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IN THIS ISSUE:

Phenylketonuria
Endometrial Adenocarcinoma
Combined Radiation and Chemotherapy

University of Minnesota Medical Bulletin

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Staff Meeting Report

Phenylketonuria (Phenylpyruvic Oligophrenia)*

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Phenylketonuria (phenylpyruvic oligophrenia) is an inborn error of metabolism almost invariably associated with mental retardation. This metabolic disturbance in phenylketonuric subjects was first noted in Norway in 1934 by Foelling,[1] who called attention to the presence of large amounts of phenylpyruvic acid in the urine of 10 of 430 institutionalized severely mentally retarded patients. The disorder has been found in most countries in the world. This condition is extremely uncommon among Jews; it has been reported among Negroes. Its incidence in the United States is estimated as 1 in 25,000, giving an approximate total of 6,400 cases in this country.[2] Severe mental retardation with an I.Q. below 50 is present in 95 per cent of the reported cases, with less than 1 per cent of patients having I.Q.'s greater than 70.[2] Phenylketonuria accounts for 0.5 to 3 per cent of institutionalized mental defectives.[3]

The disease is transmitted by a single autosomal recessive gene, its incidence in the offspring of two heterozygous parents being 1 out of 4. Limited capacity to metabolize phenylalanine has been reported in heterozygotes,[4] but phenylketonuria and excretion of other abnormal products of phenylalanine metabolism are not present in heterozygotes fed large amounts of phenylalanine.

Of infants and children wth phenylketonuria, 80 per cent characteristically have fair skin, blue-eyes, and blond hair, but a few patients have been reported to have dark hair, pigmented skin and dark eyes.

*This report was given at the Staff Meeting of the University of Minnesota Hospitals on May 23, 1958.
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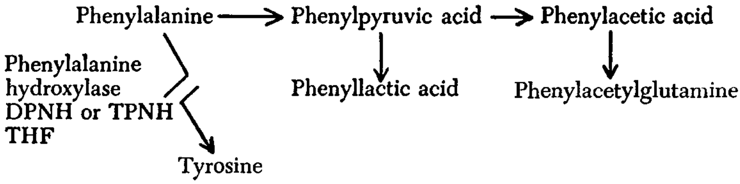
The skin frequently (20% of cases) demonstrates eczema, dermatographia, hyperhydrosis, and increased sensitivity to sunlight.[5] Other allergic manifestations such as asthma are more common in phenylketonuric patients than in the general population.[6] Physical development is generally normal, but a tendency toward microcephaly has been reported.[7]

Neurologic manifestations are variable, nonspecific abnormalities that do not differ essentially from those found in groups of children retarded from other causes. Approximately one-third of patients have no associated neurologic abnormality. The common neurologic manifestations are irritability, restlessness, clumsy movements, muscle hypertonicity, and hyperactive tendon reflexes. Tremors and choreiform movements, ankle and knee clonus, and repetitive movements of the head and extremities may occur. Seizures, particularly of the petit mal or myoclonic type, are frequent in early life in 25 per cent of all phenylketonuric children,[5] the convulsive episodes usually subsiding after these children reach ten years of age. Electroencephalographic abnormalities, seen in most phenylketonuric patients, are not specific for this disorder, since the EEG's of these patients, even with no history of seizures, are similar to the inter-seizure patterns of patients with epilepsy.

The congenital metabolic defect in phenylketonuria is an inability to convert phenylalanine to tyrosine. Following Foelling's work, Jervis[8] in 1940 demonstrated the associated accumulation of phenylalanine in the blood of these patients and postulated the defect in the conversion of phenylalanine to tyrosine. Later, Jervis[9] described the failure of formation of tyrosine from phenylalanine when incubated with liver extract obtained from phenylketonuric patients. Mitoma and coworkers[10] and Wallace and coworkers[11] independently demonstrated the absence of a specific phenylalanine hydroxylase in phenylketonuria. Kaufman[12] has shown that the enzymatic hydroxylation of phenylalanine to tyrosine requires the presence of two enzymatic fractions:* Fraction one is found only in the liver and is absent in phenylketonuric patients, while fraction two is found in many other organs besides the liver and is present in phenylketonuric patients. Reduced diphospho- or triphosphopyridinenucleotide (DPNH or

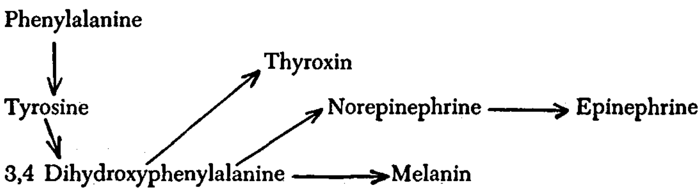
*Phenylalanine hydroxylase activity has been shown to be absent in fetal rat liver near term, due to lack of fraction one.[14]

TPNH) is required for this enzymatic hydroxylation,[12] in which, tetrahydrofolic acid (THF) also participates.[13] The pathway of phenylalanine metabolism in phenylketonuria, with major excretory metabolic intermediates, is:



Phenylpyruvic acid, phenyllactic acid, and phenylacetic acid are excreted in the urine of untreated phenylketonuric subjects, but of these, only small amounts of phenylpyruvic acid have been observed in the blood of such subjects.[15] There is practically no phenylpyruvic acid excretion with phenylalanine levels below 15 mg/100 cc of blood. During the first six weeks of life phenylpyruvic acid excretion may not be apparent in the phenylketonuric infant despite a high phenylalanine blood level,[16] although it has been observed in infants as early as five and ten days of age.[17]

Tyrosine, not as essential amino acid in the normal person, is an essential amino acid in phenylketonuric patients because of the defect in the hydroxylation of phenylalanine to tyrosine. It is therefore not surprising that some manifestations of tyrosine deficiency are seen in these patients. Tyrosine is a precursor of a number of physiologically important substances that lead to the formation of melanin, epinephrine, norepinephrine, and thyroxin:



The light pigmentation observed in phenylketonuria provides clinical evidence of the melanin deficiency; Weil-Malherbe and Bone[18] have reported decreased circulating plasma epinephrine levels in these patients; hypothyroidism, however, has not been demonstrated in phenylketonuria.

The relationship of the defect in phenylalanine metabolism to the cerebral abnormalities is still unclear. Intoxication by high blood phenyalanine levels or any of its abnormal metabolites has been considered by many but is unproven. Himwich and coworkers[19] in 1940 showed reduced cerebral oxygen uptake in phenylketonuric subjects. Recent studies of brain slices have demonstrated a significantly decreased oxidative phosphorylation in the presence of phenylpyruvic acid.[20] The relationship of the decrease in epinephrine and norepinephrine synthesis to the mental retardation has not been explored.

The diagnosis of phenylketonuria is dependent upon demonstrable elevation of phenylalanine, with values generally ranging from 15 to about 60 mg/100 cc of blood. For screening purposes determination of phenylpyruvic acid in the urine is of primary importance. This test is carried out by the addition of 1 ml. of 5 per cent ferric chloride solution to 3-5 ml. of a slightly acidified urine; the appearance of a deep olive green color indicates a positive reaction. Because of the correlation of phenylalanine values in the blood with phenylpyruvic acid values in the urine, a negative ferric chloride test may be seen occasionally in phenylketonuria.

A new approach to the problem of phenylketonuria was made in 1952 when Bickel and coworkers in Birmingham, England, began using phenylalanine-low diets to treat phenylketonuric patients.[21] Amino acid analysis of food proteins usually shows the phenylalanine content to be in the range of from 4 to 6 per cent.[22] The preparation of a phenylalanine-low protein was essential for maintaining adequate nutrition. Through phenylalanine absorption by charcoal, a phenylalanine-low casein hydrolysate was prepared. Fat and carbohydrates were added in suitable amounts, as well as a balanced mineral and vitamin mixture. From 15 to 30 mg/kg/day of phenylalanine had to be provided to keep these patients in positive nitrogen balance and to promote weight gain. With this phenylalanine intake, blood phenylalanine levels could be controlled at or near the normal range.

The reports of the effect of this phenylalanine-low diet by Bickel and coworkers[21,23] are very encouraging. Improvement in some of the associated manifestations of phenylketonuria, such as eczema, seizures, and electroencephalographic abnormalities, was most striking. A prolonged attention span and improvement in manageability were noted. Increase in pigmentation was universally observed with darkening of hair. Rate of mental development also appeared to increase slightly while these patients were on the phenylalanine-low diet. Bickel's original report dealt with children two years and older who had evinced severe mental retardation by the time dietary therapy was started. Armstrong and Tyler[24] in 1955 noted even more encouraging results in the treatment of very young children with the phenylalanine-low diet, and Horner et al.[25] reported normal mental development in a two-year-old phenylketonuric child who had been on dietary therapy since six weeks of age. A recent report by Hsia and coworkers[26] about lack of improvement of older phenylketonuric patients after one year of dietary control may indicate critical age limitations; their trial period, however, may not have been sufficient.

During the past two and one-half years, studies of 31 phenylketonuric children and adults have been made at the University of Minnesota with the support of the McClure Medical Research Fund in collaboration with the State School and Colony at Faribault, Minnesota. These data have shown, among other things, that no relationship exists between the individual's I.Q. and the phenylalanine blood level, and that the latter may, in fact, vary considerably from day to day (Table 1).

TABLE 1

MENTAL DEVELOPMENT AND PLASMA PHENYLALANINE LEVELS OF THIRTY-ONE UNTREATED PATIENTS WITH PHENYLKETONURIA

No.	Patient	Age	I.Q.	Phenylalanine Plasma Level mg. % (range)	
1	♀	7 yrs.	63	32.8	(19.3-32.8)
2	♂	5 yrs.	30	26.0	(22.3-26.0)
3	♂	2 "	37	35.5	(35.5-38.5)
4	♀	2 "	60	25.7	
5	♀	4 "	30	31.5	(18.1 - 31.5)
6	♂	5 "	10	23.5	(23.5-24.7)
7	♂	6 wks.	Normal	40.0	(33.0-57.7)
8	♀	16 mos.	37	35.0	(35.0-55.3)
9	♂	7 yrs.	15	29.9	(27.9-32.1)
10	♂	6 mos.	50	52.0	(22.4-52.0)
11	♀	10 yrs.	50	29.3	(15.3-29.7)
12	♂	7 "	30	15.0	(15.0-21.0)
13	♂	10 "	13	27.8	
14	♂	6 "	Idiot	19.8	
15	♀	11 "	"	29.0	(29.0 - 37.2)
16	♂	11 "	"	37.0	
17	♂	15 "	"	26.8	
18	♀	8 "	30	31.7	(28.9 - 31.7)
19	♂	18 "	12	45.7	
20	♂	17 "	23	42.6	
21	♂	52 "	Idiot	40.1	
22	♀	11 "	"	45.3	
23	♂	72 "	30	54.5	(30.1 - 54.5)
24	♂	25 "	19	35.6	
25	♂	58 "	46	30.3	
26	♂	11 "	32	29.9	
27	♀	48 "	25	33.9	
28	♀	14 "	Idiot	24.6	
29	♀	33 "	10	18.6	
30	♂	34 "	Idiot	20.7	
31	♀	66 "	26	22.8	

Experience has also been gained at the University Hospitals with phenylalanine-low diets. During the last two and one-half years seven phenylketonuric children were carefully evaluated prior to diet therapy by physical and neurological examination, EEG, psychological testing, and phenylalanine blood determination. Of these seven, two have been kept on the phenylalanine-low diet for two years, one for one and one-half years, and four for approximately six months.

The initial diet consisted of casein-hydrolysate (obtained from Allen and Hanbury in England, prepared according to the specifications of Bickel and coworkers) fortified with fat, carbohydrate, minerals, and a vitamin mixture. Phenylalanine blood determinations, made repeatedly during the initial period, showed that blood phenylalanine usually returned to normal range within ten days of the start of the diet, at which time 15-30 mg/kg/day of l-phenylalanine were added to the diet.

Several problems were encountered in patients during the first three to six months on this dietary therapy. Diarrhea, a common difficulty due to the lack of bulk in the preparation, was relieved by adding Cellu flour to the daily formula. Weight gain was poor for a variable period, particularly in the children under one year of age; this was partly due to inadequate caloric intake because of the poor taste of this material. It was noted that the young infant's requirement for phenylalanine was different from that of the toddler, and that at least 25 mg/kg/day of phenylalanine had to be provided for the infant below six to eight months of age in order to keep him in positive nitrogen balance and promote adequate weight gain. Severe neutropenia with bone marrow agranulocytosis was observed in one six-month old infant approximately three weeks after the start of the phenylalanine-low diet. This complication of unknown cause resulted in no clinical manifestations but did not respond when this child was again placed on a normal diet for three weeks. In accordance with the wishes of the parents this infant was again given the phenylalanine-low diet and he spontaneously recovered from his neutropenia within one month (Fig. 1).

Data from repeated psychological testing of the three children who have been on the phenylalanine-low diet for at least one and one-half years reveal considerable and consistent improvement in rate of mental development, improvement that has become increasingly apparent following the first year of dietary therapy. Patient appeared perfectly normal and had a normal EEG at age eight weeks when he was first given the phenylalanine-low diet. He still had a

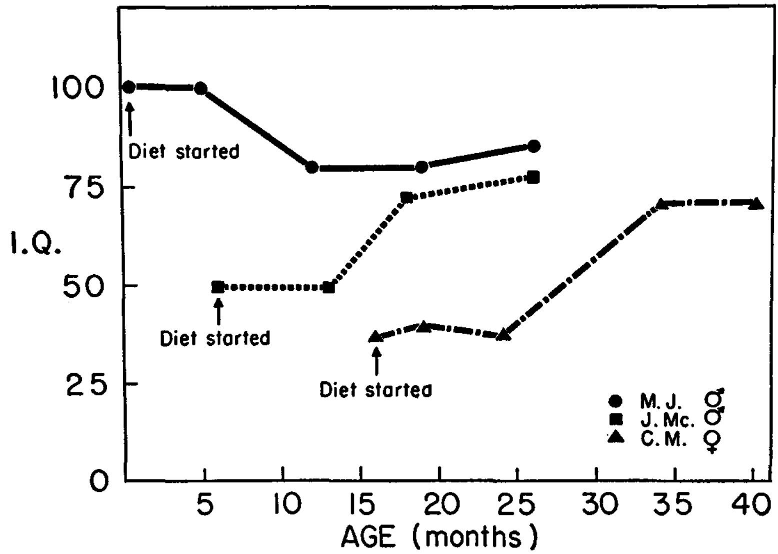


Fig. 1. Mental Development of Three Patients with Phenylketonuria on Phenylalanine-Low Diet.

normal EEG at three months when, because of poor weight gain and hypoproteinemia, he was returned to a normal diet. EEG abnormalities that had appeared within four weeks disappeared again one month after this child was restarted on the phenylalanine-low diet. This child was tested at frequent intervals and has scored I.Q.'s of 80 and 78, and the most recent determination gave him a score of 85 at 26 months. He appears perfectly normal in neuromuscular and mental development but shows some retardation in speech, the significance of which at his age is not clear.

The other four children have been on the phenylalanine-low diet for too short a time (six months) to allow any conclusions, but at present all seem to be doing well physically, with adequate weight gain.

The degree of dietary control in these children was variable; control was particularly difficult in one child, but even in his case improvement was noted. It is still impossible to tell whether the degree of dietary control is related to the rate of improvement.

Two phenylalanine-low formulas, now commercially available, can be used in treating phenylketonuria:

1) KETONIL® (Merck, Sharp and Dohme)

Ketonil® supplies other amino acids while allowing restriction of phenylalanine. There is an adequate allowance of minerals; but no vitamins are present in the mixture, because of their instability. The dietary mixture is synthesized from an acid casein hydrolysate from which phenylalanine has been removed and to which supplementary amino acids, minerals, and choline have been added.

The usual procedure is to feed Ketonil® as the exclusive source of protein for about three weeks, after which small amounts of other protein foods are introduced to provide the daily phenylalanine requirement. Fat and carbohydrate supplementation in the form of vegetable oils, Dextri-maltose,® or sugar must be made to meet the daily caloric requirement; vitamins are routinely prescribed.

Patients should receive 5 Gm/kg of basal mixture daily which will provide 3 Gm/kg of protein. One gram of basal mixture equals 2.5 calories. The caloric requirement of a patient is 60 to 90 calories/kg/day. Thirty-five per cent of the calories from fat and 50 per cent of the calories from carbohydrate will complete the daily formula. Fifteen mg/kg of phenylalanine are allowed in the daily diet after the second to third week on the formula for patients older than two years of age; 30 mg/kg for infants.

The average contents of the dietary preparation include the following amino acids and minerals per 100 Gm. as listed below:

l-arginine	.630	Gm.	aluminum	.370	mg.
l-histidine	1.1	Gm.	calcium	1.1	Gm.
l-isoleucine	3.6	Gm.	chloride	1.2	Gm.
l-leucine	8.6	Gm.	copper	1.2	Gm.
l-lysine	6.2	Gm.	iodine	.5	mg.
d-methionine	.320	Gm.	iron	.160	Gm.
l-methionine	1.5	Gm.	magnesium	.240	Gm.
l-threonine	3.8	Gm.	manganese	.1	mg.
dl-tryptophan	.650	Gm.	phosphorus	1.8	Gm.
l-tyrosine	3.3	Gm.	potassium	1.8	Gm.
l-valine	4.6	Gm.	sodium	2.7	Gm.
glycine	6.0	Gm.	sulfur	3.0	Gm.
choline	.590	Gm.			

2) LOFENALAC® (Mead-Johnson)

The main constituent as a protein source is a special protein hydrolysate that has been carbon treated to remove the phenylalanine. This treatment has also destroyed tryptophan, methionine, and tyrosine; thus additional amounts have been added. The vitamins and minerals included in the product have been calculated to equal the levels commonly present in cow's milk formulas used for infant feeding.

Lofenalac® contains Dextri-maltose,® casein hydrolysate, corn oil, arrowroot starch, calcium gluconate, mineral salts, l-tyrosine, dl-tryptophan, dl-methionine, and vitamins.

It is again the usual procedure to feed Lofenalac® exclusively for two to three weeks before adding other solid foods. Caloric requirement is again calculated on the basis of 60-90 calories per kilogram. One gram of mix provides 5 calories, 0.15 Gm. protein, 0.18 Gm. fat, 0.57 Gm. carbohydrate. Water in the proper amount need only be added to this mix.

The average contents of the dietary preparation include the following per 100 Gm. of mix as listed:

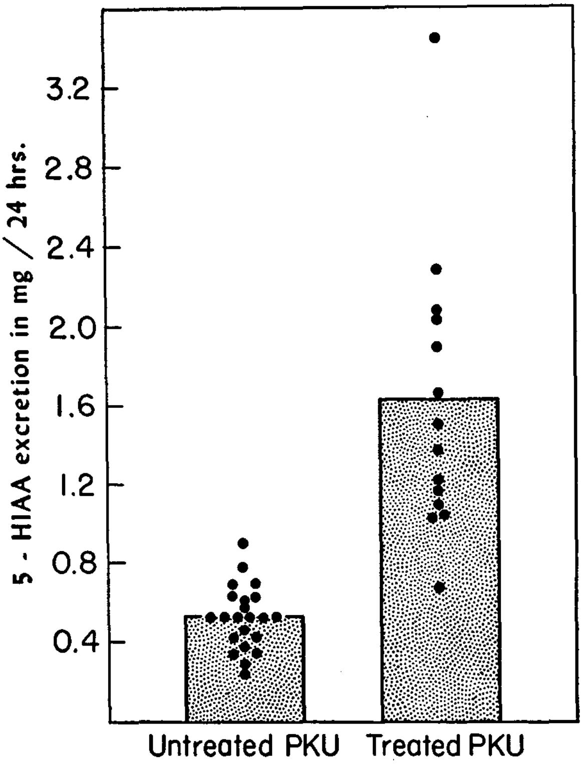
protein equivalent	15 Gm.
fat	18 Gm.
carbohydrate	57 Gm.
mineral (ash)	4.2 Gm.
moisture	2.0 Gm.
l-phenylalanine	86 mg.
l-methionine	460 mg.
l-tyrosine	430 mg.
dl-tryptophan	320 mg.
l-leucine	1.4 Gm.
l-isoleucine	720 mg.
l-valine	1.3 Gm.
l-threonine	890 mg.
l-lysine HCl	2.0 Gm.
l-aspartic acid	1.5 Gm.
l-glutamic acid	4.6 Gm.
l-arginine HCl	460 mg.
l-cystine	86 mg.
l-histidine	360 mg.
monobasic potassium phosphate	900 mg.
dibasic potassium phosphate	500 mg.
calcium hydroxide	700 mg.
magnesium oxide	100 mg.

Additional foods are supplied to provide a daily phenylalanine intake of 15 mg/kg for children above two years and 10 mg/kg for infants below two years although individual variations are sometimes necessary depending on the level of plasma phenylalanine. Phenylalanine accounts for from 4-6 per cent of the amino acids of the various food proteins. Exact values for phenylalanine content of a variety of foods are available now.[27,28,29,30]

While the alterations of phenylalanine metabolism in phenylketonuria have been studied by many investigators in the past, a disturbance in tryptophan metabolism was only recently demonstrated: Armstrong and Robinson's report in 1954 of a probable decreased excretion of 5-hydroxyindolacetic acid in phenylketonuric patients[31] was confirmed by Pare, Sandler, and Stacey[32] observing low 5-hydroxyindolacetic acid excretion with correspondingly low serotonin blood levels in such patients. The probable disturbance of tryptophan metabolism involving its pathway to serotonin was further investigated by the authors in untreated and treated phenylketonuric patients. The normal metabolic pathway of tryptophan through serotonin is as follows:

Tryptophan
↓
5-Hydroxytryptophan
↓
5-Hydroxytryptamin (serotonin)
↓
5-Hydroxyindolacetaldehyde
↓
5-Hydroxyindolacetic acid

Measurements of urinary excretion of 5-hydroxyindolacetic acid were made in phenylketonuric subjects to determine changes in the metabolism of tryptophan. The excretion of 5-hydroxyindolacetic acid was observed in 11 phenylketonuric children on normal diets and during administration of phenylalanine-low diets. In these phenylketonuric children with high phenylalanine blood levels, 5-hydroxyindolacetic acid excretion was 0.55 mg. per 24 hours. Lowering of phenylalanine below 8 mg. per 100 cc. of blood on phenylalanine-low diets resulted in a 5-hydroxyindolacetic acid excretion of 1.6 mg. per 24 hours (Fig. 2). The excretion of 5-hydroxyindolacetic acid is significantly lower in untreated phenylketonuric patients than in normal children of comparable age. Only infants below three months of age demonstrated excretion values for 5-hydroxyindolacetic acid as low as as those observed in untreated phenylketonuric subjects. (Fig. 3).



5 - HIAA excretion in treated and untreated phenylketonuria

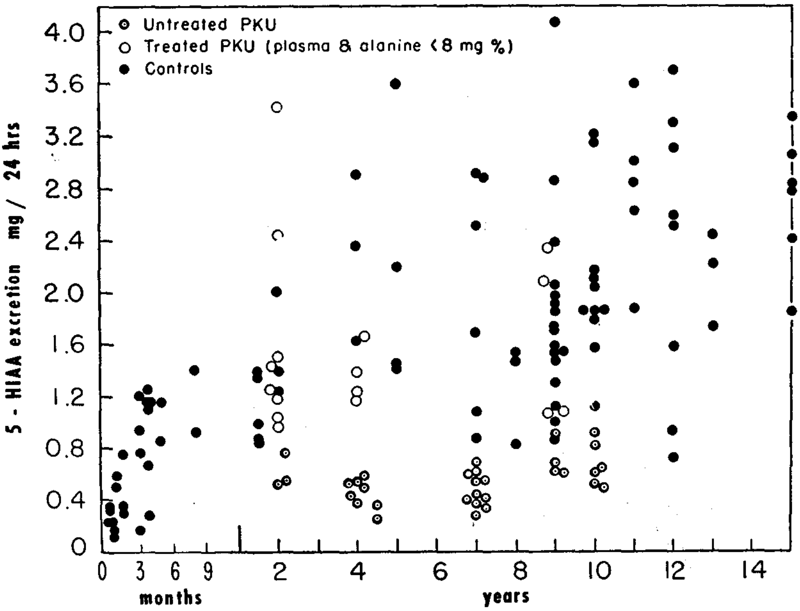
Fig. 2

When 5-hydroxytryptophan was infused in amounts of 10-20 mg. into untreated phenylketonuric children, some improvement in the electroencephalographic pattern (expressed by a decrease in spike activity and voltage) was observed during the infusion period.

The significance of these findings is not clear. The reported disturbance in tryptophan metabolism may be significant for the limitations in mental development, since serotonin is an endogenous intermediate and, theoretically, may be important for the development and function of the brain.

Conclusions:

Evidence provided in the literature and our own observations indicate the beneficial effect of early phenylalanine restriction on the



5 - HIAA excretion in controls, untreated and treated phenylketonuric children.

Fig. 3

developmental limitations of phenylketonuric subjects. The mechanism of this effect is unknown at the present time. Although this disease is fairly uncommon, it must be diagnosed early for possible prevention of mental retardation and the catastrophe of life-long institutionalization. Routine screening with ferric chloride of the urine of every infant seems to be advisable at monthly intervals during the first three months of life. The relationship of inadequacies in epinephrine, norepinephrine, and serotonin synthesis to the cerebral abnormalities of phenylketonuric subjects reguires further study.

REFERENCES

1. Foelling, A.: Ueber die Ausscheidung von Phenylbrenztraubensäure in dem Harn als Stoffwechselanomalie in Verbindung mit Imbezillitaet, Ztschr. f. Phys. Chemie 227:169, 1934.
2. Jervis, G. A.: Phenylpyruvic Oligophrenia, Proc. of the Assn. for Research in Nervous and Mental Disease 33:259, 1954.

3. Jervis, G. A.: Genetics of Phenylpyruvic Oligophrenia: A Contribution to Study of Influence of Heredity on Mental Defect, J. Ment. Sci. 85: 719, 1939.
4. Hsia, D. Y.-Y. and Driscoll, K. W.: Detection of the Heterozygous Carriers in Phenylketonuria, Lancet 2:1337, 1956.
5. Jervis, G. A.: Phenylpyruvic Oligophrenia; Introductory Study of 50 Cases of Mental Deficiency Associated with Excretion of Phenylpyruvic Acid, Arch. Neurol. & Psychiat. 33:844, 1937.
6. Strem, E. and Berendes, H.: Unpublished Observations.
7. Penrose, L. S.: Phenylketonuria: A Problem in Eugenics, Lancet 1: 949, 1946.
8. Jervis, G. A.; Block, R. J.; Bolling, D.; Kanze, E.: Chemical and Metabolic Studies on Phenylalanine; Phenylalanine Content of Blood and Spinal Fluid in Phenylpyruvic Oligophrenia, J. Biol. Chem. 134:105, 1940.
9. Jervis, G. A.: Phenylpyruvic Oligophrenia: Deficiency of Phenylalanine Oxidizing System, Proc. Soc. Exper. Biol. & Med. 82:514, 1953.
10. Mitoma, C.; Auld, R. M.; and Udenfriend, S.: On the Nature of the Enzymatic Defect in Phenylpyruvic Oligophrenia, Proc. Soc. Exper. Biol. & Med. 94:634, 1957.
11. Wallace, H. W.; Moldave, K.; and Meister, A.: Studies on Conversion of Phenylalanine to Tyrosine in Phenylpyruvic Oligophrenia, Proc. Soc. Exper. Biol. & Med. 94:632, 1957.
12. Kaufman, S.: The Enzymatic Conversion of Phenylalanine to Tyrosine, J. Biol. Chem. 226:511, 1957.
13. Kaufman, S.: The Participation of Tetrahydrofolic Acid in the Enzymatic Conversion of Phenylalanine to Tyrosine, Biochim. et biophys. acta 27:428, 1958.
14. Reem, G. H. and Kretschmer, N.: Development of Phenylalanine Hydroxylase in Liver of Rats, Proc. Soc. Exper. Biol. & Med. 96:458, 1957.
15. Jervis, G. A.: Studies on Phenylpyruvic Oligophrenia: Phenylpyruvic Acid Content of Blood, Proc. Soc. Exper. Biol. & Med. 81:715, 1952.
16. Armstrong, M. D. and Binkley, E. L. Jr.: Studies on Phenylketonuria: V. Observation on a Newborn Infant with Phenylketonuria, Proc. Soc. Exper. Biol. & Med. 93:418, 1956.
17. Berendes, H.: Unpublished Data.
18. Weil-Malherbe, H. and Bone, A. D.: The Conentration of Adrenalin in Human Plasma and its Relation to Mental Activity, J. Ment. Sc. 101: 733, 1955.
19. Himwich, H. E. and Fazekas, J. F.: Cerebral Metabolism in Mongolian Idiocy and Phenylpyruvic Oligophrenia, Arch. Neurol. and Psychiat. 44:1213, 1940.
20. Walaszek, E. and Abood, L. G.: Disturbances in Neural Metabolism Resulting from Urinary Products of Phenylketonurics, Fed. Proc. 15: 195, 1956.
21. Bickel, H.; Gerrard, J.; and Hickman, E. M.: Influence of Phenylalanine Intake on Phenylketonuria, Lancet 2:812, 1953.

22. Block, R. J. and Bolling, D.: *The Amino Acid Composition of Proteins and Foods,* Springfield, Illinois, Charles C Thomas, 1951.
23. Bickel, H.; Gerrard, J.; and Hickman, E. M.: The Influence of Phenylalanine Intake on the Chemistry and Behaviour of a Phenylketonuric Child, Acta paediat. 43:64, 1954.
24. Armstrong, M. D. and Tyler, F. H.: Studies on Phenylketonuria: Restricted Phenylalanine Intake in Phenylketonuria, J. Clin. Invest. 34: 565, 1955.
25. Horner, F. A., Streamer, C. W.; Clader, D. E.; Hassel, L. L.; Binkley, E. L., Jr.; and Durmas, K. W. Jr.: Effect of Phenylalanine Restricted Diet in Phenylketonuria II. A.M.A. Am. J. Dis. Child. 93:615, 1957.
26. Hsia, D. Y.-Y.; Knox, W. E.; Quinn, K. V.; and Paine, R. S.: A One Year Controlled Study of the Effect of Low-Phenylalanine Diet on Phenylketonuria, Pediatrics 21:178, 1958.
27. Elvehjem, C. A.: Problems in Meeting Amino Acid Requirements, J. Am. Dietet. A. 32:921, 1956.
28. Elvehjem, C. A.: Amino Acid Balance in Nutrition, J. Am. Dietet A. 32:305, 1956.
29. Wertz, A. W.; Ruttenberg, P. K.; French, G. P.; Murphy, G. H.; and Guild, L. P.: Amino Acid Content of Foods, J. Am. Dietet. A. 32: 926, 1956.
30. Murphy, D. M.; Kline, B. E.; Robbins, R. N.; Teply, L. J.; and Derse, P. H.: Amino Acids in Nine Frozen Vegetables, J. Agric. and Food Chem. 5:608, 1957.
31. Armstrong, M.D. and Robinson, K. W.: On the Excretion of Indole Derivatives in Phenylketonuria, Arch. Biochem. 52:287, 1954.
32. Pare, C. M. B.; Sandler, M.; and Stacey, R. S.: 5-Hydroxytryptamine Deficiency in Phenylketonuria, Lancet 1:557, 1957.

Staff Meeting Report

Adenocarcinoma of the Endometrium with Special Reference to Vaginal Involvement*†

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Introduction

A systematic study of adenocarcinoma of the endometrium treated on the gynecological services at the University of Minnesota Hospitals has shown secondary vaginal involvement to be a common problem complicating the therapy and prognosis of this disease. Because the medical literature contains few detailed reports on this subject, it is desirable to review the experience of a large center for the treatment of gynecological malignant disease in managing this problem.

In 1939 a definite plan was initiated at the University of Minnesota Hospitals to study the effectiveness of x-ray, radium, and simple surgery in the treatment of this disease. Bias in selection was virtually eliminated by applying a single method of treatment to as many of the total patients with this disease as possible for a chosen time period. Results (reported previously[1,2]) were evaluated in terms not of the number of cures among the patients treated but in terms of the number of cures in the entire group presenting itself for treatment during this time. Regardless of the combination of treatments studied, each patient was offered (unless contraindications were present) the most effective single therapy, namely panhysterectomy and bilateral salpingo-oophorectomy. The study also evaluated the effectiveness of x-ray and intracavitary radium when added to the basic surgical treatment. Experience had demonstrated that there was no justification for exposing patients to x-ray and intracavitary radium alone, since these were clearly inferior when applied without removal of the uterus.

In the procedure observed during 1939 and 1940, patients with endometrial adenocarcinoma began therapy with 3,000 tissue roentgens of deep x-ray to the mid-pelvis via five or more fields over a four-week period. This was followed immediately by 50 to 65 mg.

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of intracavitary radium distributed in the uterus as shown in Figure 1. The radium was left in place for 100 hours. Six weeks after radium therapy, a total hysterectomy and bilateral salpingo-oophorectomy were performed. In 1941 routine x-ray therapy was discontinued, and in mid-1946 the routine use of radium was abandoned, so that

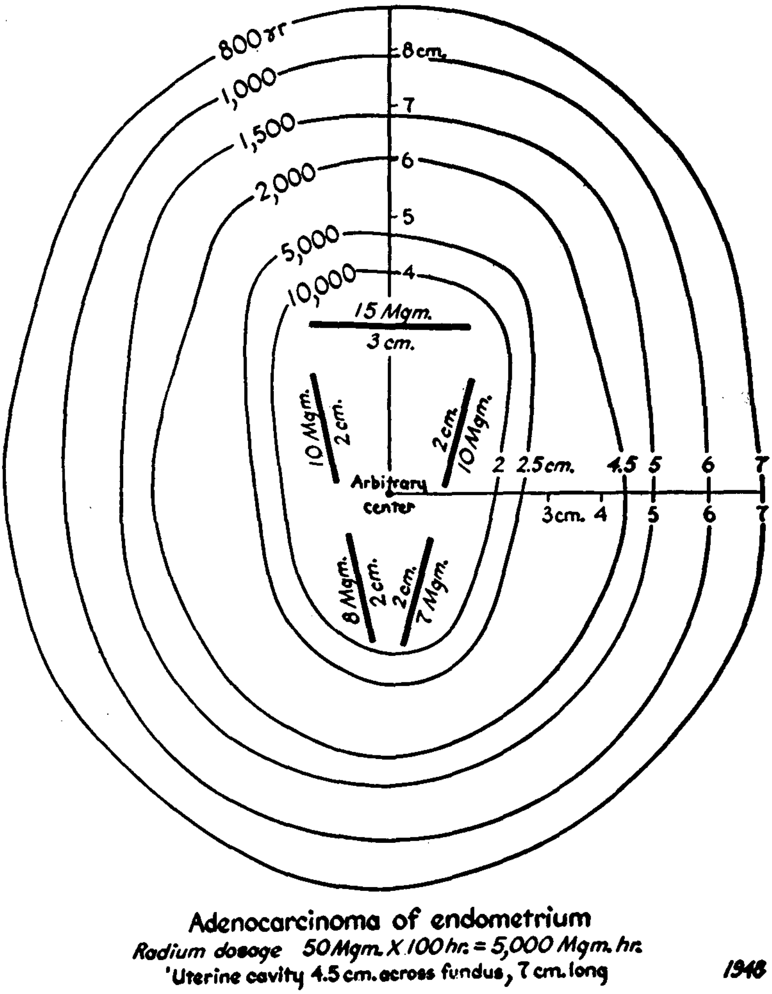

Fig. 1. Compound isodose curve showing intensity of radium radiation from multiple intracavitary source used in treating adenocarcinoma of the endometrium.

from mid-1946 to the present the sole routine treatment has been surgical. Occasional minor modifications of the planned therapy have not significantly influenced the overall results.

Study Analysis

Cases Studied

The data reported here were gathered by studying all patients with adenocarcinoma of the endometrium admitted to the Gynecology Service of University of Minnesota Hospitals from January 1, 1939 to December 31, 1953. No patients with questionable lesions were included. Microscopic evidence for endometrial and vaginal adenocarcinoma is available for each patient. The progress of all patients with vaginal lesions was followed to death or to January 1, 1958.

The lesions were classified as "reportable" or "nonreportable." The reportable group includes patients admitted to the Hospitals either with no previous treatment for adenocarcinoma of the endometrium or after negligible attempts at therapy which did not interfere with the planned treatment in use at the time of admission. The patients in this group are further divided into those in whom secondary vaginal adenocarcinoma developed before primary treatment and those in whom it developed after primary treatment. The nonreportable group contains patients who were referred here for further treatment after having had definitive therapy for the primary tumor elsewhere. Many of these were referred specifically because of vaginal lesions.

Incidence

During the period studied, 324 patients with diagnoses of primary adenocarcinoma of the endometrium were admitted to the Hospitals. The division into reportable and nonreportable groups as outlined above is shown in Table 1. Of the total number, 47 or 14.5 per cent had secondary vaginal adenocarcinoma. This incidence is compared in Table 2 with those reported by other authors for similar groups of patients. Of 51 patients treated elsewhere and referred here during this period for completion of therapy or because of recurrence, 15 or 29.6 per cent had secondary vaginal involvement. Compared with the incidence of 11.7 per cent (32 of 273) in the patients with no treatment before referral; this indicates an abnormally high degree of secondary vaginal involvement among the nonreportable group.

Signs and Symptoms

A secondary vaginal lesion developed in 18 patients after treatment of the primary endometrial lesion at University of Minnesota

TABLE 1

SECONDARY VAGINAL ADENOCARCINOMA CLASSIFIED ACCORDING TO TIME OF APPEARANCE WITH REFERENCE TO PRIMARY TREATMENT

	Total Patients		Number Patients
Reportable	273	Vaginal recurrence after primary treatment at UMH	18
		Vaginal adenocarcinoma on admission to UMH. No previous treatment	14
Nonreportable	51	Primary treatment elsewhere. Vaginal adenocarcinoma on admission to UMH.	15
	324		47

Hospitals. Of these, 12 were asymptomatic when the recurrence was discovered on routine follow-up examination. Six patients had had symptoms for one to seven weeks before presenting themselves for pelvic examination; four of these six had vaginal bleeding, one had bladder pain, and one complained of a vaginal discharge.

Of the 14 patients with vaginal involvement prior to primary treatment, 13 had a history of vaginal bleeding and 12 complained of multiple signs and symptoms. Weight loss, back, abdominal or pelvic pain, vaginal discharge, and edema of the lower extremities were the other signs or symptoms in order of frequency, most of these being indications of a far advanced malignant process.

Only two of the 15 patients referred because of a secondary

TABLE 2

INCIDENCE OF SECONDARY VAGINAL ADENOCARCINOMA IN SIMILAR GROUPS OF PATIENTS

Author	Total Patients	Number with secondary Vaginal Tumor	Per Cent
Meigs[3]	206	25	12.1
Way[4]	102	14	13.7
Strachan[5]	30	5	16.7
Finn[6]	266	14	6.4
Prem	324	47	14.5

vaginal lesion after primary treatment elsewhere were without signs or symptoms when the vaginal lesion was discovered. Twelve of the remaining 13 had had signs or symptoms for the preceding two weeks to 12 months. Bleeding was the presenting complaint in 11 of the 13 symptomatic vaginal recurrences in this group.

Interval Between Primary Treatment and Vaginal Recurrence

Among the 259 patients with no vaginal involvement prior to primary therapy for endometrial carcinoma at University of Minnesota Hospitals, the first vaginal recurrence was discovered in all but one of the 18 reportable patients within three and one-half years of the primary treatment. Thus it is unlikely that an initial vaginal lesion will develop after the present date in a patient who received primary treatment before December 31, 1953—the terminal date of the period of study. In the single exception in this group a small lesion, which proved to be adenocarcinoma, developed to the right of the urethral meatus 78 months after original treatment. The median time between original treatment and diagnosis of the secondary vaginal lesion was ten months. Among this group lesions in the vaginal vault appeared earlier than those lower in the vaginal tube.

In twelve of the 15 patients in the nonreportable group secondary vaginal lesions developed within three and one-half years of initial treatment. In the other three, secondary vaginal lesions appeared 44, 74, and 107 months respectively after primary therapy, the median interval in this entire group being 13 months. Again, lesions in the vault appeared earlier than did lesions in the lower vagina.

Site of Recurrence

The 47 patients with vaginal involvement in this study are tabulated according to general location suggesting mode of spread in Table 3. Thirteen of the vaginal lesions occuring after primary treatment here or elsewhere were described on the patients' records as being in the vault. If five other lesions simply designated as located in the upper one-third of the vagina are included, 18 of 33, or nearly 55 per cent, were vault recurrences. Eleven other leisons were located in the lower anterior vagina, most of them within 1-2 cm. of the urethral meatus in the midline or slightly eccentric. Three lesions were in the posterior lower vagina just inside the hymenal ring. One lesion involved the vault and lateral lower vagina.

TABLE 3
SECONDARY VAGINAL LESIONS CLASSIFIED ACCORDING TO GENERAL LOCATION SUGGESTING MODE OF SPREAD

Type of Vaginal Involvement	Reportable: Initial ℞ UMH	Reportable: Vaginal lesion pre-℞	Nonreportable. Initial ℞ Elsewhere	Totals
1. Discontinuous lesion	9	9	4	22
2. Vaginal vault	8		10	18
3. 1 & 2	1			1
4. Continuous extension		3	1	4
5. 1 & 4		2		2
Totals	18	14	15	47

About one-half of the patients with vaginal involvement prior to primary treatment had multiple lesions. Although lesions were observed in almost every area in the vagina, the majority were located in the vault and beneath the urethral meatus, and several were located elsewhere in the lower vagina and vulva.

Cervical Extension

A high incidence of extension of the primary endometrial tumor to the cervix was found among the reportable patients who had a vaginal lesion before or after primary treatment. The diagnosis of cervical extension was made by gross and microscopic examination of the cervix after differential curettage or panhysterectomy. In some, clinically identifiable tumor protruded from the cervical canal. The incidence of cervical extension among the reportable cases is shown in Table 4. Included in the group with isthmic tumor were three lesions that did not involve the cervix but diffusely covered the endometrial cavity, stopping abruptly at the level of the internal cervical os, and one early lesion solely confined to the isthmic portion of the uterus. In six other cases there was not sufficient information in the records to indicate whether or not the uterine isthmus or cervix was involved. Only one patient was definitely known to have no tumor in the cervix or the isthmus.

Classification of the nonreportable patients as to cervical or isthmic extension was not possible because the gross specimen or accurate gross and microscopic description of the location of the tumor in the uterus was not available.

TABLE 4

Reportable Patients with Vaginal Lesions Secondary to Adenocarcinoma of the Endometrium with Cervical or Isthmic Extension

	Total Cases	Cervical Extension	Isthmic Tumor	Neither	Not known
Tumor in vagina after original treatment	18	11	3	1	3
Tumor in vagina before original treatment	14	10	1	0	3
Totals	32	21	4	1	6

Influence of the Type of Primary Treatment

The program of planned therapy as previously outlined provided an ideal opportunity to determine the effect of various types of treatment on the incidence of vaginal recurrence. The results tabulated in Table 5 show no significant difference in the incidence of vaginal recurrence in patients treated during the three planned program periods.

TABLE 5

Incidence of Secondary Vaginal Adenocarcinoma in Reportable Patients Related to Period of Planned Therapy

	Total No. Patients Seen	Vaginal Tumor Prior to Primary Rx UMH	No Vaginal Tumor Prior Rx UMH	Vaginal Recurrence After 1° Rx UMH	Per Cent Recurrence
1939-40[a]	39	4	35	1	2.8
1941-46[b]	117	3	114	8	7.0
1947-53[c]	117	7	110	9	8.2
Totals	273	14	259	18	7.0

a. An attempt was made to treat as many patients as possible with full course of deep x-ray, intrauterine radium, and panhysterectomy with bilateral salpingo-oophorectomy.
b. Same as "a" with exception of deep x-ray
c. As many patients as possible treated by panhysterectomy and bilateral salpingo-oophorectomy only.

Influence of Primary Treatment on Site of Vaginal Recurrence

All patients with vaginal recurrence after initial treatment were studied to determine if the type of treatment received for the primary lesion influenced the location of the subsequently developed vaginal lesion. The results are shown in Table 6.

TABLE 6

INFLUENCE OF PRIMARY TREATMENT ON SITE OF VAGINAL RECURRENCE AMONG PATIENTS WHO DID NOT HAVE DEMONSTRABLE VAGINAL TUMOR AT TIME OF ORIGINAL DIAGNOSIS

Type of Primary Treatment	Total No. Treated UMH	Reportable: Upper ½ of Vagina	Reportable: Lower ½ of Vagina	Nonreportable: Upper ½ of Vagina	Nonreportable: Lower ½ of Vagina
X-ray — Radium — Surgery	27	0	3a	0	0
Radium — Surgery	61	1b	6d,h	1f	0
Surgery only	118	7c	1c	7	3e
X-ray — Radium	6	0	0	0	1
Surgery — X-ray	7	0	0	2	0
Radium only	27	0	0	1g	0
None	13	0	0	–	–
Totals	259	8	10	11	4

a. One post-op x-ray; one post-op intravaginal radium for prophylaxis
b. 10-month interval between radium and surgery
c. Wertheim radical hysterectomy
d. 12 years between radium and surgery in one patient
e. One subtotal hysterectomy
f. Inadequate dose of radium 3 years before subtotal hysterectomy
g. 44 months after token dose radium; continuous extension of tumor over cervix to vaginal vault
h. One patient also had vault involvement.

These data show that the incidence of upper vaginal recurrence is reduced when preoperative intracavitary radium is used in adequate dosage. Incidences of vaginal recurrence for each type of primary treatment can be calculated from this table for the whole period of the study. The true relationship between rates of recurrence for the various types of primary treatment is not apparent in Table 6, since the group receiving surgery alone is favorably weighted with patients who are better risks and those with less advanced lesions.

Since the total number of nonreportable patients given primary treatment is not known, it is not possible to calculate vaginal recurrence rates according to type of treatment for this group.

TREATMENT OF SECONDARY VAGINAL LESIONS

The treatment of vaginal recurrence in the 33 patients in whom the secondary lesions developed only after the initial treatment here or elsewhere was more standardized than was apparent at first glance: Interstitial implantation of radon seeds usually in one plane in and around the lesion was the treatment of choice when the lesion was located where the urethra, bladder, or rectum would not be seriously endangered. When the use of radon seeds was contraindicated, screened short-focal-distance surface radiation was employed. This radiation therapy was usually administered from a linear source in a dental wax mold fitted to the vagina, a 2 x 3.5 cm. rubberized cylindrical container, or a Kaplan colpostat. Vault lesions were usually treated with the cylindrical container or colpostat tightly packed with gauze against the vaginal apex to prevent displacement. The dental wax mold was favored when lesions were located beneath the bladder or urethra or in the rectovaginal septum. Standard tables were used to calculate the quantity of element and the time necessary to administer a cancerocidal dose to the depth of the tumor. In the earlier years of the study period, 7-8 erythema skin doses were administered to the depth of the tumor and a short distance beyond. More recently this dosage has been increased to 10 or 12 erythema skin doses without increasing radiation damage to normal surrounding tissue.

The vaginal lesions of three patients were not treated. One other patient with a very small lesion had no further treatment after an excision biopsy. Seven patients received 3,000 tissue roentgens of deep x-ray given diffusely to the mid-pelvis in addition to screened surface or interstitial radiation. When x-ray was given, a perineal field was included if the lesion was in the lower vagina. Two other patients were treated with combinations of the above methods.

Of the 14 reportable patients with secondary vaginal tumor found on first admission, 10 either were not treated or were treated with token amounts of radium. Of these 10, three had the uterus partially or completely removed, and one of these three patients was given x-ray therapy as well. Four received intensive radiological treatment consisting of intrauterine and vaginal radium with or without deep x-ray or radon seeds. One patient after this type of radiation therapy was operated upon but the tumor was found inoperable.

Results of Treatment

Reportable Patients

Of the 18 patients with secondary vaginal lesion after primary treatment at University Hospitals, six have survived five or more years after first such recurrence. One of these six patients was alive with presumed mediastinal metastasis after treatment of three separate recurrences of vaginal involvement. The others were without clinical tumor at death or at the last examination. The patient who has survived the longest was alive and well 205 months after vaginal treatment. The minimum five-year survival for this group after discovery of the first vaginal lesion is 33 per cent, and after primary treatment, 50 per cent.

Only one of 10 patients who had vaginal tumor when first seen, and who was not treated or incompletely treated radiologically or surgically, survived beyond seven months. She died 15 months after total hysterectomy and two courses of vaginal radium. The only long-term survivors were four patients who underwent intensive intrauterine and intravaginal radium treatments with or without the addition of external radiation. These four survived 26, 31, 56, and 78 months, respectively. All eventually died of tumor or are presumed to have died with tumor present. The five-year survival rate for this group is shown in Table 7.

TABLE 7

FIVE YEAR SURVIVALS OF PATIENTS WITH SECONDARY VAGINAL ADENOCARCINOMA

	Group Total	Five-Year Survivals: Following Primary ℞	Five-Year Survivals: Following ℞ of First Vaginal Recurrence
Reportable			
Primary ℞ at UMH	18	9 (50%)	6[a,b] (33%)
Vaginal lesion on admission to UMH	14	1 (7%)	1 (7%)
Nonreportable			
Primary ℞ elsewhere	15	6 (40%)	5[c] (33%)
Totals	47	16 (34%)	12 (26%)

a. 4 living without tumor, 1 dead without tumor, 1 living with presumed tumor
b. Two additional living without tumor at 23 and 53 months
c. 3 alive, 2 dead, all without tumor

Nonreportable

The five-year survival rate for the 15 patients who received primary treatment elsewhere and were referred to University of Minnesota Hospitals with vaginal recurrence is also shown in Table 7. Of the five survivors, two died of unrelated causes 66 and 144 months, respectively, after treatment of the vaginal lesion. The other three are well and clinically free of tumor 60, 82, and 110 months, respectively, after vaginal treatment. The five-year survival rate following primary treatment is also shown.

TABLE 8

FIVE-YEAR SURVIVAL RELATED TO TIME INTERVAL BETWEEN PRIMARY TTEATMENT AND VAGINAL RECURRENCE

Time of Recurrence	Patient Classification	Total Number	5-Year Survival After Vaginal Recurrence Number	Per Cent
< 24 months	Reportable	13	4[a]	25
	Nonreportable	11	2	
> 24 months	Reportable	5	2[b]	56
	Nonreportable	4	3	
Totals		33	11	33

a. One additional patient alive without tumor 53 months after vaginal recurrence
b. Two others alive—one with tumor at 10 months, one without tumor at 23 months after vaginal recurrence

FACTORS INFLUENCING PROGNOSIS

Time interval between primary treatment and vaginal recurrence

The five-year survival rate as related to the time interval between the primary treatment and the vaginal recurrence is shown in Table 8. The prognosis appears better among those in whom vaginal lesions developed not earlier than two years after primary treatment.

Location of lesion

Although the groups are too small to warrant absolute conclusions, study of five-year survival among patients who developed a vaginal lesion after primary treatment as related to location of the vaginal lesion shows the prognosis to be better when the site of recurrence was the lower vagina. Of the 13 patients with vaginal metastasis in the lower one-half of the vagina, seven, or 55 per cent, have survived five years or more after vaginal treatment. Of the 19 with the lesion located in the upper one-half of the vagina only four, or 21 per cent, survived more than five years after such treatment. A single patient with involvement of the upper and lower vagina survived less than five years.

Type of treatment

The five-year survival rate as related to the type of treatment of the vaginal recurrence developing after primary treatment is shown in Table 9.

TABLE 9
FIVE-YEAR SURVIVAL AS RELATED TO TYPE OF TREATMENT OF VAGINAL RECURRENCE AMONG PATIENTS WITHOUT VAGINAL LESIONS PRIOR TO PRIMARY THERAPY

Method of Treatment	No. of Patients	Five-Year Survival	Per Cent
Radon seeds	12[a]	7	59.5
Screened contact			
Radiation via dental wax mold	7[b]	3[e]	42.9
Rubber cylinder	7[c]	0	0
Colpostat	2[d]	0	0
Excision	2	1	50
None	3	0	0
	—	—	—
Totals	33	11	33

a. One had x-ray and one a vaginal mold in addition.
b. Four had x-ray in addition.
c. One had surgical excision and one had x-ray in addition.
d. One had x-ray in addition.
e. Two had x-ray in addition.

Discussion

Finn[6] and Speert[7] reported that 69 per cent and 75 per cent, respectively, of recurrences of adenocarcinoma of the endometrium are discovered within two years after initial therapy. Of the secondary vaginal adenocarcinomas that developed after treatment of the primary lesion, 69.7 per cent did so within two years and 90 per cent within three and one-half years. Vaginal recurrences appeared in three patients 74, 78, 107 months, respectively, after primary therapy. Late vaginal recurrences have also been reported by others. Finn[6] reported three cases of vaginal recurrence five, seven, and eleven years after primary treatment. Meigs[3] and Strachan[5] mentioned patients with a five- and a nine-year interval, respectively, between primary treatment and vaginal recurrence. In six of Dobbie's[8] patients vaginal lesions developed four to seven years after primary therapy.

Although the patient previously treated for endometrial adenocarcinoma should recognize by virtue of her experience the significance of vaginal bleeding, she does not always immediately report to her physician this common sign of vaginal recurrence. Furthermore, a study of recurrences of endometrial carcinoma by Finn[6] indicated a 1 per cent per year recurrence rate after three years. These data and experiences demonstrate the necessity for frequent planned follow-up examinations of all patients for at least three years after primary treatment and ideally at periodic intervals for life.

Our experience shows that routine periodic pelvic examination of the treated patient will demonstrate the vaginal recurrence before it becomes symptomatic. The number of patients is too small to permit us to determine statistically if early discovery significantly increases the five-year survival rate, but it is reasonable to believe that it does.

Secondary vaginal adenocarcinoma may be found in any area of the vagina or subjacent tissue. 1) Lesions in the lower two-thirds of the vagina are usually submucosal and may or may not ulcerate the surface; they are discontinuous or true metastatic lesions because they are located some distance from the primary tumor but identical to it histologically. These lesions probably result from retrograde lymphatic or venous spread from the cervix. When the cervix is involved, viable tumor cells may gain entrance to the paracervical lymphatic and venous channels. When interference with the natural route of drainage from the paracervical tissues occurs, migration through the communicating channels to the lower vagina is probable.

2) Following hysterectomy the tumor often recurs at the vaginal apex or vault adjacent to the removed cervix, frequently in the scar of the vaginal incision. Two modes of spread may be responsible for this type of recurrence: Either the lesion may arise in the line of resection as a true implant of viable tumor cells spilled during hysterectomy, or tumor cells in lymphatic or venous channels surrounding the cervix may implant and grow in the vaginal incision when these channels are interrupted at hysterectomy. Lesions in this location, referred to as vaginal "vault" or "cuff" recurrences, are usually polypoid, they bleed easily, and grossly they often resemble granulation tissue.

3) A third type is seen in the untreated patient in whom the malignant process is far advanced, with vaginal involvement resulting from contiguous spread over the cervix to the vagina. Often the entire cervix and upper vagina are replaced by clinically obvious tumor. Patients exhibiting the "contiguous extension" that characterizes this pattern of spread often have metastatic lesions in the vagina as well.

This study indicates that vaginal recurrence is more frequent among patients who have an original cervical or isthmic spread of the endometrial tumor. This type of involvement can usually be dignosed before treatment by a careful differential or fractional curettage when gross tumor is not visible on the cervix or in the cervical canal.

Although cervical extension is usually a manifestation of advanced tumor, Leissner[9] has stated that cervical spread may occur when the primary lesion is superficial and localized in the uterine isthmus. When the cervix is involved with tumor, lymph and venous vessels in the paracervical tissues that communicate with pelvic and vaginal venous and lymphatic channels may contain viable tumor cells. This probably accounts for the generally inferior results of standard treatment of endometrial adenocarcinoma when cervical extension is present as reported by Kottmeier,[10] Randall and associates,[11] and Taylor and Becker.[12]

The techniques reported here for the treatment of vault recurrences produced a 21 per cent five-year survival rate. This relatively poor rate suggests that clinically undetectable tumor may have existed beyond the reach of the maximum local vaginal radiation therapy administered. This would be expected if vault recurrence were the result of venous or lymphatic spread rather than direct implantation into the line of resection of viable tumor cells spilled from the uterus at hysterectomy.

When the uterus and cervix are treated by intracavitary radium before surgery, the incidence of secondary vaginal lesions is the same as among those treated by simple surgical methods alone, but the lesions are more likely to recur in the lower vagina than in the vaginal vault. This indicates that the vaginal vault and paracervical tissues receive sufficient radiation to prevent the vault lesion but not sufficient to destroy tumor cells in lymphatic or venous channels at slightly greater distances from the vault. These cells subsequently migrate toward the pelvic walls and lower vagina to produce clinically detectable lesions. Our figures show the prognosis is better following treatment of the lower vaginal lesions, suggesting that the intracavitary radium is partially effective in preventing pelvic as well as vault recurrence.

If these arguments are valid, irradiation of the upper vagina and cervix with radium doses similar to those given for carcinoma of the cervix should be administered preoperatively to the patient with extension of endometrial adenocarcinoma to the lower uterine segment or cervix. Irradiation thus distributed will produce a greater effect in the lateral pelvis and lower vagina than will irradiation given from intrauterine sources alone. When administered to patients with cervical or lower uterine extension, this treatment should increase the five-year survival rate while decreasing the incidence of subsequent vaginal involvement.

Prophylactic postoperative radiological treatment of the vagina has reduced the number of secondary vaginal lesions after surgical treatment. This type of treatment may take several forms: Finn[6] suggested the use of low voltage x-ray by means of the intravaginal cone for this purpose. Postoperative screened surface radium irradiation of the vaginal vault as employed by Kottmeier,[10] or of the entire vaginal tube as recommended by Dobbie[8] is more popular than x-ray. Dobbie[8] reported an incidence of vaginal recurrence of 10-11 per cent in two groups, one treated by hysterectomy alone and one by hysterectomy plus postoperative external x-ray to the pelvis; but in a third group, receiving routine postoperative intravaginal radium treatment instead of x-ray, the incidence decreased to 2.4 per cent. Although the incidence of vaginal recurrence was reduced in the group treated with intravaginal radium, the five-year survival rates in all three groups were nearly identical.

The main disadvantage to the postoperative use of radium intravaginally is the resulting stenosis, which makes the upper vagina unavailable for subsequent direct examination.

Finn,[6] observing no prolonged survival after irradiation of recurrent vaginal lesions, advocated radical or ultra-radical surgical removal of these lesions if cures were to result. Routine radical surgical attack on vaginal recurrences, however, is unrealistic since the patient with this disease is often elderly, obese, diabetic, and hypertensive. Moreover, the high applicability rate, nearly complete absence of morbidity, and the excellent results obtained with local radiation treatment reported here and by Dobbie[8] indicate that radiological attack is the treatment of choice for the secondary vaginal lesion. Radical surgery should be considered only if the vaginal lesion is too large or extensive to benefit from radiation therapy and if the patient represents a reasonable operative risk. Recently at the University of Minnesota the interstitial implantation of radon seeds has been increasingly favored over other methods in the treatment of secondary vaginal lesions.

The treatment of the patient with vaginal metastasis or extension before primary therapy is discouraging. Surgical attack short of ultra-radical procedures gives poor results. Occasionally hysterectomy, if the uterus is small and mobile, plus radiation treatment applied to a solitary vaginal lesion may be indicated. Intensive intrauterine, vaginal radium therapy and deep x-ray have produced good but merely palliative results.

Dobbie[8] has reported a 30 per cent five-year survival rate among patients in whom secondary vaginal lesions occurred after being clinically free of tumor three months after primary treatment. The minimum five-year survival rate of 33 per cent reported here following treatment of the first vaginal recurrence exceeds any previously described.

Since two additional patients in our series are alive and without evidence of recurrence 23 and 53 months after vaginal treatment, the five-year survival rate may eventually reach 40 per cent. These results indicate that vaginal recurrence following treatment for adenocarcinoma of the endometrium need not be fatal.

The prognosis for the patient who has adenocarcinoma of the endometrium with extension or metastasis to the vagina prior to primary treatment is poor, however. Among the small group of patients treated here palliative therapy in the form of intensive radium radiation gave the best results.

Summary

1. Of 324 consecutive patients with adenocarcinoma of the endometrium treated at the University of Minnesota Hospitals between 1939 and 1953, 14.5 per cent have had secondary vaginal tumor either when first seen or after primary treatment.

2. Among these patients the most commonly observed sign of vaginal recurrence is vaginal bleeding.

3. Although an occasional secondary vaginal lesion may appear many years after primary treatment, 90 per cent occur within three and one-half years after primary therapy.

4. Secondary vaginal lesions may be found in any area of the vagina. They may result from retrograde lymphatic or venous spread, implantation of viable cells, or continuous growth from the endometrium over the cervix to the vagina.

5. Extension of the tumor into the cervix and/or lower uterine segment predisposes to secondary vaginal lesions. It is important that this fact be recognized and that appropriate changes from standard therapy be applied accordingly.

6. Preoperative x-ray and intracavitary radium, either singly or in combination, do not seem to alter the total incidence of secondary vaginal lesions but do appear to influence their location. After treatment by hysterectomy alone, secondary lesions tend to occur in the vault, whereas following preoperative treatment with intracavitary radium, they recur more frequently in the lower vagina.

7. The treatment of choice for the recurrent vaginal lesion is interstitial implantation of radon seeds if the pelvic organs are not

endangered by such treatment. Where such danger is probable, screened surface radiation is used. If the tumor be excessively large, preliminary x-ray treatment may be used to reduce it.

8. Treatment of the vaginal recurrence as outlined has produced a minimum five-year survival rate of 33 per cent. Lesions discovered more than two years after primary therapy and those in the lower vagina seem to offer a better prognosis than do others.

9. A plan is outlined for treating the patient with adenocarcinoma of the endometrium in whom vaginal recurrence is most likely to develop. This treatment is designed not only to reduce the incidence of vaginal recurrence but also to increase the five-year survival for the whole group of patients with endometrial adenocarcinoma.

References

1. McKelvey, John L.; Holly, Roy G.; and Prem, Konald A.: Adenocarcinoma of the Endometrium, Am. J. Obst. & Gynec. 64A:295, 1952.
2. McKelvey, John L. and Prem Konald A. *Progress in Gynecology,* Vol. III, pp. 465-475, New York, Grune and Stratton, 1957.
3. Meigs, Joe V.: Adenocarcinoma of the Fundus of the Uterus: A report concerning the vaginal metastases of this tumor, N. Eng. J. Med. 201: 155, 1929.
4. Way, Stanley: Vaginal Metastases of Adenocarcinoma of the Uterus, J. Obst. & Gynec. Br. Emp. 58:558, 1951.
5. Strachan, Gilbert I.: Vaginal Metastases in Carcinoma of the Body of the Uterus, Proc. Roy. Soc. Med. 24:118, 1930.
6. Finn, W. F.: Time, Site, and Treatment of Recurrences of Endometrial Carcinoma, Am. J. Obst. & Gynec. 60:773, 1950.
7. Speert, H.: The Time of Recurrence of Endometrial Carcinoma, Am. J. Obst. & Gynec. 57:547, 1949.
8. Dobbie, B. M. Wilmott: Vaginal Recurrences in Carcinoma of the Body of the Uterus and their Prevention by Radium Therapy, J. Obst. & Gynec. Br. Emp. 60:702, 1953.
9. Leissner, H.: Studies on the Classification of Carcinoma of the Uterus, Acta Radiol. 78 Supplement, 1950.
10. Kottmeier, Hans L.; *Carcinoma of the Female Genitalia,* Baltimore, Williams and Wilkins Co., 1953.
11. Randall, J. H.; Mirik, D. F.; and Wilken, E. E.: Endometrial Carcinoma, Am. J. Obst. & Gynec. 61:596, 1951.
12. Taylor, H. C. and Becker, W. F.: Carcinoma of the Corpus Uteri, Surg. Gynec. & Obst. 84:129, 1947.

Staff Meeting Report

Metabolic Effects of Radiation and Chemotherapy On Mammary and Ascites Carcinomata*

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Introduction

The eradication of cancer, which is the ultimate goal of researchers in this field, can only be accomplished by understanding the mechanisms involved in the origin and growth of cancer cells. Information of this nature is being sought in our laboratories using transplanted mammary and ascites carcinomata in mice. Analyses have been made of various organic compounds in the cells of these tumors, with special consideration of the effect of irradiation on the nucleotides in acid-soluble extracts from tumor homogenates. It is hoped that this work, some of which will be considered here, will assist in a better understanding of metabolic processes involved in malignant disease, and of changes in these processes brought about by such agents as radiation and chemicals, which are also being used for cancer treatment.

Survival data on cancer patients subjected to radiation or chemotherapy for control of the disease indicate the value of these methods of treatment. It is believed, however, that these survival figures can be improved by continued investigation of treatment methods. Part of our research has been devoted specifically to studying the effects of combined administration of chemicals and radiation. Data from these investigations, are also expected to yield basic information on the biological phenomena associated with cancer metabolism.

I. Investigation of the Metabolism of Cancer Cells

In studying the metabolism of malignant tumors, we have been

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concerned with the biochemical processes that lead to the uptake of radioactive phosphorus (P^{32}) into organic compounds of the transplanted mammary carcinoma and ascites carcinoma in mice. A decrease in the uptake of P^{32} into deoxyribonucleic acid (DNA) has been observed after the administration of a dose of roentgen rays as low as 250 r (roentgens).[1,2] This effect gradually increases with doses up to a maximum at about 1500 r for mammary carcinoma, resulting in 50 per cent inhibition. Further increases in the x-ray dosage do not result in greater inhibition of the P^{32} uptake. Similar inhibition of DNA synthesis by radiation has been observed in normal regenerating tissues such as liver.[3] Beltz and associates recently reviewed the effects of radiation on nucleic acid synthesis.[4]

The incorporation of P^{32} into other cellular components of the mammary carcinoma, such as ribonucleic acid, phospholipids, and phosphoproteins, does not appear to be affected by irradiation. Furthermore, the specific activities of the P^{32} of complete homogenate are not significantly different in irradiated and nonirradiated tumors. The specific activity of the acid-soluble extract from the homogenate of irradiated tumors, however, appears to be lower than that of a comparable extract from nonirradiated tumors. Further evidence of this difference has appeared from the analyses in the fractions obtained by eluting the extracts from a Dowex 1 column (anion exchange resin) by formic acid and ammonium formate buffer. While the patterns of the fractions obtained appeared much the same qualitatively, certain differences became apparent when sufficient material was available for quantitative determination of the specific activity of the individual fractions. Some of this difference in specific activity may have been due to phosphorus compounds which passed through the column without the addition of the eluent. Certain fractions eluted by formic acid showed higher specific activities in the irradiated than in the nonirradiated samples. One interpretation of these results is that these substances have not been utilized in the biosynthesis taking place in irradiated tissues.

Considerable effort has been made to identify the fractions obtained from the acid-soluble extracts by column chromatography, facilitated in many cases by analyses of ultraviolet absorption spectra and paper partition chromatography.

Materials and Methods

Inbred mice of the Z strain were inoculated with mouse mammary carcinoma in the dorsal aspect of the right hind leg. Two to three weeks later, when the tumors were about 1 cm. in diameter, each animal in one group was placed on a treatment cone shielded with lead in such a way that only the leg bearing the tumor was irradiated with 4500 r (dose rate 150 r per minute) while the rest of the animal was protected.[5] Animals in the control group were immobilized in a similar cone for the same time, but were not subjected to irradiation. All animals both irradiated and nonirradiated were given an intraperitoneal injection of P^{32} as inorganic disodium phosphate in physiological saline solution (1 microcurie per gram body weight). Each animal was killed by cervical dislocation exactly 160 minutes after this injection, whereupon the tumors were immediately excised and frozen in liquid nitrogen. The irradiated tumors were homogenized, together with four volumes of 0.6 N perchloric acid, as were the nonirradiated tumors. After setanding half an hour in the cold temperature, the homogenates were centrifuged at 1450 g, and the residue was washed with 0.2 N perchloric acid equal in volume to that of the original homogenate. The original extracts, together with the washings, were neutralized with ice-cold 5 N potassium hydroxide.

After the neutralized extract had stood in the cold for at least 30 minutes, the potassium perchlorate was centrifuged out. Aliquots of the neutralized extracts were taken for the determination of inorganic phosphorus,[6] total phosphorus,[7] nitrogen,[8] and radioactivity. The radioactivity was assayed by transferring 2 ml. from each fraction to an aluminum cup which was placed over a thin end-window Geiger tube.

The extracts from each of the irradiated and nonirradiated tumors were then fractionated on identical Dowex 1 columns (22 cm. long and 1 cm. in diameter) according to the method of Hurlbert and associates.[9] The two columns were fed by siphons from the same mixing flask in which the concentration of formate ions was gradually increased. The rate of flow was adjusted in each column so that a 6 ml. fraction was collected every 25 minutes.

The radioactivity and ultraviolet absorption (260 microcuries) of each fraction were determined, the resulting values being plotted against the volume of eluate through the column (Fig. 1). The fractions corresponding to eack peak were pooled, and ten samples from each were ashed with sulfuric and nitric acids. From the determinations of radioactivity and phosphorus content the specific activity was calculated. (No correction was made for self-absorption during counting, and the specific activity of each sample was recorded as a percentage of that of the inorganic phosphorus.)

To test the resproducibility of the method, identical extracts were

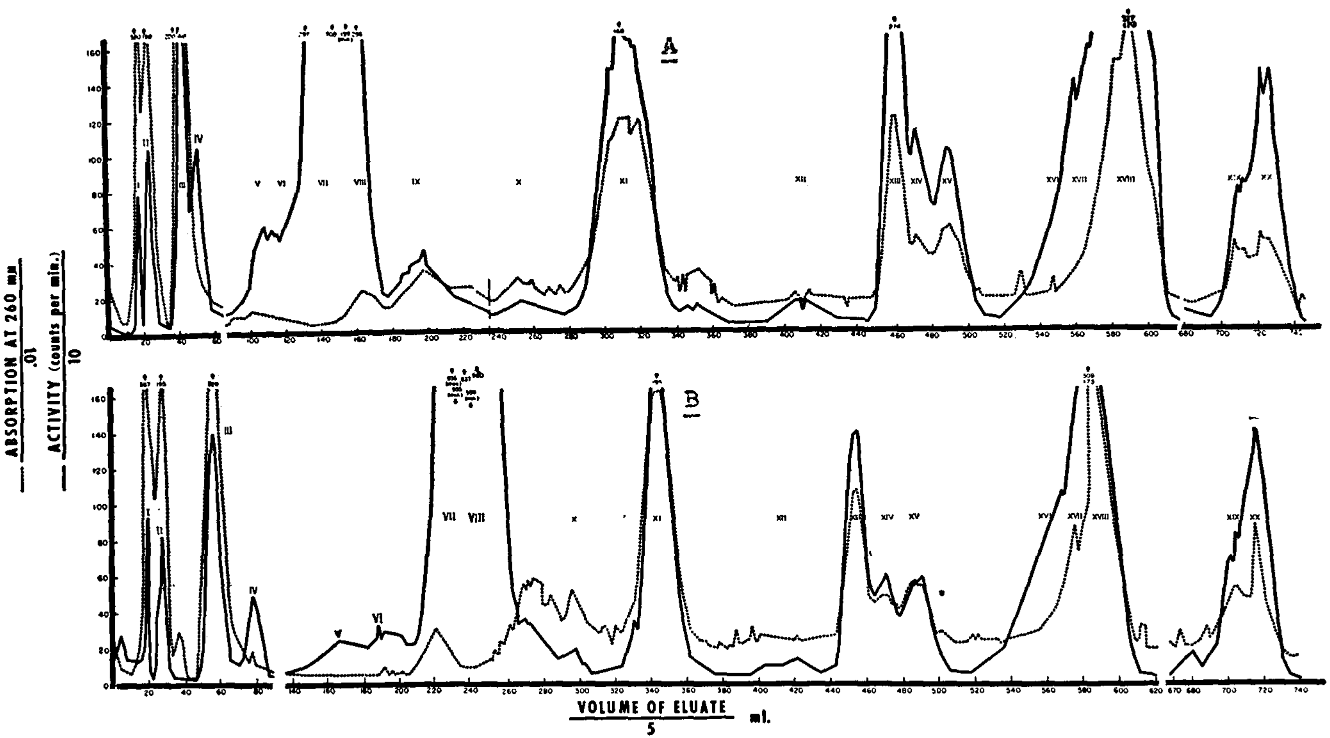


Fig. 1. Radioactivity (solid lines) and Ultra-Violet Absorption (dotted lines) of Acid-Soluble Phosphorus Compounds of Irradiated (A) and Non-Irradiated (B) Mouse Mammary Carcinoma (L_3). (See Table 1 for identification of peaks)

placed on both columns; extremely high agreement was obtained in measurements of radioactivity and ultraviolet absorption of the various fractions collected.

Results

A. *Nucleotides from Mouse Mammary Carcinoma.* Results of a study with a tumor (L_3) are shown in Table 1. Irradiation produced no significant change in the relative specific activities (r.s.a.) of CMP, DPN, AMP, GMP, and UDPGA.* The failure to obtain real differences in r.s.a. of GMP and DPN may have been due to the small amount of material available for analyses. The r.s.a. of the whole acid extract appears to be decreased slightly by irradiation, with the decrease most evident in r.s.a. of ADP, UDPAG, UDPG, GDP, CTP, ATP, GTP and UTP.

Another mammary tumor (A_3) gave similar results, but after further transplantation its response to irradiation appeared to change. Table 2 shows that after three transplantations, the r.s.a. of the total acid soluble phosphorus was lowered by irradiation, as was the r.s.a. of the ADP, UDPAG, and GTP. After the fourth and fifth transplantations, the r.s.a. of the total acid soluble phosphorus was apparently increased by irradiation, as was the r.s.a. of ADP, UDPAG, UDPG, UDPGA, and ATP. The reason for this change in behavior after repeated transplantation is being investigated.

B. *Nucleotides from Ehrlich Ascites Cells.* Results obtained using the mammary carcinoma might be criticized on the basis that changes observed had resulted from altered metabolism in tissue cells other than those of the tumor itself. To check this possibility, the procedure was repeated with Ehrlich ascites tumors. Mice of the Z strain bearing the ascites tumors were treated with 1000 r total body irradiation, after which they were injected intraperitoneally with P^{32} in physiological saline solution (30 microcuries per animal). Three hours later as much of the ascitic fluid as possible was withdrawn by peritoneal puncture. Cells from both irradiated and nonirradiated groups were centrifuged and washed twice with physiological saline

*Abbreviations used:

AMP adenosine mono-phosphate	GTP guanosine tri-phosphate
ADP adenosine di-phosphate	HMP hexose mono-phosphate
ATP adenosine tri-phosphate	IMP inosine mono-phosphate
CMP cytidine mono-phosphate	UMP uridine mono-phosphate
CDP cytidine di-phosphate	UDP uridine di-phosphate
CTP cytidine tri-phosphate	UTP uridine tri-phosphate
DPN diphosphopyridine nucleotide	UDPAG uridine di-phosphate acetyl-glucosamine
TPN triphosphopyridine nucleotide	UDPG uridine di-phosphate glucose
GMP guanosine mono-phosphate	UDPGA uridine di-phosphate glucuronic acid
GDP guanosine di-phosphate	

TABLE 1

Relative Specific Activities of Acid-Soluble Phosphorus Compounds of Irradiated and Non-Irradiated Mouse Mammary Carcinoma (L_3)

Total Acid-Soluble Material			Non-Irradiated	Irradiated	
Nitrogen to Phosphorus ratio			2.28	2.30	
Relative Specific Activity (counts per minute per μg P/specific activity of inorganic phosphorus)			68.36	54.6	
Effluent Passed Through the Column					
Nitrogen to Phosphorus ratio			7.1	7.02	
Relative Specific Activity			23.9	21.56	
Nitrogen as % of total acid-soluble Nitrogen			62.0	61.6	
Phosphorus as % of total acid-soluble Phosphorus			20.0	20.2	
Relative Specific Activity of Nucleotides		Peak No. Fig. 1			p Value
Cytidine monophosphate	CMP	I	44.65	45.37	.164
Diphosphopyridine nucleotide	DPN	II	43.2	49.6	.253
Adenosine monophosphate	AMP	III	75.7	63.8	.132
Unidentified compound		IV	92.7	95.1	.498
Guanosine monophosphate	GMP	V	74.1	63.6	.216
Hexose monophosphate	HMP	VI	82.8	57.3	
Adenosine diphosphate	ADP	XI	93.5	82.5	$<$ .001
Uridine diphosphate acetylglucosamine	UDPAG	XIII	108.5	86.4	.008
Uridine diphosphate glucose	UDPG	XIV	110.9	84.0	.002
Guanosine diphosphate	GDP	XV	96.9	67.8	$<$ .001
Cytidine triphosphate	CTP	XVI	99.6	69.3	$<$ .001
Uridine diphosphate glucuronic acid	UDPGA	XVII	100.5	80.3	.438
Adenosine triphosphate	ATP	XVIII	90.6	73.7	$<$ .001
Guanosine triphosphate	GTP	XIX	98.9	78.1	.005
Uridine triphosphate	UTP	XX	100.9	79.2	.004

TABLE 2

Relative Specific Activities of Acid-Soluble Phosphorus Componds of Irradiated and Non-irradiated Mouse Mammary Carcinomata (A_3, A_4 and A_5)

Total Acid-Soluble Material	Tumor A_3* Non-Irradiated	Tumor A_3* Irradiated	Tumor A_4 Non-Irradiated	Tumor A_4 Irradiated		Tumor A_5 Non-Irradiated	Tumor A_5 Irradiated	
Nitrogen to Phosphorus ratio	1.65	1.45	1.98	2.11		—	—	
Relative Specific Activity	76.3	67.2	63.3	73.6		67.7	72.3	
Effluent Passed Through Column								
Nitrogen to phosphorus ratio	6.9	6.5	9.17	9.56		6.97	6.70	
Relative Specific Activity	34.9	31.4	31.1	31.9		23.5	25.2	
Nitrogen as % of acid-soluble nitrogen	25.6	26.7	68.2	66.5		—	—	
Phosphorus as % of acid-soluble phosphorus	6.16	5.95	14.7	14.7		11.3	12.8	
Total Nitrogen on the column	24.4 mg.	23.3 mg.	16.7 mg.	12.0 mg.		—	—	
Total Phosphorus on the column	7.5 mg.	8.9 mg.	23.2 mg.	19.3 mg.		14.6	14.4	
Relative Specific Activities					P Value			P Value
Cytidine monophosphate	—	—	74.4	61.3	.146	57.3	58.0	.558
Diphosphopyridine nucleotide	25.6	30.2	55.3	49.1	.031	40.9	65.6	< .001
Adenosine monophosphate	49.8	38.5	57.2	61.8	.044	39.6	52.5	< .001
Guanosine monophosphate	—	—	—	—		59.4	59.9	.507
Hexose monophosphate	—	—	—	—		65.6	74.3	.008
Adenosine diphosphate	81.1	70.8	64.1	78.8	< .001	66.8	93.6	< .001
Uridine diphosphate acetylglucosamine	79.3	59.2	54.8	82.0	< .001	109.1	114.5	.290
Uridine diphosphate glucose	106.8	108.9	70.4	104.7	< .001	109.0	116.8	0.82
Guanosine diphosphate	—	—	—	—		111.9	89.2	< .001
Cytidine triphosphate	—	—	—	—		95.7	98.0	.555
Uridine diphosphate glucuronic acid	—	—	79.2	121.0	< .001	63.4	93.9	< .001
Adenosine triphosphate	—	—	72.3	87.8	< .001	93.5	114.5	< .001
Guanosine triphosphate	86.7	79.5	—	—		—	—	

*Unfortunately during the fractionation of extracts of tumor A_3 a failure in the gradient elution system caused poor resolution of some of the that sufficient analytical data were not obtained for statistical evaluation.

solution. Five ml. of packed cells from each of the groups were then homogenized with 20 ml. of ice-cold 0.6 N perchloric acid, after which the homogenates were handled exactly as described earlier for the mammary carcinoma. The pattern of the elution curve obtained, while similar, is distinct from that found with mammary carcinoma (Fig. 2). While the same phosphorus compounds are present, they occur in very different proportions: The adenosine nucleotides and CDP are more prominent, while UDPAG is barely detectable. Table 3 shows that the r.s.a. of the total acid-soluble phosphorus was increased by irradiation. Similarly, the r.s.a. of GMP, CDP, and UDPG was increased by the irradiation, while that of HMP, IMP, ADP, and ATP was reduced.

TABLE 3

Relative Specific Activities of Acid-Soluble Phosphorus Compounds Of Ehrlich Ascites Cells from Irradiated and Non-Irradiated Mice

Total Acid-Soluble Material	Non-Irradiated	Irradiated
Nitrogen to phosphorus ratio	2.08	1.87
Relative Specific Activity	80.5	85.0
Effluent Passed Through Column		
Nitrogen to phosphorus ratio	4.04	5.58
Relative Specific Activity	17.7	17.3
Nitrogen as % of acid-soluble nitrogen	37.1	39.8
Phosphorus as % of acid-soluble phosphorus	19.1	13.4

Relative Specific Activities	Non-Irradiated	Irradiated	Change Relative to Non-Irradiation*	
Pentose nucleic acid	71.4	73.6		
Deoxyribonucleic acid	61.9	17.4		
Total alcohol soluble lipids	62.5	50.7		
Phospholipids (Bloor's extract)	78.7	80.0		
				Total Amt.
Phosphorus Compound D from effluent	58.4	35.6	—39%	
First peak from Dowex 1	77.2	59.5	—22.9	—15.4
Cytidine monophosphate	87.6	82.8	— 5.5	+31.6
Diphosphopyridine nucleotide	58.4	57.3	— 1.9	+23.5
Adenosine monophosphate	149.8	154.0	+ 2.8	+34.7
Guanosine monophosphate	78.4	95.6	+21.8	+31.5
Hexose monophosphate	82.0	54.1	—35.3	
Inosine monophosphate(?)	59.2	47.8	—18.9	
Triphosphopyridine nucleotide(?)	55.4	—		
Cytidine diphosphate(?)	105.9	129.5	+23.0	+89.5
Adenosine diphosphate	133.4	100.0	—25.4	—53.5
Uridine diphosphate glucose	95.6	101.5	+ 6.2	—44.5
Adenosine triphosphate	109.1	93.8	— 4.9	—71.2
Guanosine triphosphate	108.5	—		+ 8.3
Uridine triphosphate	64.9	—		—31.2

* + indicates increase
—indicates decrease

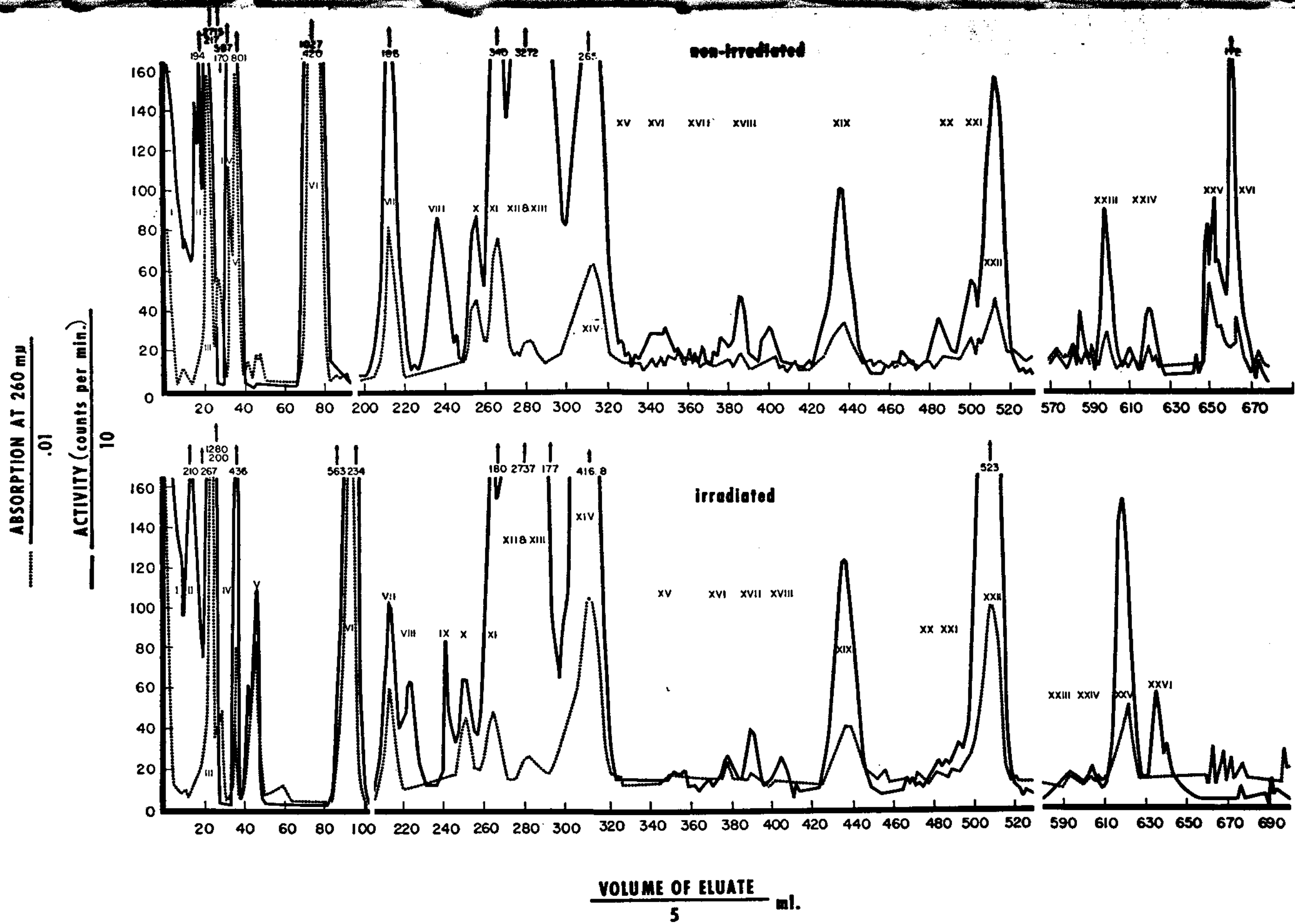


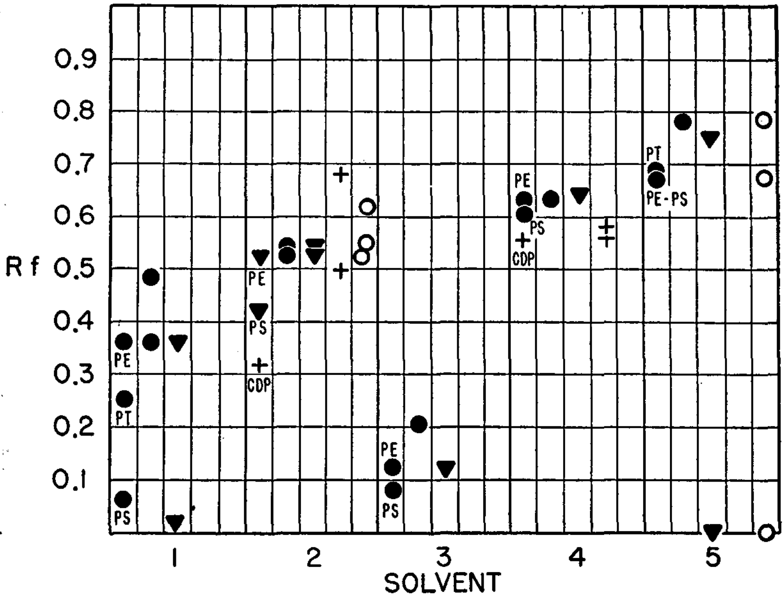
Fig. 2. Radioactivity (solid lines) and Utlra-Violet Absorption (dotted lines) of Acid-Soluble Phosphorus Compounds of Irradiated and Non-Irradiated Ehrlich Ascites Carcinoma.

Irradiation appears to bring about an increase in the amount of CDP, GMP, CMP and DPN and a decrease in the amount of ADP, ATP, UDPG, and UTP.

C. Non-ionized Phosphorus Compounds. Early in this work it became apparent that a considerable fraction of the organic phosphorus of the acid-soluble extracts was not retained by the Dowex 1 resin. The proportion of the total that was not accounted for by the above separation varied from experiment to experiment (from 10 per cent to 25 per cent), depending on the thoroughness with which the columns were washed prior to the elution of the fractions by formic acid. Hence, a standard method was adopted in which one bed volume of water was passed through the column before fractions were collected. The effluent (pH 4.5) which passed through with this washing was collected and put on a long column of Dowex 50 (cation exchange resin, 30 cm. by 1 cm. diameter). By being washed slowly with distilled water, the radioactive material was divided into four major fractions, which were studied by paper chromatography. A summary of the results of chromatography using five different solvents on the third of these fractions is shown in Figure 3. Each chromatogram was run in duplicate; after drying, one of the duplicates was inspected with ultraviolet light and any absorbing areas were outlined. The areas containing amino nitrogen were then identified by the ninhydrin reaction, and the localization of P^{32} was determined from radioautograms. The second chromatogram of each pair was examined for phosphorus compounds by the method of Hanes.[14]

The fraction that passed most freely through both anion and cation columns contained one phosphorus compound in addition to many amino acids. The second fraction to pass through the Dowex 50 contained a phosphorus compound which did not give a ninhydrin reaction, but did give a positive Fiske-Subbarow reaction on paper. The third fraction contained the major portion of radioactivity and gave a positive ninhydrin spot with an Rf value corresponding to that of O-phosphoethanolamine in four of the five solvents. A second phosphorus compound was also present. This material yielded a pink color with ninhydrin and moved slightly faster than phosphoethanolamine on the paper chromatograms, but did not separate completely. The fourth fraction to be washed through the Dowex 50 column was also highly radioactive; the phosphorus compound in this fraction did show absorbed ultraviolet light (260 microcuries), but it was too stable to give a Fiske-Subbarow reaction on the paper, and did not give a ninhydrin reaction.

SCALE DRAWINGS OF CHROMATOGRAMS OF EFFLUENT.
FRACTION C FROM DOWEX 50



Column 1 Controls PE —O-phosphoetheaolamine
PT —O-phosphothreonine
PS — O-phosphoserine
CDP — cytidine-diphosphate
Column 2 ● stained with ninhydrin reagent
Column 3 ▲ stained with Fiske-Subbarow reagent
Colunm 4 + absorption at 260 mμ
Column 5 ○ radio-autogram

Solvents
1 Phenol sat. with H_2O (10)
2 95 Ethanol-glacial acetic 60:40 v/v (11)
3 n-Butanol-glacial acetic acid-H_2O (12) 50:10.5:25 v/v
4 0.25 M Tris Buffer pH 8.0 in 50% Ethanol (11)
5 n-Butanol-amonia-H_2O 81:5:14 v/v (13)

Fig. 3

Discussion

In the experiments here reported, the time interval allowed for the reaction of the injected P^{32} after the irradiation was deliberately kept constant. It has been shown that the distribution of P^{32} among the different phosphorus compounds varies greatly with time,[15,16,17] and that in normal animals, there is a diurnal variation, which differs with different organs, in the rate of uptake of P^{32}.

It has also been shown that the uptake of P^{32} into DNA is only inhibited by ionizing radiation during a certain period of the interphase of the mitotic cycle of the cell. If the mitotic cycle of all the cells can be synchronized and the irradiation timed to coincide with the sensitive period, the uptake of P^{32} can be cut down almost 100 per cent. Thus, the failure to get more than a 50 per cent reduction in the uptake of P^{32} can well be due to the complete randomization of the mitotic cycle of the tumor cells. To minimize these complications, our experiments were performed on a uniform time schedule and always in the morning.

It should be pointed out the results of the experiments with ascites carcinoma may not be exactly comparable to those with mammary carcinoma, since the latter neoplasm received only local irradiation, whereas in the former, the total body of the host was irradiated. Moreover, some of the results observed in ascites cell preparation may have been due to the effect of irradiation on leukocytes present in the samples.*

The nature of the phosphorus compounds that are not retained by either anion or cation columns is an intriguing subject. At first the most plentiful of these was thought to be O-phosphoethanolamine, but the behavior of this compound in one of the five solvents used for chromatography has made this doubtful. In view of the recent report of the isolation by several Japanese investigators[18] of a new phospholipid, "Malignolipin," from four types of human cancer, it may be that these phosphorus compounds which were not retained on the columns are breakdown products of such a phospholipid. Moreover, the peculiar affinity of this lipid for Porphyrin III may have some bearing on the effect of hematoporphyrin on the radiosensitivity of biological systems, as will be considered in the second part of this report.

Summary

1. The phosphorus compounds in the acid-soluble extracts of mouse mammary and Ehrlich ascites carcinomata rendered radioactive by the injection of P^{32} into the host have been fractionated.

2. Irradiation of the mammary carcinoma usually causes reduction in the relative specific activity of the polyphosphonucleotides but not of the monophosphonucleotides.

3. After repeated transplantation of mammary carcinoma in inbred mice of Z strain, it appears that irradiation no longer reduces the rela-

*In order to eliminate possible effects of normal cells present in both mammary and ascites carcinoma preparations, we are presently investigating the uptake of P^{32} into HeLa cells in culture. This study is being carried out in cooperation with Dr. Leroy McLaren of the Department of Bacteriology.

tive specific activity of some phosphorus compounds but, rather, increases them.

4. In Ehrlich ascites tumors, total body irradiation of the host was observed to increase the total amount of CDP, GMP, CMP, and DPN, but to reduce the amount of ADP, ATP, UDP, and UTP. The relative specific activity of the total acid-soluble extract, as well as of GMP and CDP, was increased by total body irradiation.

5. Several phosphorus compounds that have been isolated are retained on neither anion nor cation resin columns. These appear to be closely related to the phospholipid recently isolated from human malignant neoplasms.

II. Investigation of Combined Chemical and Radiation Therapy in Treatment of Cancer

During the past four years, several departments at the University of Minnesota Hospitals have been seeking to learn how chemicals modify the effects of x-rays on various biological systems. Particular emphasis has been given to the study of porphyrins* as chemical modifiers when used in conjunction with ionizing radiations for cancer treatment.[19,20,21,22] Interest in porphyrins has stemmed primarily from the observation that regressions in the size of transplanted mouse mammary carcinoma were more pronounced when tumors were treated with radiation after the host animals were inoculated with a crude hematoporphyrin, than when tumors were treated with radiation alone.[19]

The compounds in addition to the porphyrins that have been used in our experiments include: folic acid analogues, sulfhydryl compounds, cortisone, sodium cyanide, dichlorovinyl cysteine, and ethanolamine phosphate.

Mice bearing tumors were treated in various ways with these chemicals (selected on the basis of their carcinostatic, antimetabolic, or radiation-protective properties) and with radiation therapy, in order to determine the optional dose levels and time relationship of administration of these agents. In some instances, tumors treated by combined therapy regressed faster and remained at a smaller or nonpalpable dimension for longer periods, thus definitely increasing the life span of tumor-bearing animals.

One reason for combining chemical therapy with radiation therapy is an attempt to increase the "therapeutic ratio," i.e., the ratio of the

*This work with porphyrins has been directed by Dr. Samuel Schwartz, Department of Medicine.

radiosensitivity of tumor tissue relative to surrounding normal tissue. If the therapeutic ratio could be increased by this means, it would be possible to deliver a cancerocidal dose with minimal damage to normal tissues. This may be accomplished either by sensitizing the tumor to the effects of x-rays or by preferentially protecting normal tissues.

A second reason for expecting synergistic action of chemicals and radiation in treatment of malignant tumors is evidence that alternate metabolic pathways may exist in cells, so that blocking one path by administration of a particular chemical or by radiation therapy may not produce the desired response. However, if a major pathway and alternates as well are blocked, one might achieve a specific result, such as inhibition of nucleic acid synthesis in tumors.

To determine whether or not the chemicals used are capable of altering the therapeutic ratio, their effects on the radiosensitivity of other biological systems have been studied. Observations have been made on the effects of porphyrins, sulfhydryl compounds, sodium cyanide, and dichlorovinyl cystine on the radiosensitivity of normal mice. Similar experiments have been conducted using proteolytic enzymes, cells in culture, and the bacteria, *E. Coli.*[20,23]

Materials and Methods

Mice of Z or ZBC stock, weighing from 15 to 20 Gm. were used in most of these studies. Male mice were used when available because of reported variations in the radiosensitivity of females, depending upon their phase of estrus.[24] But since so many mice (more than six thousand) were involved in these studies, it was necessary to utilize females in some instances.

Prior to its use as a modifier of the effects of radiation on mice, each compound was studied for toxic effects. The compounds were then employed in amounts well below their lethal ranges to eliminate deaths due solely to their injection. In some experiments, a total dosage exceeding the lethal dose for a single injection was administered by fractionating the amount over several days or weeks.

Irradiations were performed with a G. E. Maximar 220 X-ray machine. For studies involving total body irradiation of mice, the machine was operated at 220 KVP and 15 MA with a filter of ¼ mm. copper and 1 mm. aluminum (HVL - 0.95 mm. copper), and a focal-skin distance of 60 cm. Under these conditions, the output was 48 r per minute, as measured with a Victoreen r-meter. Full backscatter was achieved by supporting the treatment chambers with at least 10 cm. of presdwood. During irradiation, the mice were placed in a lucite box similar to the unit described by Ellinger,[25] and the average skin dose to the mice was 68 r per minute. The irradiation of mice bearing transplanted ascites or mammary tumors was carried out as described in the preceding section.

Experimental Results

1. Porphyrins

Although other porphyrins and metal complexes of these porphyrins have also been tested, hematoporphyrin has been the compound of principal interest. Studies made by Dr. S. Schwartz of the Department of Medicine, and by chemists of General Mills Research Laboratory and of Merck and Company, have shown that the hematoporphyrin prepared in their laboratories, as well as all other commercially available hematoporphyrin, is a mixture of several different porphyrins which vary greatly in their propensity for localizing in tumor tissues. At present we are attempting to measure the localization of various porphyrins in tumors and other tissues by methods employing radioactive metalloporphyrins or porphyrins labeled with radioactive carbon (C^{14}).

The purest hematoporphyrin fractions thus far available appear to localize poorly, and within one to two days after injection show but little red fluorescence in the transplanted mouse mammary carcinoma. Following administration of some of the minor porphyrin contaminants, on the other hand, considerable fluorescence persists in the tumor for a week or more. Furthermore, different preparations vary considerably in their toxic effects; many of these toxic manifestations in mice can be partially eliminated by administration of protoporphyrin.[22]

The radiosensitivity of transplanted mammary carcinoma in mice injected with hematoporphyrin has been found to vary depending on the source and relative purity of the material used. More comprehensive studies by Schwartz of mice bearing mammary or ascites carcinoma treated with porphyrin fractions prepared in his laboratories have shown similar results.[26] Other factors that appear to determine whether prior administration of porphyrins increases or decreases the radiation response of tumors are: the dose levels of the porphyrin and radiation, and the time intervals between their administration, and the sequence of their administration.[20,21]

The dose of hematoporphyrin (Mann Chemical Company) has also been shown to be an important factor in the effect of x-rays on enzymes,[20,21] paramecia,[20,26] and normal mice. Table 4 summarizes results in mice exposed to 450 r of total body roentgen irradiation three hours after receiving an intraperitoneal injection of 0.5 ml. containing 5, 1, or 0.1 mg. of hematoporphyrin. Animals inoculated with isotonic saline solution served as controls. Only 30 per cent of those receiving 5 mg. died within a 30-day period, whereas an average of 87 per cent of the mice treated with 1 or 0.1 mg. died in the same period. On the other hand, mice treated with saline solution followed by 450 r showed a 65 per cent 30-day mortality, i.e., midway between the porphyrin treated groups. Changing the time intervals between porphyrin inoculation and subsequent irradiation to more or less than three hours did not produce these large differences between porphyrin treated groups, or between these and control groups.

TABLE 4

Mortality among ZBC Mice Injected Intraperitoneally Three Hours Before Exposure to Total Body X-Irradiation (450 r)

Number of Animals	Injection	% Mortality (30 days)	± S.D.
60	5 mg. Hp	30	11
50	1 mg. Hp	92	7
20	0.1 mg. Hp	75	15
120	Saline	65	9

Other studies have shown that any protection or potentiation of the effects of total body irradiation can only be demonstrated with doses of radiation near the LD 50/30 (50 per cent lethal dose in 30 days) for the control mice. At doses near the LD100/30 for control mice (550 r), and for doses as high as 850 r, there is no difference in mortality rate between the animals treated with porphyrin and those treated with saline solution.[20]

Postirradiation injections of hematoporphyrin appear to enhance the lethal effects of x-rays. This is most pronounced for the 5 mg. dose, which was shown to protect when given three hours before irradiation.[20]

2. *Sulfhydryl Compounds*

Sulfhydryl compounds have been shown to be potent protective agents of a wide variety of biological systems if administred prior to irradiation.[27,28] We have confirmed these results by exposing groups of mice from our colony to total body irradiation 30 minutes after an intraperitoneal injection of equimolar amounts of cysteine, glutathione, or cysteamine. Results of studies in which mice were exposed to either 450 r or 550 r are shown in Table 5.

TABLE 5

Mortality and Weight Changes in ZBC Mice Injected Intraperitoneally 30 Minutes Prior to Total Body X-Irradiation

Injection	450 roentgens No. of Animals	450 roentgens % Mortality	450 roentgens Ave. % Weight Change (15 days)	550 roentgens No. of Animals	550 roentgens % Mortality	550 roentgens Ave. % Weight Change (15 days)
15 mg. Cysteine	30	13	+ 4	30	85	— 14
86 mg. Glutathione	20	10	— 2	20	75	— 18
11.5 mg. Cysteamine	30	7	+ 8	30	15	+ 2
Saline	30	70	— 16	30	100	—

These compounds were also found to protect tumors from radiation effects, for animals treated with them prior to irradiation of their tumors did not survive nearly as long as did animals treated with radiation alone (Fig. 4). The treatment was divided into 3 parts, indicated by

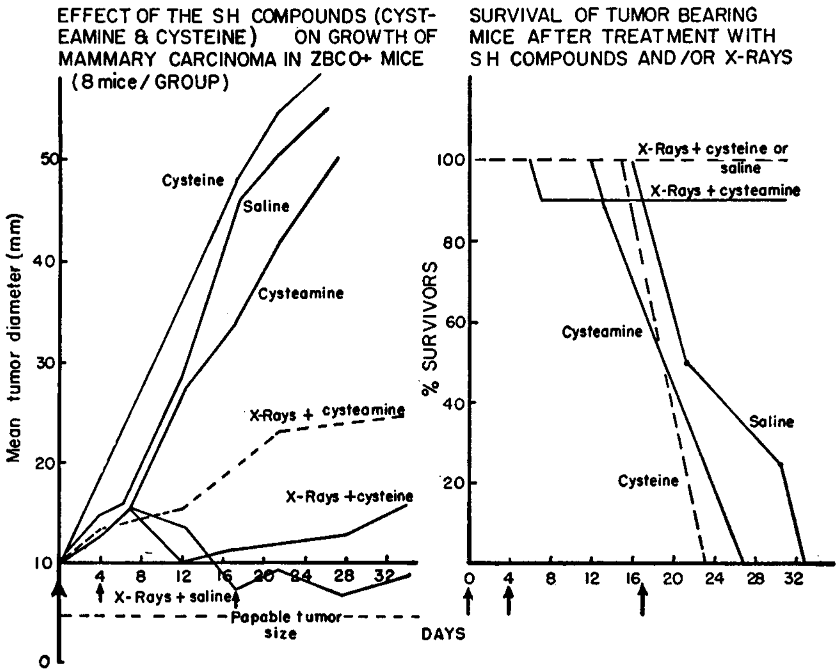


Fig. 4

arrows on the time scale. Each treatment consisted of an intraperitoneal injection of isotonic saline or 0.25 mole of a sulfhydrye compound 30 minutes prior to 1500 r of x-rays to the tumor. The average tumor sizes in mice receiving the sulfhydryl compounds increased more rapidly after x-ray treatment than did sizes of comparable tumors in mice inoculated with saline solution.

It was of interest to learn whether or not the combined administration of a sulfhydryl compound and hematoporphyrin might affect the radiosensitivity of biological systems. If the modes of action of these compounds were related in such a way that their combined administration could enhance the therapeutic ratio, one might then use them effectively in cancer treatments. Table 6 shows results of intraperitoneal administration of hematoporphyrin (Mann Chemical Company)and cysteine or cysteamine to groups of mice prior to total body irradiation (450 r). The porphyrin was injected three hours before, and the sulfhydryl compound one-half hour before, x-ray exposure. The action of the sulfhydryl compounds increased the protection normally afforded by 5 mg. of hematoporphyrin, whereas it was antagonistic to the action of 1 mg. of the porphyrin.

We have also investigated the effect of cysteine on the incorporation of P^{32} into components of tumor cells *in vivo*. Our studies have failed to show that cysteine produces any changes in P^{32} uptake into either irradiated or nonirradiated tumors.

Recently we have become interested in another sulfhydryl compound, dichlorovinylcysteine. This compound, thought to be the

TABLE 6

MORTALITY AND WEIGHT CHANGES IN ZBC MICE INJECTED INTRAPERITONEALLY WITH HEMATOPORPHYRIN (HP) 3 HOURS BEFORE AND SULFHYDRYL COMPOUNDS 30 MINUTES BEFORE TOTAL BODY X-IRRADIATION (450 r)

Injection	Number of Animals	% Mortailty (30 days)	Avg. % Weight Change (15 days)
1 mg. Hp	20	75	— 10
1 mg. Hp plus 7.5 mg. Cysteine	20	75	— 5
1 mg. Hp plus 15 mg. Cysteine	20	30	+ 5
5 mg. Hp plus 15 mg. Cysteine	10	20	+ 2
5 mg. Hp plus 11.5 mg. Cysteamine	10	10	— 4
Saline	20	60	— 15

toxic factor in soy bean meal, has been shown by McKinney and associates[29] to induce aplastic anemia in cattle, similar to that caused by radiation. These findings have been confirmed by Schultze, who has reported in addition that this material is much more toxic to cattle than to rats when administered on the basis of weight.[30] We have found that mice can readily tolerate an intraperitoneal injection of 25 mg/kg of body weight, and that one-fifth of this amount is capable of affording considerable protection from total body x-irradiation. In other experiments, we have found that 5 mg/kg of body weight is capable of inhibiting the growth of ascites carcinoma but not of mammary carcinoma. Further investigations with this compound are currently in progress.

3. *Folic Acid Analogues**

Results of many of our studies have indicated that the folic acid antagonists used in conjunction with x-ray therapy for treatment of malignant tumors in mice provide a better control of tumor growth than is possible with either agent alone. Studies in our laboratories involving the effect of folic acid analogues on ascites tumors have been reported recently by Kim.[31]

Since the development of the first folic acid antagonist by the Lederle group[32] for use by Farber and associates[33] in clinical investigations of acute leukemia in childhood, many investigators have sought to use these 4-amino derivatives in treatment of various forms of cancer. Higgins and Woods[34] reported that aminopterin restricted the growth of transplanted mammary tumor in mice during the period of its administration. Several investigators have studied the effect of aminopterin on the growth of sarcoma-180 in mice[35,36] and have shown that partial inhibition of tumor growth is possible through simultaneous administration of folic acid. Taylor and Carmichael[37] have shown that this analogue causes preferential inhibition of mouse mammary tumor in embryonated eggs. Sugiura and associates[38,39] found that folic acid analogues inhibited the growth of melanoma and mammary and ascites carcinomata in mice.

No extensive investigation has been made of the use of folic acid analogues as an adjunct to radiation therapy in cancer treatment. Thomas[40] recently reported good results when using aminopterin and radiation therapy in the treatment of a cancer patient. However, his results in limited trials on tumors in mice were very discouraging because of the high mortality among treated animals.

Inasmuch as the folic acid analogues and ionizing radiations have

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*We are indebted to Lederle Laboratories for supplying these compounds.

similarities in their effects on biological structures, we felt it worthwhile to explore their possible synergistic action in carcinostasis. Such synergistic action, possibly related to the inhibition of major and alternate metabolic pathways, might eliminate the refractoriness known to develop in tumors after repeated treatment with either agent alone.

The effect of folic acid antagonists is to inhibit transmethylation, which provides a block in the synthesis of purine bases and nucleic acids. Ionizing radiations also interfere with the synthesis of nucleic acid. The sites of action of these agents have not yet been clearly demonstrated, but several metabolic steps in the synthesis of nucleic acid appear to be involved.

The results in Table 7 indicate that each of the analogues administered 30 minutes prior to exposure of 450 r appeared to potentiate the effect of the radiation (total body). Aminopterin, the most effective, is also the most toxic of the group. At 550 r (total body), a dose at which hematoporphyrin was ineffective, each of the analogues again provided a slight potentiation of the radiation effect—potentiation in terms of the 50 per cent survival time, since all animals succumb to this treatment.

TABLE 7

Mortality in Z Mice Injected Intraperitoneally 30 Minutes Prior to Total Body X-Irradiation

Injection	450 r			550 r	
	No. of Animals	% Mortality (30 days)	Days for 50% Mortality	No. of Animals	Days for 50% Mortality
250 μg. Aminopterin	20	100	7	20	6
250 μg. Aminoteropterin	20	70	9	20	8
250 μg. Methotrexate	20	70	12	20	10
250 μg. An-Fol-A	20	70	12	20	7
Saline	20	60	15	20	12

The response of mammary tumors to radiation therapy 30 minutes after injection of analogues is shown in Figure 5. The treatment was divided into three parts, indicated by the arrows on the time scale, each treatment consisting of an intraperitoneal injection of 250 micrograms of the analogue and 1500 r to the tumor. As this figure indicates, tumor growth was definitely inhibited by the combined therapy.

The effect of chemical treatment combining hematoporphyrin

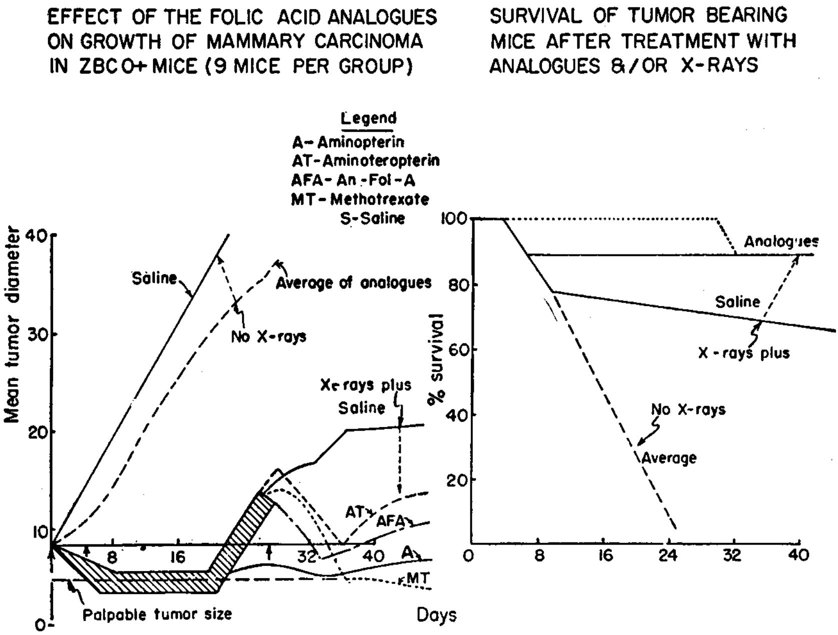


Fig. 5

(Mann Chemical Company) and aminopterin on the radiosensitivity of mice exposed to total body irradiation was also studied. One milligram of hematoporphyrin was given two hours before, and 250 micrograms of the analogue 30 minutes before, exposure to 450 r. The rate of 30-day survival of this group was no different from that of a group treated with the analogue and radiation. Under the conditions of the study, no synergistic action of these two compounds was found.

Preliminary studies have been carried out on the effect of folic acid analogues administered prior to tumor irradiation on the uptake of P^{32}. Thus far we have not been able to demonstrate any significant differences between groups so treated and groups receiving an injection of saline solution prior to tumor irradiation.

4. Sodium Cyanide

Cyanide is believed to protect *in vivo* systems from the effects of ionizing radiations by creating tissue anoxia through interference with the function of cytochrome oxidase. Large doses of hematoporphyrin administered to mice have been shown to be protective, and it has been postulated that the mechanism of action is similar to that of cyanide.[20]

As a test of this postulate, several experiments have been conducted in which sodium cyanide or a combination of sodium cyanide and hematoporphyrin (Mann Chemical Company) was administered to ABC mice weighing 30 Gm. before and after a total body exposure to either 450 or 550 r. Table 8 shows the results of a study in which 0.025 mg. of sodium cyanide was injected intraperitoneally 30 minutes after preparation of the solution. These data indicate that distinct protection is afforded by cyanide when administered prior to exposure, particularly in the case of the 450 r dose. This protection is enhanced by the addition of 5 mg. of hematoporphyrin. When both compounds were administered (porphyrin at three hours and cyanide at one-half hour before x-ray exposure) no deaths resulted. Under these conditions, the two effects are synergistic. When 1 mg. of hematoporphyrin was given with cyanide, it was apparent that at this concentration of porphyrin the actions are not additive. These observations agree with the observation discussed earlier, i.e., that the ultimate survival of mice exposed to combined hematoporphyrin and x-ray treatment depends upon the dose of porphyrin administered.

Also of interest in this table is the 50 per cent difference in mortality observed for mice receiving cyanide before and after 550 r. Inasmuch as the group receiving saline solution and 550 r have a

TABLE 8

MORTALITY AMONG ABC MICE INJECTED INTRAPERITONEALLY BEFORE OR AFTER TOTAL BODY X-IRRADIATION

Injection	Time of Injection	Dose of Radiation	No. of Animals	% Mortality (30 days)
NaCN	30 min. before	450 r	30	7
NaCN	30 min. after	450 r	30	33
5 mg. Hp plus NaCN	3 hours before 30 min. after	450 r	10	0
1 mg. Hp plus NaCN	3 hours before 30 min. after	450 r	10	50
Saline	30 min. before	450 r	30	53
NaCN	30 min. before	550 r	10	40
NaCN	30 min. after	550 r	10	90
Saline	30 min. before	550 r	10	70

mortality of 70 per cent, which lies almost midway between the mortalities exhibited by the cyanide groups, it appears that cyanide administered prior to 550 r total body irradiation is protective, whereas if administered after irradiation it renders the animals more radiosensitive. This is comparable to the effect of administering 5 mg. of hematoporphyrin in similar circumstances.

Attempts to show differences in the uptake of P^{32} into various organic compounds of the tumor cells treated with cyanide and radiation, or radiation alone, have been inconclusive.

Discussion and Summary

Any chemical, used alone or in conjunction with radiation therapy, which can be demonstrated to have a profound adverse effect on tumor metabolism with little effect on normal tissues would be of real potential value in combination therapy. Its presence in the tumor might permit the use of smaller doses of ionizing radiations, provided the compound were taken up by viable and actively growing tumor cells and not limited to necrotic tumor or pus.

A study has been made of the merit of combined chemical and radiation therapy in cancer treatment. It is evident from these results that many factors must be considered in evaluating a particular type of treatment. The effects of varying the doses of the chemicals and radiation, and of the changing time interval between their administration require further investigation.

Results of our studies with hematoporphyrin indicate that its effect on the radiosensitivity of biological systems (including tumor tissue) depends upon: (1) the dose of the compound administered, (2) the dose of radiation used, (3) the time interval between their administration, and (4) the sequence of their administration. It is thought that the radiation protection afforded mice by 5 mg. of this porphyrin may be due to the creation of a hypoxic state in the animals. The synergistic action of 5 mg. of hematoporphyrin and sodium cyanide supports the theory that both compounds inhibit the functioning of respiratory enzymes. The mechanism by which 1.0 and 0.1 mg. doses of this porphyrin potentiate the effects of radiation is not clear, but it may be that these smaller doses stimulate respiration and thus render the animals more radiosensitive. This phenomenon of diametrically opposed effects of different concentrations of hematoporphyrin on other biological systems has been reported by others.[41]

Sulfhydryl compounds are known to provide considerable protection of mice exposed to total body irradiation. Whether these materials protect tumors from radiation effects is not conclusively known, but definite evidence of tumor protection has been found. Antagonistic or synergistic action of the sulfhydryl compounds and hematoporphyrin was found to depend upon the amount of hematoporphyin used.

We have shown that the combined use of folic acid analogues and x-ray therapy provides better control of cancer growth than is possible with either agent alone. Tumors were found to regress faster with this combined treatment and remained at a smaller or nonpalpable dimension for a longer period. This treatment was also capable of increasing the survival time of tumor-bearing animals. On the other hand, mice treated with folic acid analogues plus total body irradiation exhibited a higher mortality than did comparable mice treated with irradiation alone.

It is interesting to compare the findings in folic acid analogue and sulfhydryl compound experiments. The sulfhydryl compounds afforded protection from the otherwise lethal effects of radiation and protected tumors in like manner. The analogues potentiated the lethal effects of x-rays, and likewise enhanced the radiation effect on tumors. Under the conditions of these studies, neither the sulfhydryl compounds nor analogues appear to alter the therapeutic ratio significantly. However, the analogues do potentiate the effects of x-rays on tumors and therefore they may have therapeutic value provided that normal tissues can be protected by other means. This finding appears particularly important in view of the longer survival of the animals treated with analogues.

Bibliography

1. Vermund, H.; Barnum, C. P.; Huseby, R. A.; and Stenstrom, K. W.: The Effect of Roentgen Radiation on the Incorporation of Radio-phosphorus into Nucleic Acids and Other Constituents of Mouse Mammary Carcinoma, Cancer Res. 13:633, 1953.
2. Vermund, H. and Stenstrom, K. W.: Studies of Radiation Effects in Malignant Tumors, Univ. of Minn. Med. Bull. 26:650, 1955.
3. Jardetzky, C. D.; Barnum, C. P.; and Vermund, H.: Deoxyribonucleic Acid and Phospholipid Metabolism in Regenerating Liver and the Effect of X-radiation, J. Biol. Chem. 222:421, 1956.
4. Beltz, R. E.; Lancker, J. V.; and Potter, V. R.: Nucleic Acid Metabolism in Regenerating Liver. IV. The Effect of X-radiation of the Whole Body in Nucleic Acid Synthesis in Vivo, Cancer Res. 17:688, 1957.
5. Stenstrom, K. W.; Vermund, H.; Mosser, D. G.; and Marvin, J. F.:

The Effect of Roentgen Irradiation on the Tumor Bed, Rad. Res. 2:180, 1955.

6. Ernster, L.; Zetterstrom, R.; and Lindberg, O.: A Method for Determination of Tracer Phosphate in Biological Material, Acta Chim. Scand. 4:942, 1950.
7. Fiske, C. H. and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. 66:375, 1925.
8. Ma, T. S. and Zuasaga, G.: The Micro-Kjeldahl Determination of Nitrogen, Ind. Eng. Chem. Anal. Ed. 14:280, 1942.
9. Hurlbert, R. B.; Schmitz, H.; Brumm, A. F.; and Potter, V. R.: Nucleotide Metabolism. II. Chromatographic Separation of Acid Soluble Nucleotides, J. Biol. Chem. 209:23, 1954.
10. Dent, C. E.: Study of the Behavior of Some Sixty Amino Acids and Other Ninhydrin-reacting Substances on Phenol-Collidine Filter-Paper Chromatograms, Biochem. J. 43:169, 1948.
11. Kennedy, E. P.: The Synthesis of Cytidinediphosphate Choline, Cytidinediphosphate Ethanolamine and Related Compounds, J. Biol. Chem. 222:185, 1956.
12. Benson, A. A.; Basshum, J. A.; Calvin, M.; Goodale, T. C.; Haas, V. A.; and Stepka, W.: The Path of Carbon in Photosynthesis. V. Paper Chromatography and Radioautography of the Products, J. Am. Chem. Soc. 72:1710, 1950.
13. Bergkvist, R. and Deutsch, A.: The Isolation of Uridine-5[1]-triphosphate from Muscle, Acta Chim. Scand. 8:1880, 1954.
14. Hanes, C. S. and Isherwood, F. A.: The Separation of the Phosphoric Esters on the Filter Paper Chromatograms, Nature 164:1107, 1949.
15. Barnum, C. P.; Huseby, R. A.; and Vermund, H.: A Time Study of the Incorporation of Radioactive Phosphorus into the Nucleic Acids and Other Compounds of Transplanted Mouse Mammary Carcinoma, Cancer Res. 13:880, 1953.
16. Vermund, H.; Halberg, F.; Barnum, C. P.; Nash, C. W.; and Bittner, J. J.: Physiologic 24 Hour Periodicity and Hepatic Phospholipid Metabolism in Relation to the Mouse Adrenal Cortex, Am. J. Physiol. 186: 414, 1956.
17. Barnum, C. P.; Jardetzky, C. D.; and Halberg, F.: Nucleic Acid Synthesis in Regenerating Liver, Texas Rep. Biol. & Med. 15:134, 1957.
18. Kosaki, T.; Ikoda, T.; Kotani, Y.; Nakagawa, S.; and Saka, T.: A New Phospholipid, Malignolipin, in Human Malignant Tumors, Science 127: 1176, 1958.
19. Schwartz, S.; Absolon, K.; and Vermund, H. (and collaborators): Some Relationships of Porphyrins, X-ray and Tumors, Univ. of Minn. Med. Bull. 27:7, 1955.
20. Loken, M. K.: The Effects of Roentgen Rays on Several Biological Systems with Special Reference to Their Modification by Hematoporphyrin, Univ. of Minn. Ph.D. Thesis, 1956.
21. Loken, M. K.: Porphyrins as Modifiers of the Effects of Roentgen Rays, Radiology 69:201, 1957.
23. Loken, M. K.; Mosser, D. G.; Vermund, H.; and Marvin, J. F.: The Effect of Roentgen Rays on Some Biological Systems with Special Ref-

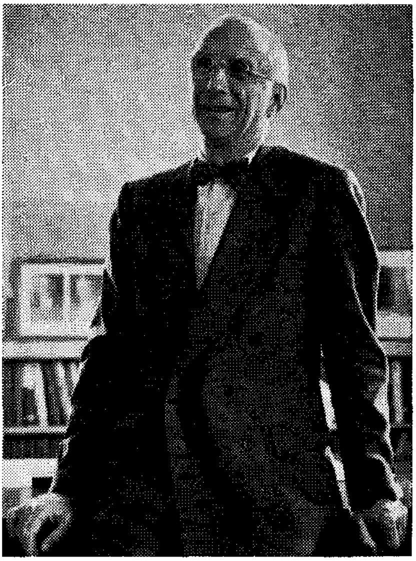
erence to Their Inactivation of Pepsin, Univ. of Minn. Med. Bull. 27: 471, 1957.
24. Rugh, R. and Clugston, H.: Radiosensitivity with Respect to the Estrus Cycle in the Mouse, Rad. Res. 2:227, 1955.
25. Ellinger, F.; Morgan, J.; and Chambers, F.: The Use of Small Laboratory Animals in Medical Radiation Biology. Part I., Naval Med. Res. Inst. 10:145, 1952.
22. Modell, J.: Some Physiological Effects of Hematoporphyrin with Special Reference to its Relationship to Radiosensitivity of Tumors, Univ. of Minn. Med. Bull. 28:385, 1957.
26. Schwartz, S.: Personal Communication.
27. Patt, H. M.: Protective Mechanisms in Ionizing Radiation Injury, Physiol. Rev. 33:35, 1953.
28. Bacq, Z. M. and Herve, A.: Ein chemischer Schutz gegen Röntgenstrahlungen, Strahlentherapie 95:215, 1954.
29. McKinney, L. L.; Weakly, F. B.; Eldridge, A. C.; Campbell, R. E.; Cowan, J. C.; Picken, J. C. Jr.; and Biester, H. E.: S-(Dichchlorovinyl) L-Cysteine: An Agent Causing Fatal Aplastic Anemia in Calves, J. Am. Chem. Soc. 79:3932, 1957.
30. Schultze, M. O.: Personal Communication.
31. Kim, Y. S.: Ascites Carcinoma in Experimental Cancer Research, Univ. of Minn. Med. Bull. 29:430, 1958.
32. Seegers, D. R.; Smith, J. M. Jr.; and Hultquist, M. E.: Antagonist for Pteroylglutamic Acid, J. Am. Chem. Soc. 69:2567, 1947.
33. Farber, S.; Diamond, L. K.; Mercer, R. D.; Sylvester, R. F. Jr.; and Wolff, J. A.: Temporary Remissions in Acute Leukemia in Children Produced by Folic Acid Antagonist, 4-Aminopteroylglutamic Acid (Aminopterin), New England J. Med. 238:787, 1948.
34. Higgins, G. M. and Wood, K. A.: The Effect of the Folic Acid Antagonist, 4-Aminopteroylglutamic Acid on the Growth of Transplanted Mammary Tumors in C_3H Mice, Proc. Staff Meet., Mayo Clin. 24:238, 1949.
35. Moore, A. E.; Stock, C. C.; Sugiura, K.; and Rhoads, C. P.: Inhibition of Development of Sarcoma 180 by 4-Amino-N^{10}-Methyl Pteroylglutamic Acid, Proc. Soc. Exper. Biol. & Med. 70:396, 1949.
36. Schoenback, E. B.; Goldin, A.; Goldberg, B.; and Ortega, L. G.: The Effect of Folic Acid Derivatives on Sarcoma 180, Cancer 2:57, 1949.
37. Taylor, A. and Carmichael, N.: Folic Acid Analogues and the Growth of Embryo and Tumor Tissue, Cancer Res. 11:519, 1951.
38. Sugiura, K.; Moore, A. E.; and Stock, C. C.: The Effect of Aminopterin in the Growth of Carcinoma, Sarcoma and Melanoma in Animals, Cancer 2:491, 1949.
39. Sugiura, K.: Merits of Ascites Tumors for Chemotherapeutic Screening, New York Acad. Sc. 63:962, 1956.
40. Thomas, S. F.: Combined Therapy, Radiation and Chemicals, Radiology 69:204, 1957.
41. Figge, F. H. J. and Peck, G. C.: Cortisone-like Effect of Hematoporphyrin and Sunlight on Anaphylaxis in Mice, Proc. Soc. Exper. Biol. & Med. 87:592, 1954.

Editorials

A Glance Backward and Forward

Editor's note: The following editorial was written by Dr. Harold S. Diehl, who retires on June 30, 1958, after 23 years as Dean of the University of Minnesota College of Medical Sciences. The faculty, alumni, and members of the Minnesota Medical Foundation wish him success and happinesss in his role as Senior Vice President for Research and Medical Affairs and Deputy Executive Vice President of the American Cancer Society.

It is with real anticipation that I accept Dr. Gault's invitation to present a few editorial comments in this final issue for the year 1957-58 of the UNIVERSITY OF MINNESOTA MEDICAL BULLETIN.



Harold S. Diehl

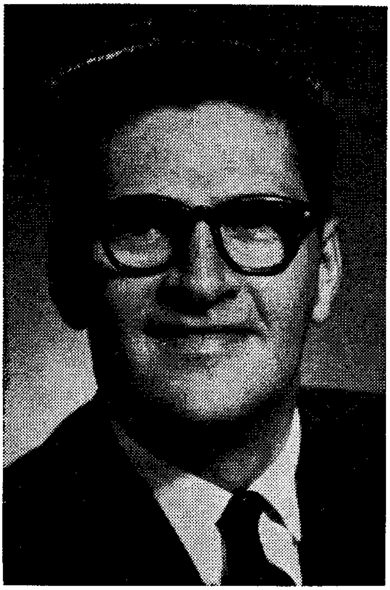
Glancing backwards: I remember vividly the beginning of the Minnesota Medical Foundation. The concept of such an organization grew out of the intimate and effective cooperation between the faculty and the alumni of the Medical School in the development of the splendid program held in recognition of the fiftieth anniversary of the Medical School. Following this event, several alumni and faculty members suggested that continuing cooperation between the alumni and the faculty of the School should be mutually rewarding and of real value in the future development of the School.

Pursuant to these suggestions, I invited some one hundred alumni to meet at the University to discuss the possibility of an organization of alumni and other friends of the Medical School to keep in close touch with the activities and the needs of the School and to give support in selected areas of special interest and importance. Acceptances of this invitation were almost unanimous; and after the thorough discussion at the meeting, I was authorized to appoint a committee to formulate a plan of organization and a program of activities. The result was the Minnesota Medical Foundation, which Dr. Erling Platou and Dr. Maurice Visscher served as President and Secretary-Treasurer, respectively, during the initially important, formative years.

Over the intervening years, which now total almost two decades, the contributions of the Minnesota Medical Foundation to the progress of our Medical School have been many. Prominent among these I might mention especially its role in initiating the Mayo Memorial Fund Drive activities, the expanding scholarship program which has been of great help to our medical students, the support that it has provided for the new expansion of the underground research facilities, and the responsibility it has assumed in publication of the BULLETIN—the means by which alumni and friends of the Medical School are kept informed of activities here. To me the increasing strength of this organization has been a matter of great personal gratification; and as I relinquish the position of Dean, I do so with a feeling of very real assurance that this splendid organization will help to underwrite to the continuing strength and development of the Medical School that means so much to us all.

—H. S. Diehl, M.D.

The New Dean



Robert B. Howard

The Board of Regents of the University of Minnesota has announced the appointment as of July 1, 1958, of an alumnus, Dr. Robert B. Howard, as Dean of the College of Medical Sciences to succeed Dr. Harold S. Diehl.

Born and reared in St. Paul, Minn., Dr. Howard developed an interest in medical science at an early age. He was educated in the St. Paul public school system and was graduated from Central High School. He was elected to Phi Beta Kappa at the University of Minnesota, graduating magna cum laude in June, 1942. He received his M.D. degree from the University of Minnesota Medical School in 1944 and began his postgraduate medical education as an intern in the Department of Internal Medicine at the University of Minnesota Hospitals. With a major in Medicine and minors in Pathology and Hemotology, he prepared a thesis entitled "Studies on the Metabolism of Iron," and earned a Doctor of Philosophy degree in June, 1952.

Dr. Howard has had a variety of assignments and has demonstrated many abilities during his years on the faculty. He served as Chief Resident of the Department of Medicine in 1948-49, then as Assistant Director of the Outpatient Department. For one year he was Consultant in Internal Medicine to the University Health Service. He was in charge of the undergraduate teaching program in the Department of Medicine in 1951. Since January, 1952, when he became Director of the Department of Continuation Medical Education, his effectiveness as an administrator and teacher has become known to the hundreds of physicians who registered for the continuation medical education courses. On October 1, 1957, he was appointed Associate Dean when Dean Diehl was granted a leave of absence. In announcing Dr. Howard's appointment as Dean, President Morrill commented, "We are fortunate to have had, within our own staff, an administrator meriting so clearly the confidence of his College of Medical Sciences colleagues and the central administration of the University.

"The deanship is a position of leadership which Dr. Harold S. Diehl has dignified and developed to the highest degree of responsibility and of professional influence. To the challenge of its future, Dr. Howard will respond with devotion and competence, I am fully confident."

Dr. Howard has been a member of many Medical School and University committees, among them the Medical School Admissions Committee, the University Press Committee, the University Radio Advisory Committee, the All-University Selection Committee for Short-Term Faculty Leaves, the Mayo Memorial Dedication Committee, and the Committee on Continuation Medical Education of the Association of American Medical Colleges. He has been a member of the Executive Board of the Minnesota Medical Alumni Association and has served as Secretary-Treasurer of the Minnesota Medical Foundation. In addition to being Editor of the UNIVERSITY OF MINNESOTA MEDICAL BULLETIN, he served on the editorial boards of *Minnesota Medicine* and *Journal-Lancet*. He is a member of the Minnesota Society of Internal Medicine, the Minneapolis Society of Internal Medicine, and the American College of Physicians.

He is married to the former Lorraine Leavitt, and the Howards, who now live in St. Louis Park, have five children, Gregory, David, Carol, Irene, and Bradley. During the summer the family lives on Bass Lake near New Richmond, Wisconsin.

On behalf of the Minnesota Medical Foundation, we congratulate Dr. Howard and pledge our support of his leadership of the College of Medical Sciences.

In Memoriam

The Minnesota Medical Foundation and the Minnesota Medical Alumni Association extend sincere sympathy to the surviving members of the families of the following alumni:

Erling S. Platou was born in Cooperstown, North Dakota, on August 30, 1896. He earned the B.S. degree in 1919, the M.B. degree in 1920, and the M.D. degree in 1921, all at the University of Minnesota. Postgraduate training in internal medicine and in pediatrics was obtained at the Long Island College of Medicine, the Minneapolis General Hospital, the Boston Floating Hospital, the New York Nursery and Children's Hospital, the Willard Parker Hospital in New York City, and concluded by a year of postgraduate work in pediatric clinics in Germany and Austria.

His death is a serious loss to the University of Minnesota and to the Medical School. Few men in the clinical practice of medicine have contributed so unselfishly of their time and talent to the ideals and objectives of their University as did Dr. Platou. His devotion to the Medical School and to the Univeristy of Minnesota from his student days until the time of his death is expressed by a continuous record of achievements, honors, and recognition given him throughout the years. He was an outstanding athlete in student days, captain of the basketball team that won the National championship, and acclaimed in a recent poll as one of the ten all time greats in this sport. He obtained a Western Conference Medal for scholarship and athletic prowess.

Dr. Platou had been president of the Northwestern Pediatric Society, the Minnesota State Board of Health, the Minnesota Alumni Association, and the Northwestern Hospital staff. He was a founder and past president of the Minnesota Medical Foundation. Dr. Platou was a superlative and stimulating clinical teacher whose weekly rounds with the students and the staff at the Minneapolis General Hospital and the University Hospitals on infectious diseases and other pediatric problems were rated as the best and most practical of teaching sessions. Always a student and a clinical investigator, he had contributed 69 publications to the literature at the time of his death, and his chapter on "Diphtheria" is currently in press.

It is said that the true qualities of a man are revealed through companionship on a hunting and fishing trip. These sports Dr. Platou dearly loved. Devotion to duty, sincerity of purpose, good sportsmanship and fairness, a gentle kindness and concern for others are qualities well known to a large number of true friends who had the privilege of his companionship on these trips.

Surrounded by the beauty of the North Woods area, Erling Platou met death as he and his family were about to leave for a day of fishing near Grand Marais. He is survived by his wife, Helen, a son John, 19, and two daughters, Nancy, 16 and Susan, 14.

Arthur Kirschbaum, Ph.D. 1936, M.D. 1943, died of coronary thrombosis on March 28th. Dr. Kirschbaum was an Associate Professor in the Department of Anatomy, University of Minnesota until 1951. At the time of his death he was Chairman of the Departments of Anatomy of Baylor University College of Medicine and of the University of Texas Dental College, Houston. He was also Consultant to the M. D. Anderson Hospital. He had gained an international reputation for his research in experimental carcinogenesis.

Louis L. Freidman, M.D. 1933, served faithfully on the clinical staff of the Department of Obstetrics and Gynecology until his death on June 14, 1958.

Herbert L. Stolpestad, M.D. 1934, met death at the hands of a mentally ill patient on May 21, 1959. A member of the Academy of General Practice, Dr. Stolpestad practiced medicine in North St. Paul and was beloved by his patients and medical colleagues.

Dr. John R. Meade, M.D. 1930, contributed to the teaching program for undergraduate and graduate students in the Ancker Hospital until his death in May, 1958.

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CUMULATIVE INDEX 1953-1958

From October, 1951, until June 1955, the name of this publication was "Bulletin of the University of Minnesota Hospitals and Minnesota Medical Foundation." In October, 1955, the name was changed to "UNIVERSITY OF MINNESOTA MEDICAL BULLETIN."

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MINNESOTA MEDICAL FOUNDATION
1939

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.