excluding hospital mortality
40.0
38.1
14.3
18.2
8.7
13.3

Fig. 7

tomy (Figure 6) and total gastrectomy (Figure 7) reveals a lower mortality rate and a greater five-year survival rate in those patients who underwent an extended gastrectomy. By "extended gastrectomy" we refer to resection of the stomach and an in-continuity dissection which includes extirpation of the entire lymph-node-bearing area, such as the spleen and splenic pedicle, celiac artery, hepatic artery, porta hepatis, and paraduodenal and retroduodenal nodes. In addition, those patients who underwent curative *partial* gastrectomy showed a definite increase in overall five-year survival rate, regardless of the extent of the dissection, when compared to those patients who underwent a curative *total* gastrectomy. Five-year survival in those patients who underwent a curative total gastrectomy with resection of other organs was 8.7 per cent, or 13.3 per cent if one excludes those patients who did not survive the operative procedure.

Figure 8 shows what happened to patients who were found to have incurable gastric cancer at time of surgery, as compared to patients who underwent curative resection. Of all palliative procedures, only palliative subtotal gastrectomy led to a survival time of as long as two years. Eighty-five per cent of patients who had had a palliative subtotal gastrectomy were dead within one year of the resection; of

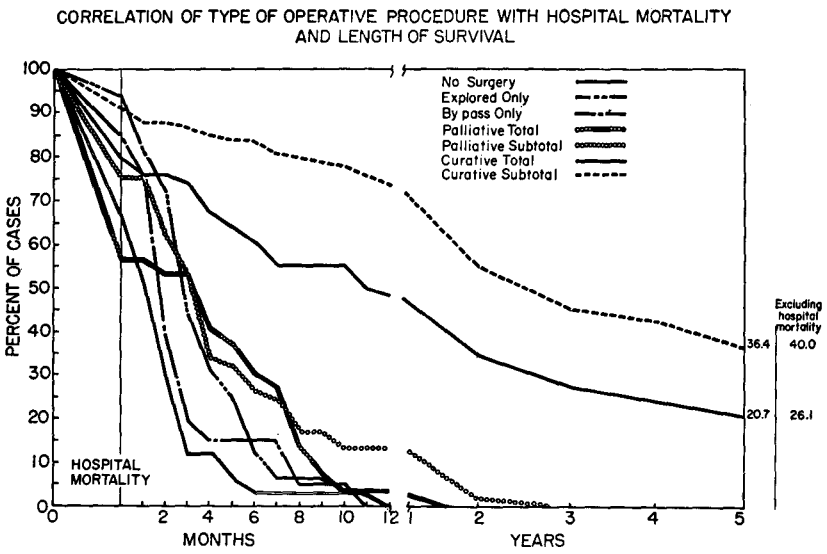

Fig.8

the remaining palliative procedure group, more than 95 per cent were dead within one year of the resection. The best means of palliation appears to be a palliative partial gastrectomy without resection of additional organs. If the surgeon finds that he is unable to follow this technique at the time of surgery, the next best procedure is to close the abdomen without attempting any further surgery. The average survival time for patients with incurable gastric cancer is 2.5 months for those who were not treated surgically; for those who underwent surgical intervention, the time varies with the nature of the procedure as follows: exploration only, 3.5 months; by-pass operations, 2.6 months; all palliative gastrectomies, 6.1 months; palliative total and subtotal gastrectomies, 6.1 months each, all curative gastrectomies, 17.5 months; curative partial gastrectomies 19.3 months, and curative total gastrectomies, 14.8 months.

From the data presented, an attempt was made to form a prognostic classification based on lymph-node involvement, extension of the lesion through the gastric wall, and size of lesion. We feel that the most important prognostic sign is the extension or lack of extension of the lesion to lymph nodes. Regardless of size of the lesion or whether it had or had not extended to involve the serosa, the five-year survival rate was significantly better in the lymph-node negative group than in the remaining three groups. But the influence of extension of the lesion through the stomach wall did have some bearing on five-year survival. If the cancer measured more than 8 cm. in diameter, or if it had extended through the wall to involve the serosa or other organs (Group b), the five-year survival rate was generally not as good as among patients with lesions or less than 8 cm. which had not extended through the serosa.

A method of predetermining the final outcome of every one-hundred patients seen at the University Hospitals is presented in Figure 9. These figures are compared with those for previous periods. During the period 1950 to 1953, of every hundred patients examined at University Hospitals for cancer of the stomach, 15 survived five years or more. Of the remaining 85 who died in five years, 11 were judged inoperable on physical examination; in 42 cases the lesion was found not resectable for cure at time of laparotomy; of the 41 patients who underwent gastric resection, 6 died in the hospital and 26 more were dead within five years. From Figure 9 it is apparent that through the years our operability rates have increased, but the percentage of curative resections since 1949 has not appreciably increased. The increase of two per 100 in five-year survivors may

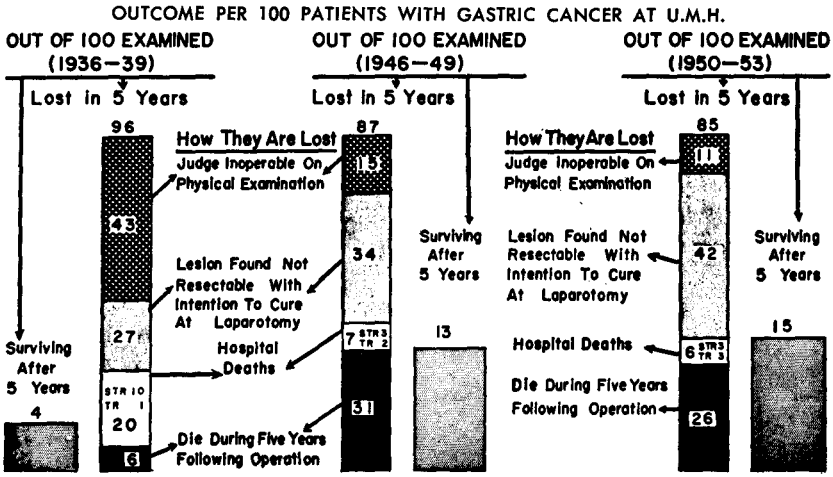

Fig. 9

be due to the more extensive operative procedure rather than to earlier diagnosis. This is verified by the fact that in only 20 per cent of all patients with gastric cancer seen at the University Hospitals was the lesion confined to the stomach alone.

From January 1, 1936, to December 31, 1953, 1,479 patients were seen at the University Hospitals with cancer of the stomach. Of this group, 157 survived five years or more (10.6 per cent). During this period 605 curative gastric resections were performed. Thus the five-year survival rate for all patients undergoing curative resections during this 18-year period is 26.0 per cent. The status of the five-year survivors at the present time is demonstrated in Table 7. Ninety-nine of the 157 patients are still living; 15 (9.1 per cent) of these 157

TABLE 7

CANCER OF THE STOMACH

UNIVERSITY OF MINNESOTA HOSPITALS, 1936-1953*

Status of 5 Year Survivors

Length of Survival	Total No.	Living	Dead
5 to 9 years	85	53	32
10 to 14 years	55	37	18
15 to 19 years	17	9	8
20 years or over	0	0	0
Total	157	99	58

*605 Curative Resections

have survived 15 years or longer, and 72 (45.9 per cent) have survived 10 years or longer. The youngest five-year survivor was a 30-year-old woman. One man, age 88 years at the time of resection, the oldest patient who survived five years, is living and well now after 5½ years—at age 93. A woman who was 84 years old at the time of resection, is now living and well at age 90.

In the period 1950-1953, four patients age 80 years or older were alive and well five years after curative gastric resections. A woman who underwent resection at age 80 years is living and well today at age 90. Similarly, a woman who was 81 years old at the time of resection survived till age 90, when she died from cerebral thrombosis. One woman, age 72 at the time of resection, is alive and well today, 13 years later. A man, age 69 years at the time of resection, is alive and well 15 years later .

Forty-one (26.1 per cent) of the 157 patients who survived five years or more were 70 years or older at time of their gastric resections; seven of these were 80 years old. This suggests that advanced age should not be considered a deterrent to surgery for cure of gastric cancer.

Summary

The University of Minnesota Hospitals' total experience with gastric cancer from 1950 to 1953 is reviewed. During that period, 327 cases of cancer of the stomach were seen at this hospital. A slight but steady increase in older patients has been noted, with a three times greater incidence of cancer in men than in women. Laboratory studies revealed that the determinations for free hydrochloric acid and occult blood in the stool are two important tests to be employed in the diagnosis of this disease. A steady increase in operability and resectability has been noted during this period compared to pervious years.

Overall five-year survival rates have increased from 3.7 per cent during 1936 to 1939 to 14.7 per cent from 1950 to 1953. Lymph-node involvement seems to be the most important single factor in determining five-year survival. Of secondary importance are the lesion's size and the extension through the gastric wall. A prognostic classification was sought that would enable the surgeon to determine the most promising candidates for curative gastrectomy. This is a problem, since lymph-node extension at the time of surgery is frequently difficult to ascertain. The extension of the lesion to the gastric wall, and the size of the lesion appear to hold some prognostic signifi-

cance and may, therefore, help the surgeon in choosing the type of operation he will perform. It was found that additional dissection—such as excision of the hepatic artery, porta hepatis, and retroduodenal and paraduodenal lymph nodes—did not increase hospital mortality and did increase the five-year survival rate.

The excision of additional organs offers no increase in cures if the cancer has already metastasized to those organs, and it results in a pronounced increased operative mortality. If the lesion has spread to the organ by direct extension, however, the possibility of five-year survival exists. Palliative total gastrectomy has little to offer the patient in length of survival time; in addition, this procedure offers a poor operative prognosis, as seen from the fact that 43 per cent of patients who underwent palliative total gastrectomy died while still in the hospital.

The increase in five-year survival rate is the result of a direct surgical attack on the lymph-node-bearing area by extending the dissection to include extirpation of all known routes of lymphatic spread of gastric cancer. This extended dissection appears to offer the highest five-year survival rate, with no additional risk to the patients on whom we have performed this operation.

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Staff Meeting Report

Reducing Sugars in the Blood of Newborn Infants*†

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Reducing substances in the blood have been determined by a variety of biochemical methods. The principle of metallic-oxide reduction by monosaccharides was first enunciated by C. A. Trommer in 1841. By 1848, Fehling and Soxhalt had used Rochelle salt to hold the cupric hydroxide in solution, allowing glucose to reduce the cupric ions to cuprous oxide. In 1874 Haines substituted glycerin for the Rochelle salt. The reagents of Fehling and Haines were used generally until 1911, when Benedict introduced his copper-reducing method, which eventually became the standard procedure for blood sugar determination.[1]

Inasmuch as there are several copper-reducing substances normally present in the blood (viz., glucose, fructose, amino acids, glutathione, ascorbic acid, phosphorylated hexoses, glyceraldehyde), it became necessary to refine the technique for specific blood glucose determination. This refinement began in 1928, when Muller first described glucose oxidase, the enzyme that specifically oxidized B-glucose to gluconic acid with the formation of hydrogen peroxide. According to Froesch and Renald,[2] the chemical reactions leading to the production of color with o-tolidine are as follows:

$$\text{B-glucose} + \text{glucose oxidase} \rightleftharpoons \text{gluconolactone} + \text{glucose oxidase } H_2$$

$$\text{Gluconolactone} + H_2O \leftrightharpoons \text{gluconic acid}$$

$$\text{Glucose oxidase } H_2 + 2\ O \rightleftharpoons \text{Glucose oxidase} + H_2O_2$$

$$H_2O_2 + \text{catalase} \rightleftharpoons H_2O + O$$

The overall reaction may be written:

$$\text{Glucose} + 1/2\ O_2 \leftrightharpoons \text{gluconic acid}$$

Glucose oxidase has been used for quantitative estimation of glucose by Keilin and Hartree,[3] who measured the oxygen uptake dur-

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on April 10, 1959.
†This study was supported in part by a grant from the Lewis R. & Maude Hill Foundation.
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ing the reaction; and by Froesch and Renald,[2] who measured the decrease in copper-reducing ability after complete destruction of the glucose by the enzyme. In both these methods the hydrogen peroxide formed was decomposed by catalase. Kestow[4] and Teller[5] showed that when (1) peroxidase is used instead of catalase and (2) a suitable chromogenic oxygen acceptor is added to the system, the color produced is a measure of the amount of glucose present. Tes-tape® and Clinistix® utilize this reaction in tests of urine.

Middleton and Griffith[6] described a method used for determining blood glucose levels in a large number of blood samples during a short period; they employed a suitably buffered solution of glucose oxidase, peroxidase, and o-tolidine at pH 5. The method of Haggett and Nixon[7] was based on that of Kestow[4] and Teller,[5] using glucose oxidase powder and peroxidase, with o-dianisidine as the chromogenic oxygen acceptor, pH 7 at 35°C. Froesch and Renald[2] found slight activity of glucose oxidase toward mannose and xylose but none toward fructose and galactose.

Both the copper-reducing and glucose oxidase methods are quantitative determinations of blood substances. They do not reveal qualitative differences in reducing substances, nor do they detect nonreducing sugars in the blood. Paper chromatography, however, exhibits a greater specificity than either fermentation or biochemical color reactions, and therefore it has become important in the qualitative determination of blood sugars.[8]

The purposes of the present studies are as follows:

1. To compare the effciency and accuracy of the glucose oxidase and copper-reduction methods (using the Nelson[9] modification of the Somogyi copper method).
2. To determine qualitatively whether or not reducing sugars other than glucose can be detected in the blood of neonates.
3. To measure the rise after feeding in total blood sugar and of blood glucose specifically on each day after birth for fiive days.

Method

I. Blood Samples and Formation of Filtrate

A. *Equipment and Reagents*

1. (K normax) blood pipets to contain 0.1 ml.
2. Pipets to contain 0.2 ml.
3. Test tubes, 10 ml. round bottom
4. Centrifuge

5. Bard-Parker scalpel blades No. 10
6. Alcohol, Band-aids,® marbles

B. *Technique*

1. The infant's bassinet is tilted feet-down for gravity advantage.
2. The heel is pricked at the medial aspect to facilitate rapid flow of blood and early healing of wound.
3. The blood is allowed to flow to 0.1 cc. mark of the blood pipet, and then blown into a 10 ml. test tube containing 1.5 cc. H_2O.
4. The pipet is rinsed by drawing the blood-H_2O mixture into it several times.
5. A Band-aid is always used to prevent excessive blood loss.
6. Then 0.2 ml. of 0.3 N $Ba(OH)_2$ is pipetted into each blood-H_2O mixture and mixed thoroughly.
7. After this mixture turns dark brown, 0.2 ml. of 5 per cent $ZnSO_4$ is pipetted into it and mixed thoroughly.
8. This mixture is then centrifuged at 1500 r.p.m. for 10 minutes.
9. The filtrate is stored in small capped bottles and is frozen for preservation.

II. Sugar Determination

A. *Glucose Oxidase Method*[4,5]

1. Equipment and Reagents
   a. Pipets, to contain 10 ml. and 500 λ (micronormax)
   b. Test tubes, 10 ml. round bottom
   c. Beckman spectrophotometer with tungsten lamp
   d. Stop watch
   e. Glucostat (Worthington Biochemical Corp.)
   f. 4N HCl (Reagent grade)
2. Technique
   a. 500 λ (0.5 ml.) of filtrate is carefully pipetted into a 10 ml. test tube.
   b. The pipet is rinsed in water and methanol, and it is air dried when used for multiple samples in succession.
   c. Next, 2.5 ml. H_2O is pipetted into each tube, using a 10 ml. serologic pipet.
   d. The enzyme is prepared by first dissolving the contents of the small vial in 50 ml. H_2O and then combining both solutions (total 51 ml.) and mixing thoroughly.

e. Twenty tubes can be used with this volume (17 samples, 2 standards of 40 and 80 mg/100 ml glucose, and 1 blank).

f. Then 2.5 ml. of enzyme is pipetted into each of the filtrate tubes at intervals of 30 seconds (total 10 minutes). One drop of 4N HCl is then placed into each tube in succession at 30-second intervals to stop the reaction. The tube is inverted to mix after adding the enzyme reagents.

g. The samples are read on a Beckman spectrophotometer using tungsten lamp at 401 $m\mu$ (slit 0.05, sensitivity 1).

B. *Copper Reduction Method*[9]

1. Equipment and Reagents

a. Beckman spectrophotometer with tungsten lamp.

b. Pipets, to contain 1 ml. and 5 ml. and 250 λ (micronormax).

c. Blood sugar tubes calibrated at 6.25 ml.

d. Boiling water bath

e. *Copper Reagent A*
25 gm. sodium carbonate (Na_2CO_3) anhydrous
25 gm. Rochelle salt
20 gm. sodium bicarbonate ($NaHCO_3$)
200 gm. sodium sulfate (Na_2SO_4) anhydrous
are dissolved in about 800 ml. of water and diluted to one liter, filtered if necessary. The solution should be stored where the temperature will not fall below 20°C. A sediment, which may form after a few days, may be filtered off without detriment to the reagent.

f. *Copper Reagent B*
15 per cent of copper sulfate ($CuSO_4 \cdot 5H_2O$) containing one or two drops of concentrated sulfuric acid per 100 ml.

g. *Arsenomolybdate color reagent*
25 gm. ammonium molybdate is dissolved in 450 ml. distilled water, and 21 ml. concentrated sulfuric acid is added. After mixing, 3 gm. $Na_2HAsO_4 \cdot 7H_2O$ is dissolved in 25 ml. water and added to the mixture. The total solution is thoroughly mixed and placed in an incubator at 37° C. for 24 to 48 hours, then stored in a glass stoppered brown bottle.

2. Technique
   a. First, 250 λ (0.25 ml.) of filtrate is carefully pipetted into the blood-sugar tubes, then the pipet is rinsed in water and methanol and is dried in air for re-use.
   b. Next, 0.25 ml. copper reagent mixture (25 parts reagent A to one part reagent B) is pipetted (using 1 ml. serologic pipet) into each tube.
   c. The contents are mixed thoroughly by shaking the tubes against the opposite hand, and the tubes are heated in a boiling water bath for 20 minutes.
   d. Then, 0.25 ml. arsenomolybdate color reagent is pipetted into each tube, and the contents are again mixed thoroughly.
   e. The samples in each tube are diluted to the 6.25 ml. mark with water and mixed by inversion.
   f. One blank and two standards (one of 40 mg/100 ml and one of 80 mg/100 ml) are prepared in the same way.
   g. The samples are read in a Beckman spectrophotometer with a tungsten lamp at 520 mμ (slit 0.1, sensitivity 1).

C. *Paper Chromatographic Method*[8]

1. Equipment and Reagents
   a. Lyophilization apparatus
   b. Whatman No. 1 paper washed for 10 days in methanol, cut to 7.5 by 18 in. with lower edge scalloped
   c. Chromatography assembly: glass cylinders 12 in. in diameter and 24 in. high, equipped with glass cover, solvent trays, and metal racks
   d. Three shallow 12 by 24 in. enamel finish photographic developing trays
   e. Separatory funnel
   f. Beaker, 400 ml.
   g. Graduate, 200 ml.
   h. Test tubes, 10 ml. round bottom
   i. Bottles, 300 ml.
   j. Pipets, to contain 1 ml. and 5 λ (micronormax)
   k. Blanket, bricks
   l. Absolute alcohol
   m. Acetone C.P.
   n. Ethyl acetate (reagent grade)

o. Photo-Fix® (Kodak)
p. Pyridine (reagent grade)
q. Silver nitrate, 1 per cent
r. Sodium hydroxide O.2 N
s. Dry ice

2. Technique
   a. Remainder of filtrate (O.7 ml.) is poured into a test tube and lyophilized.
   b. The lyophilized powder is dissolved in 50 λ absolute alcohol, care being taken to dissolve it completely.
   c. The alcohol solution of the sample is then spotted on Whatman No. 1 paper using a 5 λ pipette in the manner shown in Fig. 1.
   d. Four papers are run simultaneously in the solvent system of pyridine, ethyl acetate, H_2O in the proportions of 2: 5: 7 (using 50 ml. pyridine, 125 ml. ethyl acetate, 175 ml. H_2O).
   e. These three substances are mixed in a separatory funnel; the upper phase is used as the solvent system, the lower is used to saturate the paper that lines the inside of the tank.
   f. The papers are inserted in the tank and equilibrated with the lower phase for 24 hours.
   g. The upper phase is poured into the trough in the tank using a 400 ml. beaker and taking care to avoid spillage.
   h. The tank cover is sealed with grease, bricks are placed on top to insure seal, and a wool blanket is wrapped around the tank for insulation.
   i. After papers are run in the tank for 22-24 hours, they are dried in air.
   j. One ml. $AgNO_3$, 1 per cent, is pipetted into 300 ml. acetone.
   k. Two such mixtures (600 ml.) are poured into one large developing tray.
   l. The dried papers are dipped in this tray and allowed to dry immediately in air.
   m. While papers are drying under hood, three different trays are prepared as follows:
      (1) 500 ml. 0.2 NaOH
      (2) Kodak Photo-Fix (373 gms. in 1 liter)
      (3) Running cold tap water

n. The papers are dipped into the NaOH tray and left there until two minutes after spots have appeared.
o. Papers are then dipped into Photo-Fix tray until background clears.
p. Papers are immediately placed into running tap water tray for one hour and are dried in air.
q. The contents of the NaOH and acetone-$AgNO_3$ trays are discarded, but the Photo-Fix may be saved.

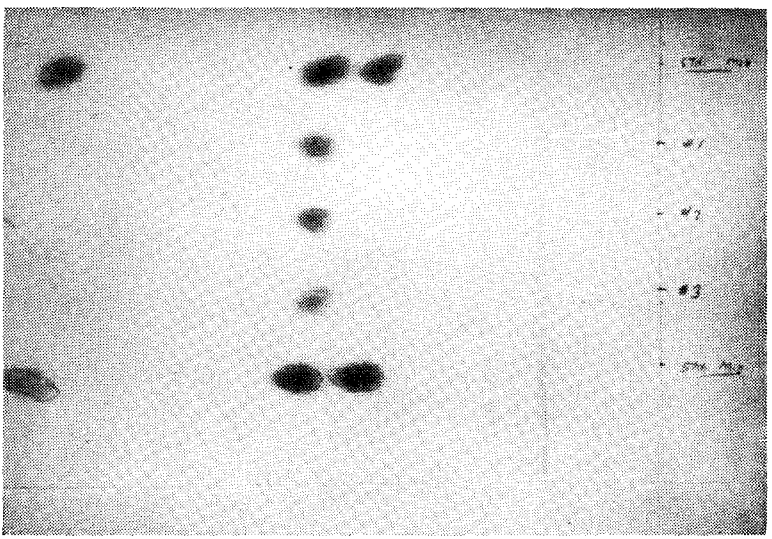

Fig. 1. This is an illustration of a sample chemotogram showing the distribution of spots from these specimens. The only spot detected is that corresponding to glucose in the standard mixture of sugar. The standard mixtures contain lactose, galactose, glucose, and xylose respectively from the base line.

Clinical Protocol

Thirty-two newborn infants at the University of Minnesota Hospitals were used in this procedure. Eleven normal females and 21 normal males were selected on the basis of age and previous oral intake: All were less than 12 hours old at the time of selection and had received nothing but glucose and water prior to the first determination on day 1. Infants with known abnormalities were omitted from this study. The neonates were arbitrarily divided into groups A and B, Group A infants being tested on days 1, 3, 5; group B, on days 1, 2,

4. On days 2 through 5, two samples from each infant were obtained —before the 10:00 A.M. feeding, and one hour after this feeding (designated A. C. (*ante cibum*) and P. C. (*post cibum*), respectively). The feeding mixture, which was constant for all infants, consisted of 1/3 evaporated milk with 5 per cent W/V Dextri-Maltose® every four hours, quantity ad lib.

Of the 32 infants studied, nine were placed in group A. Of these nine, only five gave results for day 5, due to invalid determinations and unforeseen laboratory accidents. The twenty-one placed in group B were followed completely through day 4. Infants No. 31 and No. 32 were tested on days 1, 2, and 3 as a variation in method due to time limitation.

Results

The quantitative results are presented in Tables 1 through 5. In determination of blood sugar content in newborn infants, no significant statistical difference was found between the values obtained by the copper-reduction method and those arrived at by the glucose oxidase method.

Glucose oxidase appeared in this study to be a more efficient method of blood glucose determination than was copper reduction. It proved to be quicker, and it has been shown to be as reliable and as valid as the copper method.

Glucose was found to be the only reducing sugar in the blood of newborn infants as revealed by the insignificant differences between the glucose oxidase and copper-reduction values. Further confirmation of this result was obtained by the finding of a single spot, identical to glucose, on each paper chromatogram.

The mean blood glucose levels appear to rise significantly approximately 20 mg/100 ml each day after feeding, as indicated by the difference in the average of the mean values of blood sugar before and after feeding. But this rise is transitory, since the mean antecibal blood glucose values do not rise absolutely from one day to the next. There does, however, appear to be an overall rise in ante-cibal values, when the entire period from day 1 to day 5 is considered.

It should be emphasized that individual values on aliquots vary greatly between the two methods. This variation has been interpreted as being due to minimal errors in procedure and technique, and possibly to hidden variation in the methods themselves. This points up a need to investigate further the calibration used in these methods and their inherent errors and variabilities.

TABLE 1

DETERMINATION OF BLOOD SUGAR CONTENT BY TWO METHODS IN NEWBORN INFANTS

(Values are in mg/100ml)

Infant	Day 1		Day 2 AC		Day 2 PC		Day 3 AC		Day 3 PC		Day 4 AC		Day 4 PC		Day 5 AC		Day 5 PC	
No.	C.R.*	Gl. Ox.†	C.R.	Gl. Ox.	C.R.	Gl. Ox.	C.R.	Gl. Ox.	C.R.	Gl. Ox.	C.R.	Gl. Ox.	C.R.	Gl. Ox.	C.R.	Gl. Ox.	C.R.	Gl. Ox.
1	96	16							59	43	115	43			85	19	152	64
2	80	40	31	39	35	35							47	63				
3	56	39					56	59	66	71	63	36			124	79	162	75
4	24	0	43	25	59	37							76	31				
5	107	30	54	37	45	37						43		59				
6	112	16	56	55	69	55							130	88				
7	115	21	75	53	94	53							122	69				
8	93	32	54		78	55					86	32	102	32				
9	51	45					43	47	60	74					34	49	69	76
10	16	42					32	46	99	84					36	34	45	51
11	23	41	35	36	41	56					34	53	68	73				
12	79	75	96	43							37	29	77	72				
13	31	53	94	74	31	63					41	27	65	62				
14	31	51	47	36	31	69					53	25	54	32				
15	52	47					27	47		100					56	60	38	57
16	77	58	64	52	94	93					56	58	61	59				
17	19	30					54	50	47	90								
18	42	72	38	22	23	36					35	90	36	115				
19	44		23	90	63	94					23	36	105	155				
20	15	79							23	94								
21	15	104					23	112										
22	62	49	60	54	66	60					118	80	65	63				
23	40	54	41	43	80	71					59	63	43	38				
24	36	75	54	51	78	80					54	80	60	70				
25	36	43	37	31	84	75					54	103	88	138				
26	42	52	56	92	30	87					43	35	57	73				
27	40	77	30	26	45	48					43	79	65	185				
28	40	73	36	39	62	81					39	40	72	135				
29	49	53					57	66	59	68								
30	55	57	41	40	57	64					62	50	79	80				
31	58	68	45	42	59	57	63	53	55	64								
32	69	73	45	43	53	53	57	53	64	62								
Mean	53	52	49	49	57	63	49	63	64	73	57	56	74	80	67	48	93	69

* C.R.=Copper reduction method †Gl. Ox.=Glucose oxidase method

TABLE 2

STATISTICAL COMPARISON OF BLOOD SUGAR LEVELS AS DETERMINED BY TWO METHODS DURING THE FIRST FIVE DAYS OF LIFE

		Copper Reduction Mean (mg/100 ml) ± Std. Dev. (SD)			Glucose Oxidase Mean (mg/100 ml) ± Std. Dev. (SD)		
Day 1		53	±	28.9	52	±	18.1
Day 2	AC	49	±	20.0	49	±	9.0
	PC	57	±	23.4	63	±	12.4
Day 3	AC	49	±	10.3	53	±	33.0
	PC	64	±	16.0	73	±	23.6
Day 4	AC	57	±	23.0	56	±	12.8
	PC	74	±	23.1	80	±	42.4
Day 5	AC	67	±	34.0	48	±	21.2
	PC	93	±	63.6	65	±	11.0

TABLE 3

COMPARISON OF DIFFERENCES IN VALUES OBTAINED BY COPPER REDUCTION AND GLUCOSE OXIDASE METHODS USING ALIQUOTS

		Mean of Difference of C.R.=Gl. Ox. (D)	Standard Error ($S / \sqrt{n}$)	T	N	P (Approximate)
Day 1		2.8	7.7	0.35	31	0.56
Day 2	AC	3.1	5.0	0.63	23	0.50
	PC	3.7	4.7	0.78	22	0.44
Day 3	AC	13.5	10.0	1.35	9	0.21
	PC	13.1	9.3	1.34	9	0.21
Day 4	AC	2.5	8.0	0.31	18	0.55
	PC	8.0	10.3	0.77	20	0.44
Day 5	AC	18.8	15.9	1.19	5	0.30
	PC	28.6	24.6	1.16	5	0.30

TABLE 4

Comparison of PC-AC Values for Copper Reduction and Glucose Oxidase Methods

		Mean of Difference PC-AC (D)	Standard Error (S / $\sqrt{n}$)	T	N	P (Approximate)
Day 2	C.R.	10.0	5.2	1.91	22	0.07
	Gl. Ox.	14.3	3.2	4.48	22	0.001
Day 3	C.R.	12.5	9.5	1.27	7	0.25
	Gl. Ox.	24.0	6.5	3.67	8	0.008
Day 4	C.R.	14.4	7.1	2.03	17	0.062
	Gl. Ox.	28.0	9.2	0.30	19	0.56
Day 5	C.R.	26.2	14.6	1.79	5	0.15
	Gl. Ox.	16.4	9.2	1.79	5	0.15

TABLE 5

Comparison of Ante Cibum Blood Sugar Values from Day to Day in the Individual Infants

		Mean of Difference (D)	Standard Error (S / $\sqrt{n}$)	T	N	P (Approximate)
Day 2-Day 1	C.R.	7.9	5.8	1.35	23	0.19
	Gl. Ox.	5.3	5.2	1.01	23	0.33
Day 3-Day 1	C.R.	3.4	5.7	0.60	9	0.56
	Gl. Ox.	3.6	4.7	0.77	9	0.47
Day 4-Day 2	C.R.	6.8	7.3	0.93	18	0.36
	Gl. Ox.	6.0	8.5	0.73	19	0.47
Day 5-Day 3	C.R.	23.0	17.0	1.36	4	0.27
	Gl. Ox.	5.8	7.0	0.83	4	0.46

Discussion

Methodology

The copper-reducing method has been used generally as the standard for blood sugar determination. This method lacks the desired specificity, however, since it measures total reducing substance rather than glucose. Cochrane[10] has described a method to distinguish between this total reducing substance, which he calls "apparent sugar," and "total true sugar." He found the difference between these values to be 13.4 mg. glucose. The universal use of the copper method would erase this common discrepancy, but the desired value in many cases is the blood glucose level rather than the total reducing substance. In cases in which the nonsugar-reducing substance or the nonglucose sugar is elevated or depressed, a relative hyperglycemia or hypoglycemia would be difficult to detect by means of the copper reduction method. With the glucose oxidase method, however, specific values for blood glucose can be determined.

The glucose oxidase method also lends itself better than the copper reduction method to rapid determination of many simultaneous samples. This procedure is not only quicker, and more efficient than, and apparently as valid as, the copper method, but it has the advantage of being more specific and more sensitive. Thus, while the copper method is sensitive to 0.1 mg. "apparent sugar," the glucose oxidase method is sensitive to 1 microgram of glucose. As noted above, Froesch and Renald[2] found slight activity of glucose oxidase toward mannose and xylose but none toward fructose and galactose. This apparently has little or no effect on newborn blood glucose determination.

Findings

Statistical evaluation* shows no significant difference between the values determined by the copper-reduction method and those yielded by the glucose oxidase method. This would indicate that the only reducing substance in the blood of new born infants is glucose. Further confirmation was obtained by the chromatographic study, which showed only glucose. Quantities as small as one microgram of reducing sugar were detectable on the chromatograms, corresponding, in this procedure to a blood value of 3 mg/100 ml.

This finding is of physiologic importance in the study of disorders of the central nervous system in neonates, as well as in the diagnosis and management of various carbohydrate-metabolic dis-

*The statistical evaluation was performed under the guidance of Jacob Bearman, Ph.D., Professor, Division of Biostatistics, School of Public Health.

orders. Hartmann et al.,[11] using fermentation methods, found that "under ordinary conditions of milk feedings in near-normal infants, as much as 40 per cent of the blood sugar may be galactose." In this study no galactose was found in the blood of any of the 32 infants on any day one hour after feeding. This suggests that galactose conversion to glucose is quite efficient in normal newborn infants. The reactions normally responsible for this conversion are:

$$\text{galactose} + \text{ATP} \overset{\text{Galactokinase}}{\leftrightharpoons} \text{galactose-1-PO}_4 \overset{\text{Galactowaldenase}}{\rightleftharpoons} \text{glucose-1-PO}_4$$
$$\text{Coenzyme UDP glucose}$$

Haworth and associates[13] found this metabolic pathway to be incompletely developed in normal newborn and premature infants. In 13 of 50 full-term newborn infants they found galactosuria, and of 152 samples from 26 premature infants Haworth[14] noted reducing substances in 77 (51 per cent). Glucose and xylose were occasionally observed, but lactose and galactose were the most common sugars excreted. Haworth suggested two possible bases for the glactosuria, namely, hepatic immaturity, or persistence of the ductus venosus permitting absorbed galactose to bypass the liver. Hartmann[11] explained the galactosuria by postulating that the blood galactose level exceeds the renal threshold of galactose. No determinations of sugar in the urine were made in the studies reported here. Our failure to find reducing sugars other than glucose in the blood emphasizes the low renal threshold for the nonglucose sugars.

The maintenance of blood sugar at a constant level from day to day was determined statistically by comparing values of the same infant on successive days before feeding. This indicates a maturation of the glucose metabolism mechanism in newborns. Villee[15] noted that, in general, glucose-6-phosphatase activity appears in the human fetal liver at 12-15 weeks, reaching full activity only after 20-24 weeks of development. Harris and Olson[16] were unable to detect changes in activity of glucose-6-phosphatase in portions of liver and renal cortex from children 40 hours to 13 years of age. Nemeth found glucose-6-phosphatase to be absent from fetal liver of guinea pig, making its appearance at term, and he stated that "The liver possesses at term the full complement of enzymes necessary for the formation of glucose from glycogen."[17]

All this evidence favors the hypothesis that there are present at birth the enzymes for the reversible reaction of glucose $\longrightarrow$ glycogen, which are necessary to maintain a constant level of blood glucose dur-

ing the neonatal period. The apparent overall mean rise in blood glucose from day 1 to day 5 cannot be proved statistically. The variability of individuals was great. The rise in blood glucose after feeding was only temporary and further indicates a mature enzyme system for the utilization and absorption of glucose as well as for the production of glycogen. The liver is recognized as being immature in many of its functions at birth; and the mechanism of the successive development of enzyme systems in the liver as the infant matures is still to be elaborated. This study presents evidence bearing on one small area of the problem to provide a basis for understanding future studies of the carbohydrate-metabolic disorders in the newborn period.

Summary and Conclusions

A comparison of the copper-reduction and glucose oxidase methods for blood sugar determination was studied in blood samples from thirty-two newborn infants. The use of present copper reducing methods was found to be satisfactory for accurate determinations, but the glucose oxidase method appeared to be more specific and more efficient and to lend itself better to rapid determinations of many simultaneous samples. It was shown by the use of paper chromatographic methods, that newborn blood contained no detectable (above 3 mg/100 ml) sugar other than glucose. Blood glucose levels in newborn infants showed a significant rise of about 20 mg/100 ml one hour after feeding. This rise was only transitory, however, as the day-to-day ante-cibal determinations were statistically the same. There appears to be an overall rise in fasting blood glucose levels from day 1 to day 5 of life. The variability and inherent errors of these methods indicate the need for their further study and refinement.

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Staff Meeting Report

Rapid Identification of Malignant Cells in Vaginal Smears by Fluorescence Microscopy*†

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Introduction

The fluorescent dye acridine orange (AO) was introduced by Strugger in 1940.[1] Subsequently, several investigators in botany, medicine, virology, and bacteriology showed that AO has an affinity for both the nucleic acids, but particularly for ribonucleic acid (RNA).[2,3] Actively proliferating cells, such as those present in embryonic or malignant tissue, have an increased amount of RNA as compared with their normal adult counterparts.[3] Evidence has also shown that increased cytoplasmic RNA accompanies nuclear changes in proliferating malignant cervical squamous epithelial cells,[4,5,6] and that the amount of RNA of normal squamous epithelium decreases as the cells cornify.[2]

In 1956, Bertalanffy et al.[7] described a technique in which acridine orange was used to estimate cellular RNA qualitatively and, in addition, to provide morphologic detail. The method was rapid and simple and produced polychromatic fluorescence of cervical squamous epithelium. The nuclear chromatin, which fluoresced green to yellow, provided a contrasting background for an orange nucleolus. The cytoplasm fluoresced green, orange, or intense red-orange, depending on the amount of RNA present. In gynecologic smears, the cytoplasm of certain exfoliated malignant cells fluoresced a characteristic intense red-orange. On the other hand, the cytoplasm of other malignant cells, which had presumably lost some of their RNA, fluoresced a nonspecific orange or red.[3] The procedure was said to be applicable to mass programs for detection of uterine cancer because it required neither a highly trained screener nor a long screening time.

In 1958, Bertalanffy et al.[3] reported correct identification of malignant cells in AO-stained smears from 56 of 58 patients with uterine

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on April 10, 1959.

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cancer. There has been no report, however, that gives the percentage of smears containing malignant cells which can be identified by characteristic red-orange fluorescence alone without using conventional morphologic criteria of malignancy. Such information would be important since the clinical usefulness of the AO fluorescent method depends on the recognition of a unique color. The present report discusses the results of a study of 864 AO-stained smears from 641 unselected patients, including patients during pregnancy and patients with cervicitis and with untreated or previously irradiated uterine carcinoma.

Method

Between July and December, 1958, 864 smears were collected from 641 unselected gynecologic and obstetric patients at the University of Minnesota Hospitals. There were 326 patients from the Cancer Detection Center, 172 from gynecology tumor clinic, 128 from the gynecology and obstetric clinics, and 15 from the gynecology inpatient service. Those from the Cancer Detection Center represented a group of asymptomatic women. Patients from the gynecology tumor clinic had been treated previously for cancer and were examined during this period for possible recurrence of neoplasms. Patients from the gynecology clinic had been referred by other hospital services for evaluation of routine pelvic complaints. The fifteen inpatients had various gynecologic disorders (Appendices 1 and 2).

The collection routine was adapted to existing procedures in each clinic. Smears were taken by the physician or medical student who made the initial examination. A duplicate smear was made for independent examination by the Cancer Detection Center or Gynecology Department and stained by the conventional Papanicolaou methods. Vaginal aspirations were taken from all patients, and in addition, cervical scrapings were obtained from many of the Cancer Detection Center patients. Slides from the Cancer Detection Center and inpatient service were coded so that neither the patient's name nor hospital number were known to the AO screener; those from the other clinics contained the patient's name and hospital number. Furthermore, the clinical history and diagnosis, biopsy report, and Papanicolaou diagnosis were all unknown prior to examination of the AO-stained smears.

The slides were fixed immediately in 95 per cent ethanol. Most slides were stained after three to four hours in the fixative, although some remained in 95 per cent ethanol as long as a month. Ten

slides at a time were stained by the following 13-step method, modified after Bertalanffy:[7]

1. 80 per cent ethanol: 10 dips or 10 seconds
2. 70 per cent ethanol: 10 dips or 10 seconds
3. 50 per cent ethanol: 10 dips or 10 seconds
4. Distilled water: 10 dips or 10 seconds
5. 1 per cent acetic acid: 10 dips or 10 seconds (retards subsequent fading)
6. Distilled water: 10 dips or 10 seconds
7. 0.01 per cent acridine orange (National Aniline) in pH 6 phosphate buffer: 3 minutes.

8-10. pH 6 phosphate buffer: 10 dips each or 30 seconds (decolorization)

11. O.1 *M* $CaCl_2$: 45 seconds (differentiation)
12. Mount in pH 6 phosphate buffer and coverslip
13. Read wet within one hour; slides are not permanent.

Reproducible colors in exfoliated vaginal cells were obtained consistently by this procedure, as detailed by Bertalanffy.[3] The three-minute staining time was found to be critical. It was found that three dishes of pH 6 phosphate buffer were necessary to remove excess dye from a slide. At the end of this step, all the material on a slide stained orange. After 45 seconds in O.1 *M* $CaCl_2$, nonspecific staining was removed, leaving a green to yellow nucleus with an orange nucleolus. The cytoplasm of exfoliated squamous epithelium fluoresced green, orange, brick-red or intense red-orange, while that of endocervical epithelium fluoresced bright orange. The vaginal flora (orange-staining bacteria, monilia, Trichomonas) and the yellow-green nuclei of leukocytes were observed also; erythrocytes, however, do not stain by this method.

A Spencer monocular microscope, equipped with a plain aluminized mirror, quartz condenser and yellow ocular suppression filter, was used for examination of the smears. The light source was a Zeiss-Winkel lamp containing an HBO 200 (Osram) mercury arc bulb. One 5113 and two BG 12 primary filters, which transmit near ultraviolet and blue-violet light, were used. Incident intensity was standardized by adjusting the condenser diaphragm until the background was black under high dry magnification. Slides were scanned initially under low power, only suspicious areas being scrutinized under high dry power. Staining and screening were done by an observer who had had some training in pathology but none in cytology. Ten slides were

TABLE 1

RESULTS OF ACRIDINE ORANGE FLUORESCENCE MICROSCOPY IN 641 PATIENTS

	Total	Positive No.	Positive Per Cent	Negative No.	Negative Per Cent	False Positive No.	False Positive Per Cent	False Negative No.	False Negative Per Cent	Inadequate Smears No.	Inadequate Smears Per Cent	Percent Accuracy*
Normal	326	0	0	324	99.5	2	0.5	0	0	0	0	
Gynecology Tumor Clinic	172	0	0	156	90.7	5	2.9	2	1.2	9	5.2	0 % (0/ 2)
Gynecology and Obstetric Clinics	128	1	0.8	117	91.4	3	2.3	1	0.8	6	4.7	50 % (1/ 2)
Gynecology inpatients	15	6	40	6	40	1	6.7	2	13.3	0	0	75 % (6/ 8)
(864 smears)	641	7		603		11		5		15		58.4% (7/12)

*Percent accuray $= \frac{\text{Number of correct AO positive}}{\text{Number of known positive}} \times 100$

stained and read in one hour, photography time not included. Photographs were taken on 120 Super Anscochrome film with an exposure time of five minutes. (Slides can be destained in 50 per cent ethanol and can be restained by conventional Papanicolaou methods if desired.)

A positive diagnosis of malignancy was made when the cytoplasm of several cells fluoresced an intense red-orange; all other slides were called negative, there being no intermediate category. Slides that contained only a few cells (or as in one case, only material from a rectovaginal fistula) were called inadequate.

In summarizing the results, an AO diagnosis was called correctly positive when there were corresponding clinical, biopsy, and Papanicolaou diagnosis of malignancy. A slide called negative was considered correct when all other evidence cited above was negative. The AO diagnosis was considered to be false positive when intense red-orange cellular cytoplasm was observed, but there was no clinical, biopsy, or Papanicolaou evidence of malignancy. When no intense red-orange cytoplasm was seen, but all other evidence was positive, the AO diagnosis was considered false negative.

Results

The results are summarized in Table 1. During the five-month period included in this study, clinical findings, biopsy specimens, and Papanicolaou smears indicated malignancy in 12 patients. Only 7 of the 12 (58 per cent) had positive AO diagnoses on initial vaginal aspiration (Tables 2 and 4); no cervical scrapings were obtained from these patients. Among the 12 patients there were two recurrent, postirradiated squamous cell carcinomas of the cervix, neither of which was identified by AO fluorescence. Of the five primary squa-

TABLE 2

AO Fluorescence — Correct Positives

Patient	Diagnosis
	Endometrial adenocarcinoma
	Endometrial adenocarcinoma
	Endometrial adenocarcinoma
	Squamous carcinoma of the vagina
	Squamous carcinoma of the cervix
	Squamous carcinoma of the cervix
	Undifferentiated carcinoma of the cervix

mous cell carcinomas of the cervix, three were correctly called AO positive. Three of the four adenocarcinomas of the endometrium, as well as the undifferentiated carcinoma of the cervix, yielded positive results. Thus 7 of 10 (70 per cent) primary malignancies were identified properly by AO fluorescence of cytoplasm.

Case Histories

Case 1: . Age 65 para 3-0-1-3 LMP age 42. History of vaginal spotting of several months' duration. Examination disclosed a bleeding lesion on the right wall of the vagina. Clinical diagnosis of carcinoma was confirmed by biopsy, which showed squamous cell carcinoma of the vagina. Papanicolaou smear: positive. AO smear: many positive cells.

Case 2: Age 64 para 2-0-0-2 LMP age 49. Seen in July, 1958, with history of postmenopausal bleeding. A Papanicolaou smear was interpreted as negative for tumor cells. Diagnostic dilatation and curettage in August, 1958, led to a diagnosis of endometrial polyp. The patient returned to gynecology clinic in September, 1958, with the complaint of persistent lower abdominal pain. The endometrial polyp revealed by pelvic examination was removed. The uterine corpus at this time was enlarged to the size associated with a 16-18 week pregnancy. A second dilatation and curettage was performed on October 9. Gross and microscopic diagnosis at this time was endometrial adenocarcinoma. A review of the initial Papanicolaou smear and curettings showed that malignancy had actually been present in July, 1958. An AO smear taken October 10, 1958, revealed many positive cells.

Case 3: Age 36 para 2-0-0-2 LMP November 5, 1958. The patient had been well until November, 1958, when she had a prolonged menstrual period which lasted nine days. Her private physician performed a dilatation and curettage. Microscopic sections showed squamous cell carcinoma of the cervix. Physical examination on admission to University Hospitals revealed a friable, partially necrotic, exophytic cervical tumor mass. Clinical and pathologic diagnosis (biopsy) was squamous cell carcinoma of the cervix, League of Nations (LNS) Stage II. Papanicolaou smear: positive. AO smear: few positive cells.

Case 4: Age 61 para 2-0-0-2 LMP age 45. In August, 1957, there was moderate postmenopausal vaginal bleeding for a few days. Bleeding recurred in March, 1958, and by December, 1958, a bloody vaginal discharge "soaked" several pads daily. Pelvic examination showed an enlarged, soft cervix, with dilation of the os. The uterine fundus was 1½–2 times enlarged, soft, mobile, and regular in contour. Material from a dilatation and curettage revealed an adenocarcinoma of the endometrium. Papanicolaou smear: positive. AO smear: few positive cells.

Case 5: Age 47 para 0-0-0-0 LMP 1957. This patient had prolonged and irregular vaginal bleeding in the winter of 1956-57. In May, 1957, a panhysterectomy was performed elsewhere for carcinoma of the ovary. The patient was well until July, 1958, when vaginal bleeding recurred and increased. Pelvic examination revealed that the apex of the vaginal vault was replaced by a friable, granular, necrotic tumor with cen-

tral ulceration. Biopsy: adenocarcinoma of the endometrium with extension to the vaginal vault. Papanicolaou smear: positive. AO smear: few positive cells but much lysis.

Case 6: Age 72 para 8-0-0-6 LMP age 49. This patient was well until September, 1958, when a routine pelvic examination by her private physician revealed a suspicious area on the cervix. A biopsy specimen taken from this area was found to be positive for carcinoma. Pelvic examination at the University Hospital revealed a healing biopsy site and reddened cervical os. Biopsy: Undifferentiated carcinoma of the cervix. Papanicolaou smear: positive. AO smear: several positive cells among many polymorphonuclear leukocytes.

Case 7: Age 43 para 2-0-0-2 LMP October 6, 1958. Onset of abnormal vaginal bleeding in February, 1957, following an automobile accident. Profuse menses occurred every two to three months. Pelvic examination showed the cervix to be replaced by a necrotic, ulcerating, exophytic tumor. Clinical and pathologic diagnosis: squamous cell carcinoma of the cervix, LNS I. Papanicolaou smear: positive. AO smear: few positive cells.

In 629 patients no malignant growth was present. In 15 of these, the smears were considered inadequate. Among the remaining 614 patients, in 603 (98 per cent) a correct negative diagnosis was made. Of the 11 AO false positives, six were from patients with postirradiated squamous cell carcinoma of the cervix, one was from a pregnant patient, one from a patient with a healed cervical laceration, and two were from asymptomatic patients (Cancer Detection Center). In the entire series of 641 patients 18 were called AO positive; of these, seven (44 per cent) actually had malignant tumors.

Discussion

The value of early diagnosis of uterine cancer by Papanicolaou smears is accepted, but conventional staining techniques are very time-consuming and require skilled cytoscreeners. In the past, several workers[7] have used fluorescence, in various and largely unsuccessful techniques, to correct these problems. The technical advantages of AO fluorescence are as follows: (1) The rapid six-minute staining procedure results in a polychromatic picture with a single dye. (2) The screening time is short. (3) Some malignant cells can be identified easily by characteristic flaming red-orange cytoplasm. (4) With the addition of a reasonably inexpensive ultraviolet light source and a yellow filter in the ocular, a standard laboratory microscope can be used.

AO fluorescence might be expedient for screeners already experienced in cytology, but certain refinements are necessary before a minimally trained observer can use this technique to identify uterine malignancy accurately. The striking color, however, can be recognized

even by an observer with minimal training in cytology. In two cases, in fact, many such typical cells were seen even under low power. Furthermore, the incidence of AO false positives in a normal population is low (Table 1). Certainly such a property would facilitate the operation of mass screening programs, although cells which give this reaction may not be present in all smears from patients with malignant growths.

Several factors limit the number of AO positive cells which may be found in smears from patients with uterine cancer. One of the major limitations of AO fluorescence is implied in the studies by Gross and Danziger,[4] by Foraker,[5] and by Moberger.[6] Gross and Danziger, using Brachet's methyl green-pyronine technique, found increased cytoplasmic RNA only in the immature, rapidly dividing cells in cervical squamous carcinoma. Foraker demonstrated by photometery of hematoxylin and eosin preparations that cellular basophilia (RNA + DNA) was increased in both invasive and intra-epithelial squamous cell carcinomas of the cervix. Moberger, using absorption spectrophotometry, demonstrated increased concentration of cytoplasmic nucleic acid of infiltrating cells in rapidly growing epidermoid carcinomas as compared to homologous tissues of origin and to slowly growing carcinomas. He also pointed out that some portions of a neoplasm have reduced amounts of RNA. Thus, it may be presumed that a vaginal aspiration from a patient with a malignant uterine lesion will contain only a certain proportion of AO positive malignant cells.

In this series, only two patients with correct AO positive results (Cases 1 and 2) presented with many AO positive cells in the vaginal aspiration. One of these was a patient with clinically early carcinoma (Case 1), the other a patient who had had a diagnostic dilation and curettage the day before vaginal aspiration (Case 2); thus the presence of many cells in this specimen may have resulted from their disruption by this procedure. There were only a few characteristic cells in all the other correct AO positive cases, but these had been exfoliated from grossly necrotic tumors (Cases 3, 4, 5, 6, 7). The proliferating cells that were AO positive were undoubtedly diluted by the greater number of necrotic cells present in such tumors, and such cells do not stain an intense red-orange. Restricting the use of the AO fluorescent technique to the diagnosis of early squamous cell carcinomas of the cervix, which contain large numbers of proliferating cells,[8] may increase markedly the proportion of positive cells obtained.

Other limitations of the technique are suggested by the reasons for the five false negatives in this series (Table 3). In two cases of

squamous cell carcinoma of the cervix, the slides remained in 95 per cent ethanol longer than two weeks. It was apparent at the time of staining that many cells had floated off the slide during rehydration, but since many cells still remained, a diagnosis was made. Slides from two other patients remained in ethanol for a comparable period,

TABLE 3

AO FLUORESCENCE — FALSE NEGATIVES

Patient	Source	Reason for Error	Diagnosis
	Gynecology Tumor Clinic	Positive on fourth Papanicolaou test	Endometrial adenocarcinoma
	Gynecology Tumor Clinic	2 week fixative	Squamous carcinoma of the cervix
	Gynecology Clinic	4 week fixative	Squamous carcinoma of the cervix
	Gynecology Clinic in-patient	Lysis	Squamous carcinoma of the cervix
	Gynecology Clinic in-patient	Full of cocci	Squamous carcinoma of the cervix

TABLE 4

AO FLUORESCENCE — PROPORTION ACCURACY

Squamous carcinoma of the cervix	3 / 7
Endometrial adenocarcinoma	3 / 4
Undifferentiated cancer of the cervix	1 / 1

but since they retained a few characteristic cells, the observer was able to make a correct diagnosis. All other slides were kept in ethanol less than two to three days. If the two false negative slides which were in 95 per cent ethanol longer than two weeks originally contained characteristic cells, these may have been lost during the staining procedure; thus, staining slides within two to three days after fixation may prevent loss of positive cells.

In one smear from a patient with squamous cell carcinoma of the cervix, most of the cells were lysed, as manifested by many clumps of orange staining material accompanying bare nuclei. Slides of this sort, which represent a grossly necrotic carcinoma, seldom present characteristic fluorescence. Such slides in the future should be rejected for AO diagnosis, and conventional Papanicolaou stains should be

used instead. Another squamous cell carcinoma of the cervix was missed because the entire slide was covered with orange staining cocci which obscured the exfoliated cells. Similarly, a second smear should be obtained and stained by conventional techniques. An adenocarcinoma of the endometrium, which had been called negative on three previous Papanicolaou diagnoses, was missed. Although three out of four such tumors were positive in this series, they are known for their poor exfoliative properties, and the AO fluorescent method may not be adequate to identify them.

Occasionally, AO positive malignant cells are present but are difficult to see; therefore thick areas on a slide must be screened carefully under high dry power. Conversely, bacteria which are clumped in a circular arrangement—distinguished by their uniform granular apperance and absence of a nucleus—may resemble positive malignant cells. Finally, if incident intensity is not standardized carefully so that the background is black, orange staining cells may be easily confused with malignant cells because of added cytoplasmic intensity.

Tissue cultures can be grown on coverslips and stained in the same manner as are vaginal smears. Present evidence[9] obtained from cell cultures indicates that AO fluorescence of cytoplasm reflects cellular proliferation. When human amnion cells were first cultured, they were found to be round, discrete, and sedentary, and their cytoplasm stained orange with AO. But when these cells began to proliferate, became pleomorphic, and were transplanted, their cytoplasm fluoresced an intense red-orange identical with the fluorescence of HeLa cells, a strain cultured from human cervical squamous cell carcinoma. Some investigators have expressed the belief that such cells underwent a transformation similar to that occurring in malignant cells.[10,11,12,13]

Since intense red-orange AO fluorescence of cytoplasm seems to characterize proliferating cells, it can be inferred that certain benign conditions which occur in the uterus might not be distinguished from uterine carcinoma. Bertalanffy et al.[3] stated that in AO-stained smears, certain benign uterine hyperplasias differed from malignant tumors only in their normal morphologic properties. In the present series, 49 patients had benign uterine hyperplasias, including chronic cervicitis, cervical erosion, endocervical polyp, chronic polypoid endocervicitis, vaginal papilloma, trichomoniasis, moniliasis, pregnancy, and obortion (Appendix 1). Of this group, one patient with a threatened abortion and one patient with a healed cervical laceration were called AO positive (Table 5). Certain AO-stained cells from benign hyperplasias might present intense red-orange cytoplasm, but this fact does not

TABLE 5
AO FLUORESCENCE — PROPORTION FALSE POSITIVES

Irradiated squamous carcinoma of the cervix	6 / 11
Threatened abortion	1 / 11
Healed cervical laceration	1 / 11
Normal	3 / 11

limit the effectiveness of the AO fluorescent technique. In this series, 98 per cent (603/614) of patients who had no malignant uterine lesion were correctly identified (Table 1). If necessary, conventional Papanicolaou procedures can be applied to destained AO slides for comparative diagnosis by a skilled cytologist.

Poorest results were obtained in smears from postirradiated patients (Table 1). Both cases of recurrent postirradiated squamous cell cervical carcinomas were missed, and more than 50 per cent (6/11) of the false positives were from patients in the postirradiated group. The fact that postirradiated cells may contain increased RNA[14,15] may have contributed to this result. These patients should be followed by standard procedures, however, to determine if they show an earlier incidence of recurrence than does a comparable postirradiated group.

These data suggest that an observer untrained in cytology will have the greatest probability of making a correct diagnosis in smears from tumors which contain large numbers of actively proliferating cells. The unsuspected or early squamous cell carcinoma of the cervix is such a tumor, and therefore it is the logical object of a mass screening program.[16]

Other malignant lesions may very likely be identified also by this technique. Touch preparations from normal gastric mucosa and from a gastric adenocarcinoma, for example, are strikingly different in color. Future studies are necessary to confirm the reliability of AO fluorescence in detecting malignancy in other organs.

CONCLUSIONS

Vaginal aspirations and cervical scrapings from 641 unselected gynecologic and obstetric patients were screened for malignancy with acridine orange fluorescence by an observer untrained in cytology. Presence of intense red-orange fluorescence of the cytoplasm, which reflects an increased amount of ribonucleic acid, was considered presumptive evidence of malignancy. Of 18 patients with smears called AO positive, 7 (44 per cent) actually had malignant tumors. Most

of the AO false positives (6/11) were from patients with postirradiated squamous cell carcinoma of the cervix. Present evidence indicates that the increased RNA in a cervical squamous cell carcinoma occurs in rapidly proliferating cells. Reasons for false negatives are discussed. By restricting AO fluorescent screening to detection of early carcinomas which contain rapidly growing cells, it is conceivable that greater accuracy will be obtained.

The technique is rapid and simple. It appears to be valuable in screening a normal population in that there is a low percentage of false positive results. It does not appear to be of value in screening postirradiated patients for recurrence of neoplasms. In this study, 70 per cent (7/10) of primary malignant lesions were identified properly by AO fluorescence of cytoplasm.

APPENDIX 1

DIAGNOSES IN GYNECOLOGIC AND OBSTETRIC CLINIC PATIENTS EXCLUDING CARCINOMA

Diagnosis	Number
Pelvic relaxation	32
Normal pelvis	27
Pregnancy	20
Chronic cervicitis (biopsy)	14
Cervical erosion	4
Abortion	3
Trichomoniasis	2
Moniliasis	2
Myomata	2
Atrophic endometrium	2
Cervical laceration	1
Ovarian tumor	1
Benign papilloma — vagina	1
Menorrhagia, etiology unknown	1
Atrophic vaginitis	1
Virilism	1
Sebaceous cysts — vulva	1
Leukoplakia	1
Recto-vaginal fistula	1
Atrophic vulvitis	1
Menopausal syndrome	1
Endocervical polyp and cervical erosion	1
Primary dysmenorrhea	1
Endocervical polyp	1
Chronic polypoid endocervicitis (biopsy)	1
Ectropion	1
Hydradenoma of vulva	1
Total	126

APPENDIX 2

Diagnoses in Gynecologic Patients Previously Treated for Malignancy

Diagnosis	Number
Cervical squamous cell carcinoma	126
Endometrial adenocarcinoma	31
Undifferentiated carcinoma	5
Carcinoma of the vulva	4
Ovarian pseudomucinous cystadenocarcinoma	2
Ovarian adenocarcinoma	1
Ovarian fibrosarcoma	1
Ovarian papillary cystadenocarcinoma	1
Adenoma malignum	1

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Faculty Publications

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BENSON, E. S. and HALLAWAY, B. E.: Interaction of Actomyosin and Adenosinetriphosphate as Studied with Glycerol-Extracted Cardiac Muscle Bundles, Am. J. Physiol. 194:564, 1958.

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GLENCHUR, HARRY; HAGLUND, ROGER V.; WILCOX, ROBERT H.; and HALL, WENDELL H.: Urinary Bacteriological Studies from Patients with Inlying Catheters, J. Clin. Invest. 37:897, 1958.

WEEKLY CONFERENCES OF GENERAL INTEREST

Physicians Welcome

Monday,	9:00 to 10:50 A.M.	OBSTETRICS AND GYNECOLOGY Old Nursery, Station 57 University Hospitals
	12:30 to 1:30 P.M.	PHYSIOLOGY- PHYSIOLOGICAL CHEMISTRY 214 Millard Hall
	4:00 to 6:00 P.M.	ANESTHESIOLOGY Classroom 100 Mayo Memorial
Tuesday,	12:30 to 1:20 P.M.	PATHOLOGY 104 Jackson Hall
Thursday,	11:30 A.M. to 12:30 P.M.	TUMOR Todd Amphitheater University Hospitals
Friday,	7:45 to 9:00 A.M.	PEDIATRICS McQuarrie Pediatric Library, 1450 Mayo Memorial
	8:00 to 10:00 A.M.	NEUROLOGY Station 50, University Hospitals
	9:00 to 10:00 A.M.	MEDICINE Todd Amphitheater University Hospitals
	1:30 to 2.30 P.M.	DERMATOLOGY Eustis Amphitheater University Hospitals
Saturday,	7:45 to 9:00 A.M.	ORTHOPEDICS Powell Hall Amphitheater
	9:15 to 11:30 A.M.	SURGERY Todd Amphitheater University Hospitals

For detailed information concerning all conferences, seminars, and ward rounds at University Hospitals, Ancker Hospital, Minneapolis General Hospitals, and the Minneapolis Veterans Administration Hospital, write to the Editor of the BULLETIN, 1342 Mayo Memorial, University of Minnesota, Minneapolis 14, Minnesota.