THE EFFECTS OF TUMOR ON THE NUCLEIC ACID METABOLISM OF THE HOST TISSUES

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Date Oct 6, 1961

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Abstract

A humoral factor elaborated by tumor tissue has been suggested as the etiological agent which causes the systemic effects accompanying malignant disease. Because of the important role of the nucleic acids in the metabolism of the cell, it was postulated that the tumor factor might produce the systemic effects by altering the nucleic acid metabolism of the host tissues. With these considerations in mind, a study has been made of the effect of several transplantable tumors on the incorporation of formate C^{14} and tritiated thymidine into the nucleic acids of the host tissues. Studies on the incorporation of thymidine into the host tissues have been emphasized since this compound is a specific precursor of DNA thymine. Liquid scintillation counting methods were developed in order to assay the radioactivity of the tritium-labelled thymine. Methods for the liquid scintillation counting of carbon-14 labelled purines and pyrimidines were also established.

The presence of the ascitic and subcutaneous forms of the Ehrlich tumor was found to have little effect on the incorporation of formate-C^{14} into the nucleic acids of the host tissues. In contrast an increased uptake of tritiated thymidine by the DNA of the host tissues was observed in animals bearing the Ehrlich ascites tumor, Novikoff hepatoma and the Walker 256 carcinosarcoma. This effect was particularly striking in the case of the liver and spleen of animals bearing the Walker 256 tumor.

Other investigators have isolated a substance known as toxohormone from tumor tissue which has been shown to produce certain systemic effects similar to those of tumor tissue. It was postulated that the increased incorporation of thymidine into the DNA of the host tissues might be the result of the action of toxohormone. In order to test this hypothesis, the effect of toxohormone on the incorporation of tritiated thymidine into the DNA of rat liver and spleen was studied. Crude toxohormone caused an increased uptake of thymidine by the DNA of spleen, but the results obtained for liver were equivocal. A highly purified fraction of toxohormone was prepared by ion-exchange chromatography and gel filtration. However, this fraction had no effect on the incorporation of thymidine into the DNA of rat liver and spleen. These results suggested that tumor tissue might contain a factor which stimulated DNA synthesis and which could be isolated in company with crude toxohormone.

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PUBLICATIONS

1. The Incorporation of Formate-C ^{14} into the Nucleic Acids of Rats with Regenerating Liver and Novikoff Hepatoma. J. C. Nixon and S. H. Zbarsky, Canadian Journal of Biochemistry and Physiology, **37**, 1405 (1959).

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The presence of the ascitic and subcutaneous forms of the Ehrlich tumor was found to have little effect on the incorporation of formate-C^{14} into the nucleic acids of the host tissues. In contrast an increased uptake of tritiated thymidine by the DNA of the host tissues was observed in animals bearing the Ehrlich ascites tumor, Novikoff hepatoma and the Walker 256 carcinosarcoma. This effect was particularly striking in the case of the liver and spleen of animals bearing the Walker 256 tumor.

Other investigators have isolated a substance known as toxohormone from tumor tissue which has been shown to produce certain systemic effects similar to those of tumor tissue. It was postulated that the increased incorporation of thymidine into the DNA of the host tissues might be the result of the action of toxohormone. In order to test this hypothesis, the effect of toxohormone on the incorporation of tritiated thymidine into the DNA of rat liver and spleen was studied. Crude toxohormone caused an increased uptake of thymidine by the DNA of spleen, but the results obtained for liver were equivocal. A highly purified fraction of toxohormone was prepared by ion-exchange chromatography and gel filtration. However this fraction had no effect on the incorporation of thymidine into the DNA of rat liver and spleen. These results suggested that tumor tissue might contain a factor which stimulates DNA synthesis and which could be isolated in company with crude toxohormone.

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INTRODUCTION

Malignant tumors are characterized by the ability to grow at the expense of, and in most cases lead to the death of the host organism. The autonomous growth of the tumor in the host is influenced by the defense mechanisms of the host and also by the systemic effects of the tumor on the host. The dominant aspect of this reciprocal tumor-host relationship is without question the effect of the tumor on the structure and function of the host tissues. In some cases the effect of the tumor on the host can be attributed to secondary effects such as infection, hemorrhage, and malnutrition. However, it is true that systemic effects of tumor may be evident when these secondary effects are absent (1,2). Furthermore it is known that cancer can cause death without destroying vital organs, such a cancer death has been termed a metabolic death (3). These observations have led many cancer investigators into studies of the systemic effects of tumor on the host tissues. Detailed and comprehensive treatments of this subject are found in reviews by Begg (3), Feninnger and Mider (1), Greenstein (4), Homburger (5), Nakahara and Fukuoka (6), and Goranson (7).

I. Systemic Effects of Tumors.

1. Nucleic acid metabolism of host tissues:

Several authors have shown that tumors have various effects on the nucleic acid metabolism in tissues of the host. Changes in the nucleic acid concentration of the host tissues have been found, and it has been shown that tumor tissue alters the incorporation of certain nucleic acid precursors. Cerecedo *et al.* (8-12) have

shown an increase in concentration of nucleic acids in the liver, lung, kidney, thymus and lymph nodes of sarcoma-bearing mice. These authors also found (13) that rats bearing Walker carcinoma showed an increase in the nucleic acid concentration of the spleen and the ribonucleic (RNA) concentration of bone marrow. Growth of the Murphy-Sturm lymphosarcoma, in the rat, was accompanied by an increase in the deoxyribonucleic acid (DNA) content of liver, lung, and spleen, and a decrease of DNA in the bone marrow (14,15). The RNA concentration of the host's spleen was also increased. During the development of the Jensen sarcoma in the rat a rise in the DNA concentration in the lung was observed (15). Cerecedo and associates (15) have also found that a myeloid leukemia in the mouse caused a rise in lung DNA and a decrease in DNA of the spleen. When the Murphy-Sturm lymphosarcoma, Jensen sarcoma or myeloid leukemia underwent regression each of the observed changes in nucleic acid concentration reversed and normal patterns were re-established.

Other investigators have studied the effect of tumors on the incorporation of several nucleic acid precursors into the nucleic acids of host tissues. Kelly and associates (16-18) have observed an increased rate of incorporation of P^{32} into the DNA of liver and spleen of mice and rats bearing several transplanted tumors. Similar increases in the incorporation of glycine-2-C^{14} and formate-C^{14} into the DNA of host tissues of tumor-bearing mice have been reported (19), although LePage and Heidelberger (20) found no significant difference between the incorporation of glycine-2-C^{14} into the DNA and RNA of the livers of rats bearing Flexner-Jobling carcinoma, compared to normal liver. In contrast,

it has been demonstrated (21) that the livers of rats bearing Flexner-Jobling carcinoma have a higher uptake of P^{32} into the DNA nucleotides than livers of normal rats. The results of experiments performed in this laboratory (22) have indicated that rats bearing sixth day Novikoff hepatoma had an increased incorporation of formate into the nucleic acid purines of the host tissues. On the other hand the presence of fourth day Novikoff hepatoma decreased formate incorporation into the nucleic acid purines and thymine of the host's spleen and intestinal mucosa, but had little effect on liver (23). Others (24,25) observed a stimulation of incorporation of adenine-8-C^{14} into the nucleic acids of the host tissues of tumor-bearing mice. In addition, Balis and co-workers (26,27) reported an increased incorporation of guanine and hypoxanthine into the RNA of certain host tissues of rats bearing transplanted human tumor.

Kelly and Jones (28) observed that repeated injections of tumor homogenates caused an increased incorporation of P^{32} into the DNA of tissues of normal animals. Furlong et al. (29) were able to demonstrate an increased uptake of adenine-8-C^{14} into the DNA of liver, spleen and lung when tumor homogenates were injected into mice. A similar stimulation of adenine incorporation in vitro into DNA was found (30) in a system containing normal tissue and tumor homogenates. Furlong et al. (29) concluded that tumor tissue, and to a lesser extent some normal tissues, elaborated a factor which seemed to increase the incorporation of labelled adenine into the DNA of normal tissues. More recent experiments by Furlong (31) and Furlong and Griffin (32) have indicated that

the soluble fraction obtained from the ultracentrifugation of Novikoff hepatoma homogenate possessed DNA polymerase-like activity. Inoue et al (33) have also reported that the injection of a human tumor extract caused an increased incorporation of P^{32} into rat liver DNA.

Slices of host tissues obtained from tumor-bearing rats were found (34) to incorporate tritiated thymidine and adenine-8-C^{14} to a markedly greater extent than did slices of corresponding normal tissues. The difference was most apparent in the slices of liver and spleen from the tumor-bearing rats, and was reversible since it disappeared on regression of the tumor.

Further to these observations on the effect of tumor on the incorporation of nucleic acid precursors into host tissues, Annau and co-workers (35) and Malmgren (36) have reported an increased mitotic rate in the livers of tumor-bearing mice and rats. Others (37-39) have obtained a mitosis-promoting substance from malignant tumors.

Waravdekar et al reported (40,41) that the synthesis of diphosphopyridine nucleotide (DPN) in the liver of tumor-bearing animals was depressed and suggested the existence of a humoral factor in the tissues and blood of animals. The findings of Oide (42) suggested that the decreased amount of DPN in the host tissues resulted from a high level of cortisone in tumor-bearing animals. However, Tomaru and Ono (43,44) have found that the injection of the toxohormone factor of Nakahara and Fukuoka (45) inhibited in vivo the synthesis of DPN by the liver.

The evidence obtained by other investigators indicated that the presence of tumor tissue causes a stimulation of the nucleic acid metabolism in the host tissues. In addition, the results suggested that the alteration in the nucleic acid metabolism of the host tissues might be caused by a factor produced by the tumor tissue.

2. Liver catalase:

Approximately fifty years ago a group of German workers reported (46-49) that liver catalase activity was markedly reduced in tumor-bearing humans and animals. In a series of later investigations Greenstein and co-workers (50-54) confirmed these results and established that the decrease in liver catalase was a result of the presence of the tumor and not due to any secondary effects to which the tumor may give rise. The depression of liver catalase was observed (4) in a variety of animals bearing several different spontaneous and transplantable tumors and the liver catalase activity returned to normal on extirpation of the tumor (53). It was also observed (55) that the activity was lowered to a greater degree by malignant tumors than by benign tumors and rapidly growing non-malignant tissue, such as embryo, had no effect (56). Recent reports (57,58) indicated that liver catalase was also lowered in animals bearing leukemia. Mason et al. (59) have demonstrated a depression of liver catalase in humans with cancer, but their data suggested that the effect was an indirect result of the malnutrition produced in the host. However, on the basis of the data available from animal experiments it was apparent that the lowered catalase activity in the liver of a tumor-bearing animal

was not explicable on the basis of malnutrition alone (3).

3. Other enzymes:

The study of liver catalase has occupied the dominant position in investigations of the enzyme aspects of tumor-host relations and consequently relatively little is known about the levels of other enzyme systems in the host tissues of tumor-bearing animals. The depression of kidney catalase was not as striking as that of liver; and blood catalase in tumor-bearing animals was normal (60).

The effects of tumor on other host tissue enzymes have been varied. Purr (61) observed an increase in tissue cathepsins during tumor growth and an increase in aspartic-glutamic transaminase has been found (62) in the livers of tumor-bearing rats. On the other hand reductions in the levels of carboxypeptidase (63) and D-amino acid oxidase (64,65) were found in the tissues of tumor-bearing animals. Greenstein (66) observed a slight decrease in liver arginase and a decrease in liver threonine dehydrase has been reported (67) in tumor-bearing rats.

Altered levels of some of the enzymes involved in carbohydrate metabolism have also been demonstrated. An increased aldolase content has been reported (68,69) in the serum of animals and humans with cancer. The elevation was attributed to release of enzyme from the tumor. On the other hand liver aldolase was found (70,71) to be lower in tumor-bearing animals. Several investigators (72-75) have also reported elevated serum lactic dehydrogenase levels in tumor-bearing animals and humans. Phosphorylase and glucose-6-phosphatase were found (76,77) to be

lower in the liver of tumor-bearing mice. Very little has been reported regarding investigations of the enzymes involved in lipid metabolism in the tumor-bearing host.

Alterations in serum acid and alkaline phosphatase in the tumor-bearing host have been observed for some time. Serum acid phosphatase was elevated in patents with carcinoma of the prostate (78) and serum alkaline phosphatase activity was raised in cases of bone tumor (79). These changes in the serum phosphatases appeared to be the result of release of the enzyme from the tumors.

4. Protein, carbohydrate and lipid metabolism:

The wide-spread alterations in the morphology and enzymology of the tumor-bearing host would indicate concurrent alterations in the metabolism of host tissues. Most of the attention in this field has been devoted to a study of protein metabolism in tumor-bearing animals. Some of the salient findings are presented here.

Mider et al. (80) have suggested that the tumor acts as a nitrogen trap in the animal resulting in a state of negative nitrogen balance. It was demonstrated by a number of investigators (3) that tumor tissue exhibited a high degree of incorporation of amino acids, although the evidence of Greenlees and LePage (81) indicated that the tumor protein did participate in the dynamic exchange of amino acids in the body. A decrease in the excretion of trytophan and anthranilic acid has been observed (82) in tumor-bearing mice.

Alterations in the level of plasma proteins have also been

demonstrated in patients or animals with cancer. The low level of plasma albumin has been attributed to a defective synthesis of albumin by the liver (83). However, recent work by Hradec (84) indicated that there was increased biosynthesis of albumin by the liver of tumor-bearing animals. The hypoalbuminemia in tumor-bearing animals was assumed to result from an inability of the host to meet the tumor's demand for albumin. An increase in the level of an α-globulin has been observed (85-87) in the serum of tumor-bearing rats. Elevations of this protein, to a lesser extent, were also observed in the serum of rats with regenerating liver, kidney and skin, wound-healing, pregnancy or in very young rats. It was assumed that the α-globulin fraction was directly concerned with tissue growth.

A few alterations in carbohydrate metabolism have been observed in the host tissues of tumor-bearing animals. Haven et al (88) have shown an increase in the citric acid level in the tissues of tumor-bearing rats. It has also been observed (89-91) that the livers of tumor-bearing mice and rats have a diminished ability to deposit glycogen. The presence of a tumor reduced blood sugar levels and glycosuria in diabetic animals and it has been suggested (3) that the blood glucose was shunted to the tumor. It has also been suggested (7) that diabetes places a limiting influence on tumor growth as the result of a competition for protein between gluconeogenesis on the one hand, and tumor growth on the other.

The mobilization of depot fat is apparent on gross examination of patients or animals with cancer. This mobilization is reflected by a hyperlipemia which has been well documented (3).

No ketosis or ketonuria occurs in association with the hyperlipemia indicating that the lipids were utilized to meet an increased caloric requirement. Trew and Begg (92) have observed an impairment in the incorporation of acetate into the fatty acids of adipose tissue and suggested that such a lesion might be responsible for a diminished storage of depot fat in the tumor-bearing animal. Except for the low tissue and high blood lipid content, a tumor rat appears to have an adequate metabolic system for the oxidation of fat.

Other systemic effects which have been demonstrated during tumor growth are anemia, enlargement of the liver, spleen and adrenals, and involution of the thymus. These and the other aberrations cited above serve to indicate a general alteration in the metabolism of the tumor-bearing host.

II. Mechanisms of Tumor Effects

Two theories have been advanced (7) to explain the mechanism underlying the systemic effects of tumors. One theory presumed that the tumor removed metabolites from the host tissues which were necessary for the proper host metabolism. The second theory suggested that the systemic effects were produced by some humoral substance or substances elaborated by the tumor tissue. One or both of these mechanisms could be responsible for the observed effects on the host tissues. The second hypothesis has received the most attention by investigators in this field, and has been particularly important in explaining the effect on liver catalase.

The evidence obtained regarding the depression of liver catalase activity in tumor-bearing animals suggested that this effect was caused by a circulating toxin released by the tumor. The liver catalase-depressant effect appeared to be specific for tumor tissue. Other factors such as protein depletion and X-ray which reduced liver catalase, also lowered the activity of other liver enzymes such as xanthine oxidase (3,54). Furthermore the presence of rapidly growing tissues such as regenerating liver, foetal tissue, and fast-growing benign tumors was without effect on liver catalase (54).

A correlation was observed (93,94) between the decrease in liver catalase activity and the size of the tumor and regression or removal of the tumor resulted in a return to normal hepatic catalase activity (53). The experiments of Lucke et al. (95) using parabiotic animals also substantiated the hypothesis of a circulating factor as the causal agent in liver catalase depression. Following implantation of tumor into one parabiont, an equal lowering of hepatic catalase activity was observed in both parabionts.

1. Toxohormone:

In 1948 Nakahara and Fukuoka (45) isolated a substance from tumor tissue which, when injected into normal animals, had the ability to decrease liver catalase activity. The material was obtained as the alcohol precipitate of an aqueous extract of tumor tissue and was designated toxohormone. The findings of Nakahara and Fukuoka were soon confirmed by others (96-98) and the production of toxohormone was found (45) to be a universal property of all the malignant cells tested, both carcinoma and sarcoma. It was

also soon discovered (96) that toxohormone could be isolated from normal tissues, but in much smaller amounts than from tumor tissues. Furthermore, normal tissue toxohormone preparations were found (96) to possess considerably less activity in lowering liver catalase. Further studies (99,100) revealed that toxohormone although active *in vivo*, had no effect on liver catalase *in vitro*. Toxohormone has been isolated from the urine of cancer patients (101), the ascitic fluid of animals bearing the Ehrlich ascites tumor (102), the gastric juice of patients with gastric tumors (103), and the blood of cancer patients (104).

In addition to the effect on liver catalase, it has also been shown that the injection of toxohormone resulted in changes in the non-hemin fractions in the liver of normal mice. Iijima *et al.* (105) have reported the following alterations in the liver of normal mice following the injection of toxohormone: a decrease in the ferritin fraction, an increase in the "nucleic acid iron fraction", and an increase in the hemosiderin fraction. Fukuoka and Nakahara (106) have induced thymus involution in mice by injecting toxohormone. Recently Kampschmidt *et al.* (107,108) have reported that the injection of toxohormone caused thymus involution, anemia, decreased plasma iron, depressed activity of the liver and kidney catalase, and increased weight of the liver, spleen and adrenals. It was also observed (107) that the plasma iron was up to 500 times as sensitive to toxohormone as liver catalase, and therefore was considered to be of value in testing for toxohormone activity.

The crude toxohormone preparation originally obtained by Nakahara and Fukuoka (45) depressed liver catalase activity in

doses of 50 to 100 mg per mouse. Further purification was obtained (6) by reprecipitation with one of the conventional protein precipitants resulting in a fraction active for mice in 5 mg amounts. Nakagawa et al. (109) obtained a copper sulfate precipitate of the crude fraction which was active at a dose of 1 mg. Extensive purification of toxohormone has been obtained by the use of ion-exchange chromatography. Ohashi and Ono (110) and Goranson (111) employed diethylaminoethyl (DEAE) cellulose ion-change columns and eluted the purified toxohormone with phosphate buffers of increasing acidity. Goranson (111) obtained catalase-depressing activity at a dose of 50 ug per rat. The most highly purified preparations of toxohormone have been prepared by Yunoki and Griffin (112,113) who have precipitated crude toxohormone by the addition of ether to an acetic acid extract of the acetone-dried powder of tumor tissue and purified the crude toxohormone by fractionation on Amberlite XE-64. Liver catalase-depressing activity in mice was obtained on injecting 1 µg of the most active fraction.

On the basis of the method of isolation of toxohormone and the fact that it was precipitated by protein precipitants such as trichloroacetic acid, perchloric acid and cupric sulphate, Nakahara and Fukuoka (6) assumed at the outset that toxohormone might be protein-like in nature, probably a polypeptide. Digestion of the fraction with 6N HCl yielded a number of amino acids. The original toxohormone preparation also contained about 30% nucleic acid (6), but in later experiments active toxohormone fractions free of nucleic acid were obtained (6,114). The association of nucleic

acid with many toxohormone preparations suggests that the toxohormone polypeptide is basic in character. In agreement are the findings of Ono et al. who have isolated, from tumor tissue, a basic polypeptide having toxohormone activity. This finding has been verified by Fujii et al. (115) who have isolated basic proteins from two tumors which were effective in depressing liver catalase activity and plasma iron levels in vivo but had no catalase inhibiting activity in vitro.

Toxohormone as originally isolated by Nakahara and Fukuoka (45) was non-dialyzable (6). However, further studies demonstrated that toxohormone could be converted into a dialyzable form without loss of activity (6). Ono et al. (116) subjected a toxohormone fraction to pepsin hydrolysis and isolated an active fraction from the hydrolysate as a crystalline picrate. The active picrate gave a blue color after reacting with ninhydrin for one week and on hydrolysis yielded a number of amino acids. The molecular weight of the active picrate was calculated to be close to 4000.

In a recent paper Yunoki and Griffin (113) have studied the composition and properties of a highly purified preparation of toxohormone prepared from human tumor tissue. The purified fraction represented approximately 0.002% by weight of the fresh tumor tissue. About 80% of the fraction could be accounted for as polypeptide, the remaining 20% consisting largely of phospholipid. Hydrolysis with pepsin or 6N HCl rendered the fraction inactive and the acid hydrolysate yielded the following amino acids: aspartic acid, threonine, serine, proline, glutamic acid, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine,

lysine, and arginine. Ethanolamine was also detected following acid hydrolysis. Leucine, alanine, glutamic acid and aspartic acid were present in the highest concentration and accounted for almost one half of the total amino acid composition. Further fractionation of the highly purified toxohormone preparation yielded three sub-fractions with molecular weights ranging from 4200 to 6400. The N-terminal amino acid of each sub-fraction was shown to be arginine.

The evidence indicated (6) that the action of toxohormone was to suppress the process of catalase synthesis; however, the mechanism of this action is as yet unknown. Nakahara and Fukuoka (117) postulated that toxohormone might cause underutilization of porphyrin by impairing iron metabolism, thereby impairing the synthesis of the iron-containing moiety of catalase. A similar mechanism may be invoked to explain the anemia that accompanies neoplastic disease. Ono et al. (118) have recently presented evidence which indicated that toxohormone acted by lowering iron mobilization from the iron reserves.

2. Other tumor preparations:

The kochsaft factor of Hargreaves and Deutsch (119), obtained as a boiled extract of tumor homogenate, inhibited catalase activity in vitro apparently by a binding of the iron moiety of catalase. However, kochsaft factor lacked activity in vivo, in contrast to toxohormone, indicating that they are two different substances.

Other investigators (120-122) have obtained heat stable preparations from a number of tumors which were found to stimulate

tumor growth. Enhancement of tumor growth has also been found (123,124) following the injection of fresh or lyophilized tumor homogenates. These authors suggested that the active factor present in the tumor homogenate might be a lipoprotein. Haven et al (125) have observed an increase in body weights when phospholipids prepared from tumor were fed to normal rats.

A substance named oncotrephin has been isolated from tumors and has been shown to promote the mitosis of mouse epidermal cells in vitro (126) and to accelerate the growth of L cells in tissue culture (127). Preliminary studies on the characterization of oncotrephin indicated (128) that it was a protein which might be associated with a porphyrin compound. An oncotrephin-like substance has been isolated (129) from regenerating rat liver. The subject of growth-promoting factors in tissues has been reviewed by Paschkis (130).

III. The Present Investigation.

The role of the nucleic acids and related compounds as participants and determinant factors in the biosynthesis and metabolism of cell constituents has been well established (131). The importance of the nucleic acids in protein and enzyme synthesis has also been clearly demonstrated (132). As indicated in the section on nucleic acid metabolism, the presence of tumor tissue has been found to cause certain alterations in the nucleic acid metabolism of the host tissues. The findings of Cerecedo et al (15) indicated that increases in the DNA concentration of the host tissues of tumor-bearing animals occurred more frequently than increases in

RNA concentration. The results of Kelly et al. (16-19) also demonstrated that tumor enhances incorporation of nucleic acid precursors to a greater degree into the DNA of the host tissues as compared to the RNA.

The purpose of the research reported herein, has been to study in more detail the effects of tumor tissue on the nucleic acid metabolism of hose tissues. In view of the observations of Cerecedo et al. (15) and Kelly et al. (16-19) and because of the important role of DNA in the cell it was planned to study in particular the effect of tumor on DNA metabolism in host tissues.

The results of preliminary experiments indicated that neither the solid subcutaneous form nor ascites form of the Ehrlich tumor had an effect on the incorporation of formate-C^{14} into the nucleic acid of the host's spleen, liver, kidney or lung. It has been observed by several investigators (22,23,133) that formate is rapidly excreted as expired CO_2 following subcutaneous injection. It was felt, therefore, that this nucleic acid precursor was not satisfactory for studies of the effect of tumor on host tissues. Thymidine has been shown to be a specific precursor for DNA thymine (134) and is therefore useful in determining alterations in DNA metabolism. With these considerations in mind a study was made of the effects of tumor on the incorporation of tritium-labelled thymidine into the DNA thymine of host tissues. Liquid scintillation counting methods were developed in order to assay the radioactivity of the tritium-labelled thymine. Methods for the liquid scintillation counting of carbon-14 labelled purines and pyrimidines were also established.

The results of the experiments recorded herein indicated that the presence of Ehrlich ascites tumor in a mouse caused an increased incorporation of thymidine into the DNA of the host's liver, spleen, and kidney. On the other hand, no increase in the incorporation of tritiated thymidine was observed in the host tissues of rats bearing the Novikoff hepatoma. However, a marked increase in the incorporation of tritiated thymidine into the DNA of host liver and spleen was observed in rats bearing the Walker carcinosarcoma 256. Thymidine also tended to be incorporated to a greater degree into the DNA thymine of the lung and intestinal mucosa of rats bearing the Walker tumor as compared to control rats.

The concept of a tumor toxin has been postulated (4,6,135) as an explanation of the systemic effects of tumor. Such a hypothesis seemed reasonable in view of the many clinical and experimental observations which indicated that profound systemic effects could be caused by relatively small tumor lesions. These effects appeared to be a result of the presence of malignant cells in the host and could not be explained on the basis of any anatomical aberrations caused by the tumor. An example of this phenomenon was apparent in primary tumors of the lung in which the presence of a relatively small amount of tumor tissue has been observed to cause profound cachexia, weight loss, anorexia and anemia.

Nakahara and Fukuoka (45) and others (96-98) have clearly demonstrated that toxohormone is active in depressing liver catalase. However very little work has been reported on the effect of tumor preparations on nucleic acid metabolism. Kelly and Jones (28) have shown that repeated injections of tumor homogenates

caused an increased incorporation of P^{32} into the DNA of normal liver and spleen. Furlong et al. (29,30) concluded that tumor tissue elaborated a factor which caused an increased incorporation of adenine-8-C^{14} into the DNA of normal tissues. The injection of a human tumor extract caused (33) an increased incorporation of P^{32} into rat liver DNA and a mitosis-promoting substance has been isolated (37-39) from malignant tumors. Ono and Tomaru (43,44) observed that toxohormone inhibited in vivo the DPN synthesis activity of the liver. However, no experiments have been reported on the effect of toxohormone on nucleic acid metabolism. Since it has been shown that toxohormone caused a decrease in the synthesis of the enzyme catalase, it seemed reasonable to hypothesize that such an effect could be mediated through an alteration in nucleic acid metabolism. Furthermore, the other systemic effects observed in tumor-bearing animals, or following the injection of toxohormone, might be expressions of a deep-seated and more fundamental disturbance brought about by toxohormone.

With these considerations in mind an investigation was made of the effect of toxohormone preparations on the in vivo incorporation of tritiated thymidine into the DNA thymine of rat liver and spleen. Crude toxohormone was isolated from Walker 256 tumor by the method of Yunoki and Griffin (112), and assayed by determining the depression of plasma iron levels in rats.

The injection of crude toxohormone into rats tended to increase the degree of incorporation of thymidine into the DNA thymine of spleen measured twelve hours after the injection of crude toxohormone. However the results obtained for liver were equivocal. In view of these results a study was made of the

effect of a purified preparation of toxohormone on the incorporation of thymidine-H^3 into the DNA of rat liver and spleen. A highly purified fraction of the crude toxohormone was obtained by ion-exchange chromatography on Amberlite XE-64 and Sephadex G50. However the purified preparation of toxohormone appeared to have no effect on the rate of incorporation of tritiated thymidine into the DNA thymine of liver and spleen.

These results have been discussed in relation to the findings of others. The concept of the production of growth-promoting factors by malignant and non-malignant tissues has been reviewed and the possible relationship of these factors to toxohormone has been pointed out. In addition, the significance of alterations in the nucleic acid metabolism of the host tissues of tumor-bearing animals has been discussed in relation to the systemic effects of tumor. In view of the findings obtained herein, it has been postulated that crude toxohormone may be an important factor in the etiology of the systemic effects of tumor.

EXPERIMENTAL

A. METHODS

I. Materials

1. Experimental animals:

Male Sprague-Dawley rats, female Swiss mice or female Ha/ICR mice from the colony at the University of British Columbia were used in the experiments reported herein.

2. Radioactive materials:

Sodium formate-C^{14} was obtained from Merck & Co., Ltd. The C^{14}-labelled purines and pyrimidines used in studies of the liquid scintillation counting methods, were isolated from the DNA or RNA of tissues of rats or mice which had previously been injected with sodium formate-C^{14}. Tritiated thymidine was obtained from Schwarz Bio-Research Inc.

II. Tumors Studied

In any study of the effect of tumor tissue on the metabolism of the host tissues, there are certain criteria which govern the selection of the tumors used in the studies. It is desirable to use readily transplantable tumors which will grow to a considerable size without metastasizing. Furthermore, in order to study the effect on the host tissues of humoral substances produced by the tumor, it is also desirable to select a tumor which can be implanted at a site some distance from the host tissues being studied.

The intraperitoneal Ehrlich ascites tumor is an easily

transplantable tumor which can also be grown subcutaneously. The Novikoff hepatoma can be implanted intraperitoneally and grows to a considerable size without metastasizing. The Walker carcinosarcoma 256 tumor was felt to be a very satisfactory tumor for these studies since it is an easily transplantable, rapidly growing tumor, which can be implanted into the muscle of the hind legs of the rat. Furthermore the work of Kampschmidt et al. (108) indicated that the Walker 256 tumor elaborated more toxohormone than other tumors.

1. Ehrlich ascites tumor:

The Ehrlich ascites tumor was transplanted in mice as described by Paterson (136). The tumor was transplanted at weekly intervals and the material for transplantation was prepared in the following manner. The ascitic fluid was drained from the peritoneal cavity of the donor mouse and centrifuged. The cells which sedimented were suspended in 10 volumes of Ringer's ascites buffer (137). If the ascitic fluid was sanguinous the cells were resuspended twice in buffer. Each mouse was then injected intraperitoneally with 0.2 ml. of a 1:10 suspension of Ehrlich ascites tumor cells.

2. Solid subcutaneous Ehrlich tumor:

When the Ehrlich ascites tumor cell suspensions prepared in the above manner were injected subcutaneously into mice, subcutaneous tumors formed at the sites of injection. Injection into the axillae resulted in the rapid formation of tumor masses but it was difficult to isolate and excise these tumors. Injec-

tion of the tumor cell suspension under the skin of the anterior abdominal wall gave rise to subcutaneous tumors which quickly eroded through the abdominal wall into the peritoneal cavity. The anterior thoracic region was found to be a satisfactory site for the subcutaneous injection of the tumor cells. Subcutaneous tumors growing in this region showed gross evidence of necrosis on the 16th day of tumor growth, but none up to the 12th day of tumor growth. Each mouse was injected bilaterally in the anterior thoracic region with 0.1 ml of a 1:10 Ehrlich ascites tumor cell suspension.

3. Novikoff hepatoma:

The Novikoff hepatoma has been maintained in Sprague-Dawley rats by weekly intraperitoneal injection of 0.5 ml of a 1 in 5 dilution of a minced tumor cell suspension in physiological saline.

4. Walker carcinosarcoma 256 tumor:

The Walker 256 tumor has been maintained in Sprague-Dawley rats by transplantation at approximately two week intervals. Two-tenths ml of a 1 in 5 dilution of a minced tumor cell suspension in physiological saline was injected into the muscle of the medial aspect of both hind legs. The intramuscular site was chosen because a large amount of tumor tissue could be obtained. Subcutaneous Walker 256 tumor was found to ulcerate rapidly and the intraperitoneal tumor metastasized rapidly to other organs. Twelve to fourteen day Walker 256 tumor was used, in the experiments described herein, since rats bearing the intramuscular Walker 256 tumor began to die about 15 days after implantation of

the tumor. No gross evidence of metastasis was observed in rats bearing 12-14 day intramuscular Walker tumor.

III. Determination of Liver Catalase

The activity of liver catalase was determined by a modification (138) of the method of Douhce and Shanewise (139). In this method an excess of hydrogen peroxide is allowed to react with an aliquot of liver homogenate for 5 minutes and the unreacted peroxide determined iodimetrically. The liver catalase activity was expressed as the first order reaction rate constant (K) for an amount of liver which gave color with the Folin-Ciocalteau reagent equivalent to 0.01 mg of tyrosine.

IV. Determination of Plasma Iron

In those experiments in which the plasma iron was determined, the animals were injected intraperitoneally with 5 mg of sodium pentabarbitone in 0.1 ml of water, and 8 mg of sodium heparin in 0.1 ml of water. The animals were exsanguinated by withdrawing blood from the inferior vena cava using a syringe. If the heparin was omitted it was more difficult to withdraw blood and the serum obtained usually showed evidence of hemolysis. The plasma iron was assayed by the method of Henry et al. (140), in which the iron is removed from protein and the protein precipitated by treatment with hot trichloroacetic acid. The iron in the supernatant is reduced by hydrazine sulfate and the ferrous iron determined colorimetrically by reaction with sulfonated bathophenanthroline.

V. Isolation and Determination of Purines and Pyrimidines.

The nucleic acid purines and pyrimidines of the tissues were isolated and determined in the following manner. The lipids were extracted by homogenizing in 95% ethanol, ethanol-ether (3:1), and ether. The fat-free tissues were dried and the nucleic acids extracted in sodium chloride solution as described by Bendich et al. (141). RNA and DNA were separated by the procedure of Tyner et al. (142) and the DNA was then hydrolyzed with perchloric acid as described by Marshak and Vogel (143). The hydrolyzate was neutralized by the addition of 50% potassium hydroxide, chilled, and the precipitated potassium perchlorate removed by centrifugation. The purines and pyrimidines, contained in the supernatant fluid, were separated by ion-exchange chromatography on columns of Dowex-50 (200-400 mesh), H^+ form. The elution was carried out first with 20 to 30 ml of distilled water, followed by 1.5N HCl. The eluate was collected in fractions of 2 to 3 ml using an automatic fraction collector. The optical density, at approximately 260 mμ, of the eluate from the Dowex-50 column was continuously measured by a GME model 10 ultraviolet absorption meter and recorded by a Rustrak miniaturized automatic chart recorder. The fractions containing the individual pyrimidines and purines were pooled, evaporated to dryness and the residues were dissolved in 0.1N HCl and made up to a known volume. The absorption spectrum of each base was determined using a Beckman DK-2 ratio recording spectrophometer, and the concentrations determined from the molar extinction coefficients.

VI. Radioactive Counting Procedures

1. Gas flow counting:

In those experiments in which the incorporation of formate-C^{14} into the nucleic acid purines and pyrimidines was studied, the radioactive counting was done with a windowless gas flow counter connected to a scaler as described (23).

2. Liquid scintillation counting:

The radioactivity of the tritium-labelled thymine was determined using an automatic Tri-Carb liquid scintillation spectrometer, (Packard Instrument Co., La Grange, Ill.). Liquid scintillation counting began in 1950 when it was reported (144,145) that dilute solutions of fluorescent substances in aromatic solvents could be used with photomultiplier tubes to detect radiation. Beta particles striking the fluorescent substance cause emission of photons which are detected by the photocathode face of the photomultiplier tube and give rise to electrical pulses which are amplified electronically. The photomultiplier voltage is varied by changing the tap setting of the instrument. In general a tap setting is selected which will give maximum counting efficiency. After amplification the pulses are selected by the pulse height analyzer which, by appropriate settings of the discriminators, is able to select the pulses produced by the beta radiation of carbon-14 or tritium. Upon leaving the analyzer, the pulses are fed into the red and green scalers. By means of an electronic timer and a digital printer the data are printed out on paper. The instrument is equipped with an automatic sample changing device which handles up to 100 standard 5 dram

glass vials.

In order to eliminate thermal pulses arising from the photomultiplier tube, the use of cooling and a coincidence arrangement of two photomultipliers is necessary. The automatic sample changer, [illegible] photomultipliers and preamplifiers are kept in a freezer at -3°C. Two photomultipliers "look" at the sample vial containing the solution of radioactive sample and liquid scintillator and therefore legitimate light pulses are seen simultaneously by both. An electronic coincidence circuit is provided to pass such pulses and to reject thermal pulses which occur at random in the two photomultipliers and rarely chance to coincide in time. In all the counting procedures reported herein, the counts were recorded on the red scaler of the instrument at selector position 2, the AA' discriminator set at 10V, the B discriminator set at 50V and the C discriminator set at 100V.

B. RESULTS

I. Liquid Scintillation Counting Procedures.

The advent of liquid scintillation counting (144,145) provides a method which in many cases allows greater ease in the preparation and counting of C^{14}- and tritium-labelled samples. Furthermore this method yields a higher counting efficiency (146) for tritium as compared to other conventional radioactive assay techniques.

Although methods for the liquid scintillation counting of several biologically important compounds have been described (147), little information was available concerning liquid scintillation systems for the counting of C^{14}-labelled purines and pyrimidines, and tritiated thymine. Davidson and Feigelson (146) have reported methods for counting C^{14}-labelled bases in aqueous solution. Since in the experiments reported herein the isolated purines and pyrimidines were dissolved in 0.1N HCl, the counting methods were designed so as to allow the most efficient determination of radioactivity in 0.4 ml aliquots of these solutions. In most cases this volume contained a significant amount of radioactivity.

1. Procedures for counting C^{14}-labelled purines and pyrimidines:

Davidson and Feigelson (146) have reported a satisfactory method for counting C^{14}-labelled purines in which the bases are first dried in the vials and then dissolved in ethylene glycol. A scintillation system containing the following components is then added to each vial:

p-dioxane	:	6 parts by volume
anisole	:	1 part by volume
1,2 dimethoxyethane	:	1 part by volume

2,5 diphenyloxazole (PPO) is dissolved in the solvent system to a concentration of 1.2%; 1,4-bis-2-(5-phenyl oxazolyl)-benzene (POPOP) to a concentration of 0.05%.

Davidson and Feigelson (146) designated this system Polyether-611. The ratio of Polyether-611 to ethylene glycol was 4:1. In view of these findings, experiments were performed to determine the reliability of the ethylene glycol-Polyether-611 system. Serial amounts of adenine-C^{14} in 0.1N HCl were dried in the counting vials. The residues were then dissolved in 1.25 ml of ethylene glycol as described by Davidson and Feigelson (146) and 5 ml of the Polyether-611 scintillator system were added to each vial. The vials were cooled to -3°C and counted at the optimum tap setting of 4.

The results are shown in Figure 1. It is seen that there is proportionality between the amount of adenine-C^{14} present and the radioactivity detected. If the ethylene glycol was omitted from the system the agreement between duplicate samples was poor and there were also variations on repeated counting of individual samples.

Using a similar counting system varying aliquots of cytosine-C^{14}, uracil-C^{14}, guanine-C^{14} and thymine-C^{14} were counted and the results plotted in Figures 2 to 5. Again there is proportionality between the amount of base present and the radioactivity detected.

Preliminary experiments had indicated that adenine-HCl

might have a quenching effect if the concentration of the compound was increased beyond the quantities shown in Figure 1. Furthermore Davidson and Feigelson (146) have observed that as little as 1 mg of adenine per vial reduced the counting efficiency by about 10%, whereas up to 4 mg of guanine per vial had no quenching effect. Therefore samples of the purines and pyrimidines were prepared containing constant amounts of radioactivity but increasing amounts of non-radioactive carrier. The radioactivity was determined using the Polyether-611-ethylene glycol system. From Figure 6 it is seen that the quenching effect of adenine-HCl is quite pronounced as the amount of base is increased from 0.036 mg to 4,036 mg. The quenching effect of guanine-HCl is much smaller and quenching by the pyrimidine bases is negligible (Figure 7 and 8).

Since aqueous solutions are soluble in the Polyether-611 system, it was decided to substitute water for ethylene glycol in the counting system. Serial aliquots of the bases dissolved in 0.1N HCl were dried, the residues dissolved in 1 ml of water and 4 ml of the Polyether-611 scintillator system added. The samples were counted at tap 4 and the results are shown in Figures 9 to 13. In all cases except uracil there is proportionality between the amount of base present and the radioactivity determined. The lack of linearity with uracil is attributed to a slight coloration of the uracil solution which caused color quenching as the amount of uracil was increased.

Table I

Comparison of counting efficiency of water and ethylene glycol systems for C^{14}-labelled purines and pyrimidines.

	Counts per Minute	
	Water	Ethylene glycol
Thymine-C^{14}	424	351
Uracil-C^{14}	252	58
Cytosine-C^{14}	56	56
Guanine-C^{14}	176	176
Adenine-C^{14}	843	817

On comparing the counts obtained from equal quantities of these bases using the water and ethylene glycol systems as shown in Table I, it is evident that the ethylene glycol system has a lower efficiency for thymine-C^{14} and uracil-C^{14}. However, there is no significant difference in the efficiency of the two systems for C^{14}-labelled cytosine, guanine and adenine.

In the procedures described above the HCl solutions of the purines and pyrimidines were dried in the counting vials and the residues dissolved in water or ethylene glycol before adding the scintillator system. Since drying the samples in the vial required a considerable amount of time it was felt that the procedure could be simplified by eliminating the drying step. However, 0.4 ml of 0.1N HCl was found to be immiscible with 4 ml of Polyether-611 at -3°C.

A system was then sought which would allow the 0.1N HCl and Polyether-611 solutions to remain miscible at -3°C. Several authors (148-155) have described methods in which hyamine hydrox-

ide (p-(diisobutyl-cresoxethoxyethyl)-dimethyl benzyl-ammonium hydroxide) has been used as a solvating compound for the liquid scintillation counting of biological materials. It was felt therefore, that the addition of hyamine hydroxide to the counting system might allow miscibility of the 0.1N HCl and Polyether-611 solutions. Serial amounts of the C^{14}-labelled base dissolved in 0.1N HCl were pipetted into the counting vial and dried in vacuo. The residues were then dissolved in 1 ml of 0.1N HCl. One-half ml of hyamine hydroxide, prepared by the method described by Eisenberg (154), was added to each vial, followed by 4 ml of the Polyether-611 system. The solutions were miscible at -3°C. The use of an automatic syringe was found to be a convenient method for dispensing the hyamine hydroxide and organic solvents into the counting vials. Tap 5 was found to be the optimum voltage setting for this system. From Figures 14 to 18 it is seen that there is proportionality between the amount of C^{14}-labelled compound added and the radioactivity determined. The lack of linearity in the uracil curve is attributed to color quenching.

In order to determine whether hyamine hydroxide and 0.1N HCl have a quenching effect on carbon-14 in the system containing 4 ml of Polyether-611, a series of samples were prepared in which the volume of these solutions in the counting vials was increased independently of one another from 0.2 ml to 1.0 ml. From Figure 19 it is apparent that increasing the volume of 0.1N HCl or hyamine hydroxide solution causes a decrease in the counting efficiency of the system.

Experiments were performed to determine the effect of increasing the volume of Polyether-611. A series of vials were prepared containing 0.4 ml of a solution of adenine-C^{14} in 0.1N HCl, 0.5 ml of hyamine hydroxide and increasing amounts of Polyether-611. The samples were counted at tap 5. From Figure 20 it is seen that there is a sharp increase in the counting efficiency of the system as the volume of Polyether-611 is increased to 5 ml. The addition of more than 5 ml of Polyether-611 has little effect on the efficiency.

It is felt, therefore, that the following is a suitable counting system for C^{14}-labelled purines and pyrimidines:

0.4 ml of a solution of C^{14}-base in 0.1N HCl.

0.5 ml of hyamine hydroxide.

5 ml of Polyether-611 containing 1.2% PPO and 0.05% POPOP.

Maximum counting efficiency at tap 5.

On comparing the ethylene glycol-Polyether-611 and hyamine hydroxide-Polyether-611 systems using equivalent amounts of adenine-C^{14} as reference, it was found that the former system had a counting efficiency 1.5 times that of the latter. This increase in efficiency was offset by the greater ease of preparation of the samples containing hyamine hydroxide and, therefore, the latter system was considered to be the more suitable.

Kinnory et al (155) have described a counting system containing toluene, ethanol, hyamine hydroxide and PPO which was suitable for the liquid scintillation measurement of radioactivity in a variety of biological materials. It was felt that such a

system might be satisfactory for the liquid scintillation counting of C^{14}-labelled purines and pyrimidines. The system of Kinnory et al. (155) was slightly modified to give the following counting system:

0.4 ml of a solution of C^{14}-labelled purine or pyrimidine in 0.1N HCl.

1.0 ml of hyamine hydroxide.

3 ml of absolute ethanol.

4 ml of toluene containing 0.63% PPO and 0.0262% POPOP.

Maximum counting efficiency at tap 5.

The addition of POPOP in this concentration increased the efficiency by about 8%. However, the hyamine hydroxide, Polyether-611 system was found to be about 10% more efficient than the hyamine, hydroxide, ethanol, toluene system for the C^{14}-labelled bases.

2. Procedures for counting H^3-labelled thymine:

In attempting to find a suitable method for the liquid scintillation counting of thymine-H^3, procedures were employed similar to those described for the C^{14}-labelled purines and pyrimidines. The thymine-H^3 was prepared by hydrolysing thymidine-H^3 with perchloric acid (143). The excess perchloric acid was precipitated as potassium perchlorate and carrier thymine was added to the solution of thymine-H^3. The thymine was purified by ion-exchange chromatography (23) and the fractions containing the thymine were pooled and dried. The residue of thymine-H^3 was dissolved in 0.1N HCl to a concentration of about

1 mg of thymine-H^3 per ml. Increasing volumes of thymine-H^3 dissolved in 0.1N HCl were pipetted into the counting vials. The solutions were evaporated in vacuo and the residues dissolved in 1 ml of ethylene glycol. Four ml of the Polyether-611 system were added to each vial. In another series 1 ml of water was substituted for the ethylene glycol. The samples were cooled to $-3^{\circ}C$ and counted at the optimum tap setting of 7 in the liquid scintillation spectrometer. From Figure 21 it is evident that there is little difference in the efficiency of the water and ethylene glycol counting systems. Furthermore, there is fairly good proportionality between the amount of thymine-H^3 per vial and the radioactivity detected, particularly in the region up to 1 mg of thymine-H^3. This region is of particular importance, since the amounts of thymine that are counted in the experiments described herein fall within this range. The counting efficiency of the ethylene glycol system for thymidine-H^3 was found to be 4.4%, that of the water system 4.7%, using the stated activity of a commercially obtained sample of tritiated thymidine as an approximate standard.

In agreement with the experiments on C^{14}-labelled purines and pyrimidines, hyamine hydroxide was found to facilitate the preparation of the samples of thymine-H^3. One-half ml of hyamine hydroxide solution was added to 1 ml of the 0.1N HCl solutions of thymine-H^3, followed by 4 ml of the Polyether-611 system. The samples were counted at tap 7. From Figure 22 it is seen that there is good proportionality between the amount of thymine-H^3 in the series of vials and the radioactivity detected. The results shown in Figure 19 also indicate that 0.1N HCl and hyamine

hydroxide have a slight quenching effect on this system containing thymine-H^3. In a counting system containing 0.4 ml of a standard solution of thymidine-H^3 in 0.1N HCl, 0.5 ml of hyamine hydroxide and 4 ml of Polyether-611, the counting efficiency was found to be 2.1%. Increasing the volume of Polyether-611 caused a gradual increase in the counting efficiency as shown in Figure 23.

Using the hyamine hydroxide, ethanol, toluene system as outlined previously the counting efficiency for H^3-labelled thymine was found to be about 40% higher than the hyamine hydroxide Polyether-611 system. Increasing the volume of the toluene-scintillator solution up to 6 ml caused a sharp increase in the efficiency (Figure 24). However, when 6 ml or more of the toluene solution were added, the solutions separated into 2 phases when cooled to $-3^{\circ}C$. Increasing the volume of the toluene-scintillator solution beyond 6 ml had little effect on the counting efficiency except for a slight decrease when 15 ml of the solution were added.

As a result of the above experiments the system used routinely in these studies for counting tritium-labelled thymine in the liquid scintillation spectrometer is as follows:

0.4 ml of a solution of the thymine-H^3 in 0.1N HCl.

1 ml of hyamine hydroxide.

3 ml of absolute ethanol.

5 ml of toluene containing 1.63% PPO and 0.0262% POPOP.

Maximum counting efficiency at tap 7.

Fresh absolute ethanol was found to be necessary for the system. If the absolute ethanol was exposed to the air for a few days and then used in the counting system, the solutions in the vials separated into two phases. This feature was attributed to the absorption of water by the absolute ethanol. The relative counting efficiency of this system for thymidine-H^3 is 3% using an approximate standard of tritiated thymidine. From Figure 25 it is seen that there is good proportionality between the amount of thymine-H^3 per vial and the radioactivity detected.

In order to correct for radioactive decay, variations in the counting efficiency of the instrument and for variations in the concentration of the hyamine hydroxide, duplicate 0.4 ml aliquots of a standard thymine-H^3 solution in 0.1N HCl were counted with each series of vials and the necessary corrections made. Since the solutions containing all the components of the counting system become discolored on standing, the vials containing the standard thymine-H^3 were prepared anew with each series of vials.

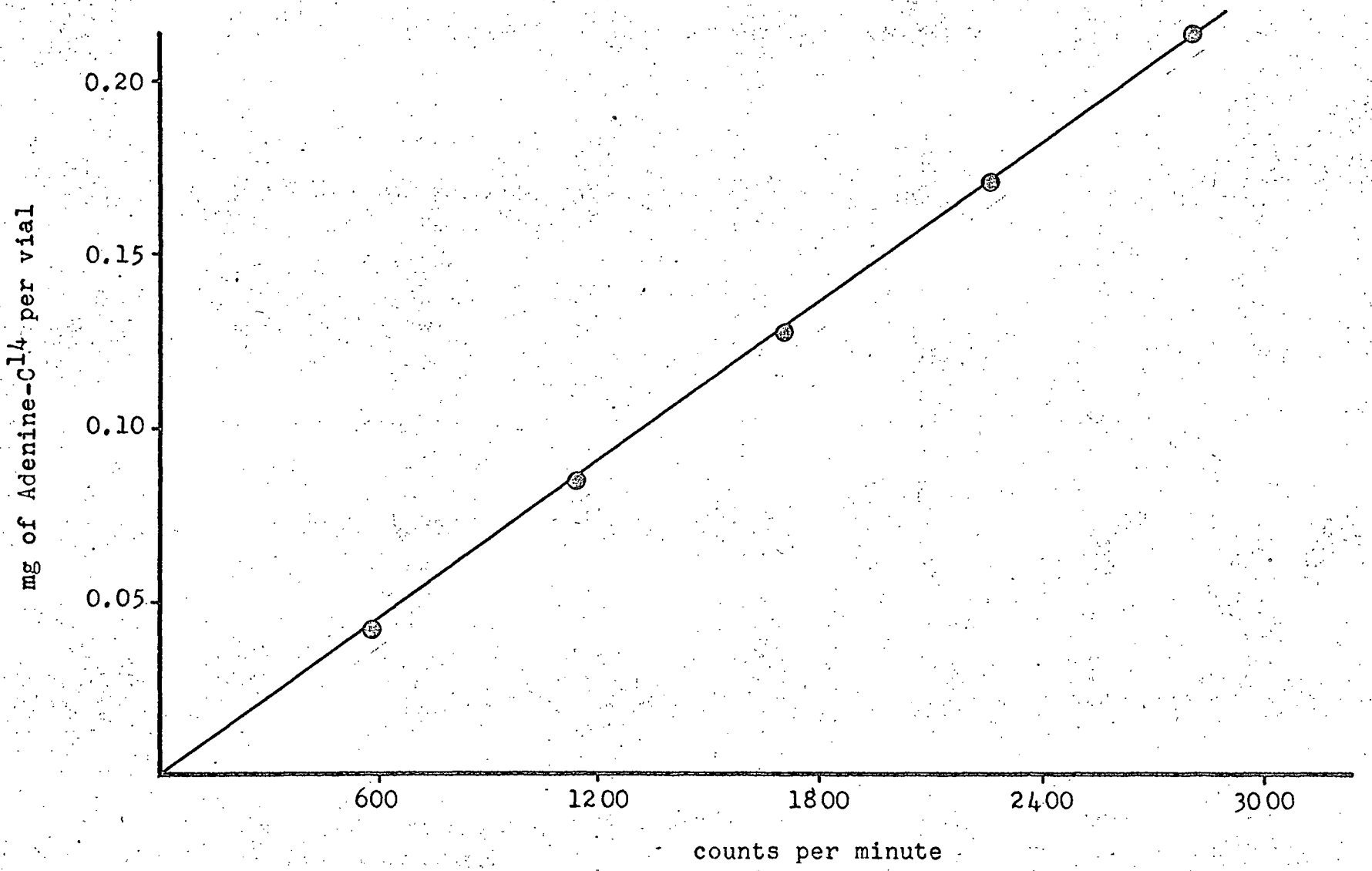

Figure 1. Counts per minute obtained from Adenine-C^{14} dissolved in 1.25 ml of ethylene glycol and 5 ml of Polyether-611.

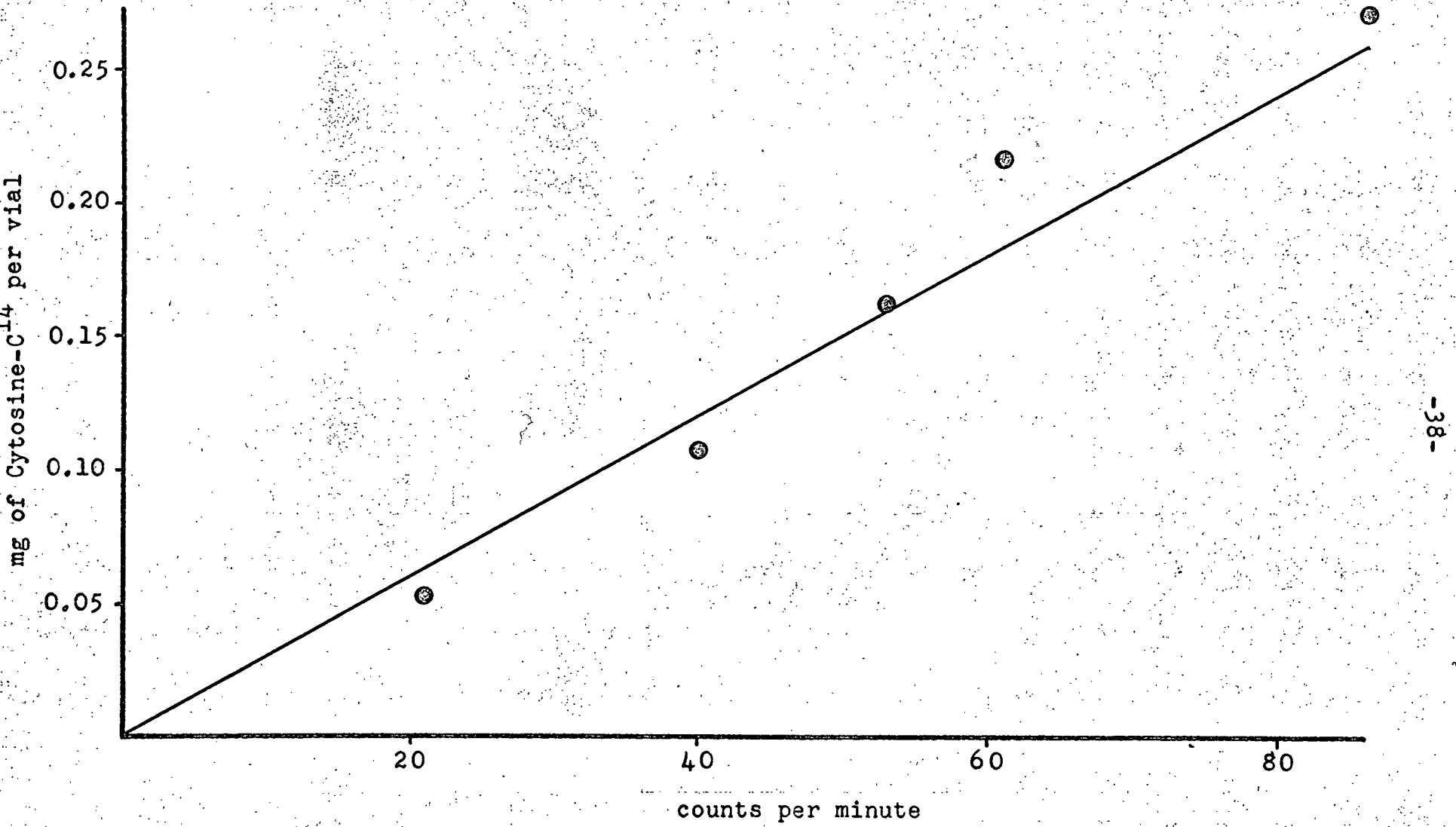

Figure 2. Counts per minute obtained from Cytosine-C^{14} dissolved in 1.25 ml of ethylene glycol and 5 ml of Polyether-611.

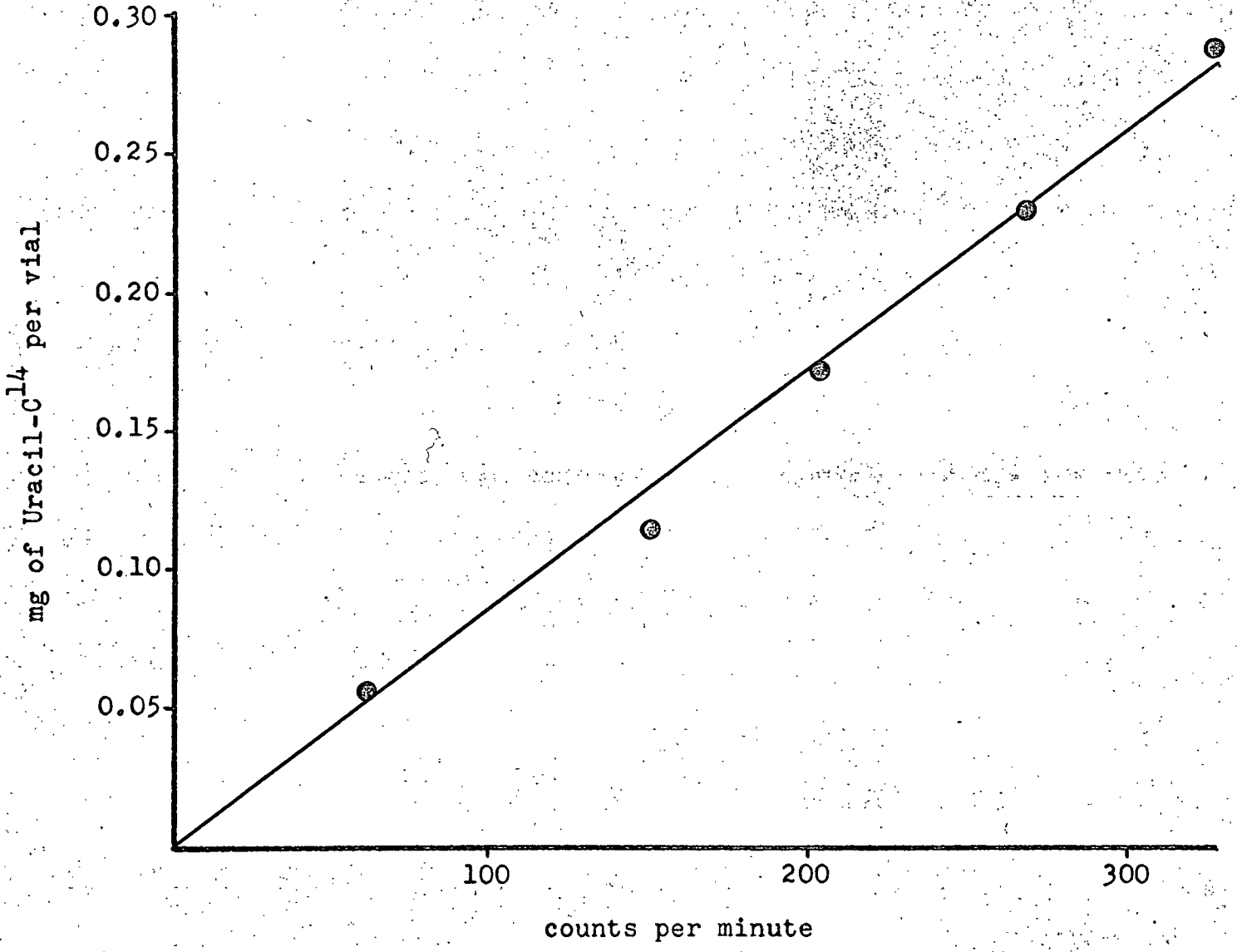

Figure 3. Counts per minute obtained from Uracil-C^{14} dissolved in 1.25 ml of ethylene glycol and 5 ml of Polyether-611.

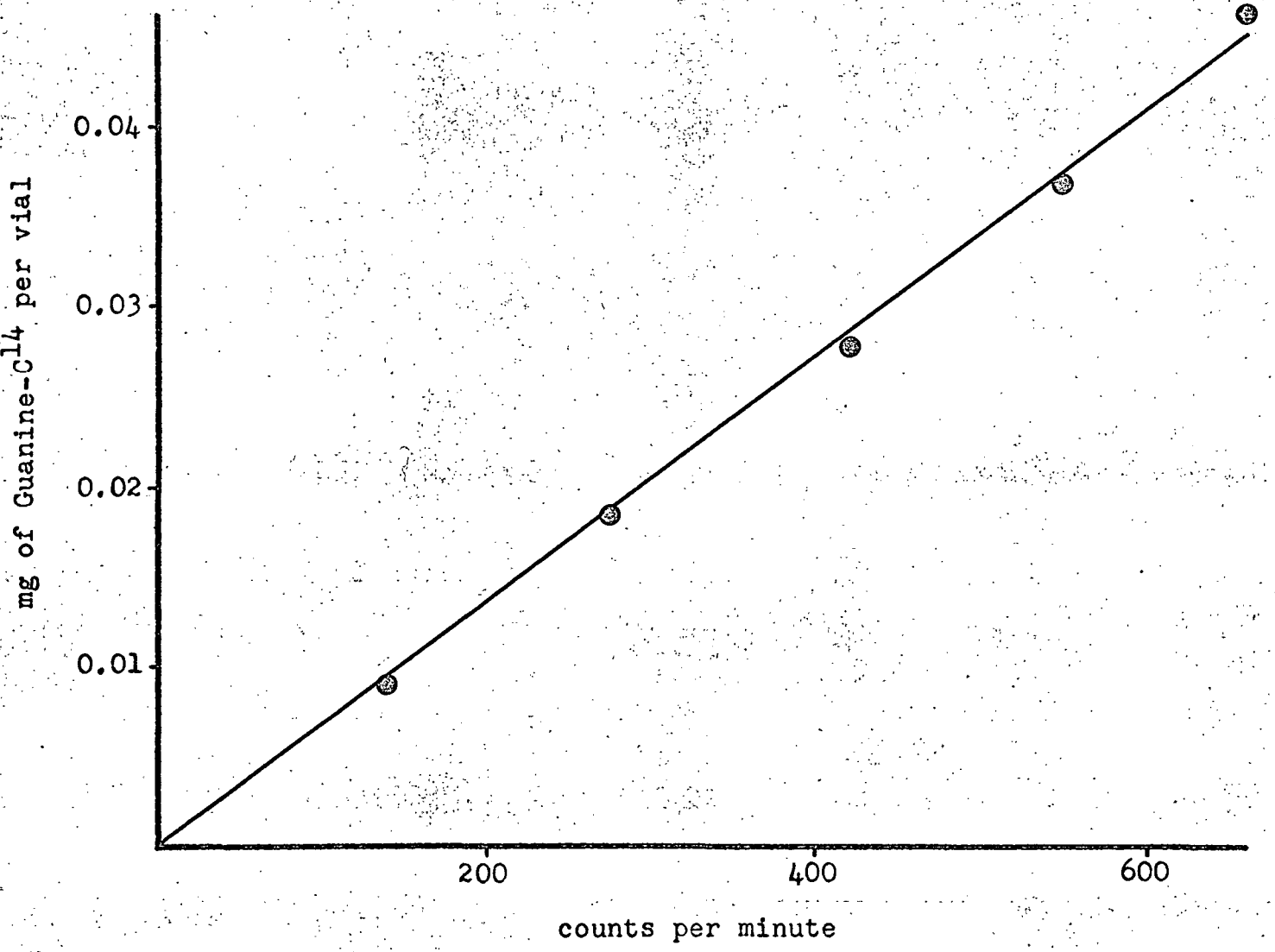

Figure 4. Counts per minute obtained from Guanine-C^{14} dissolved in 1.25 ml of ethylene glycol and 5 ml of Polyether-611.

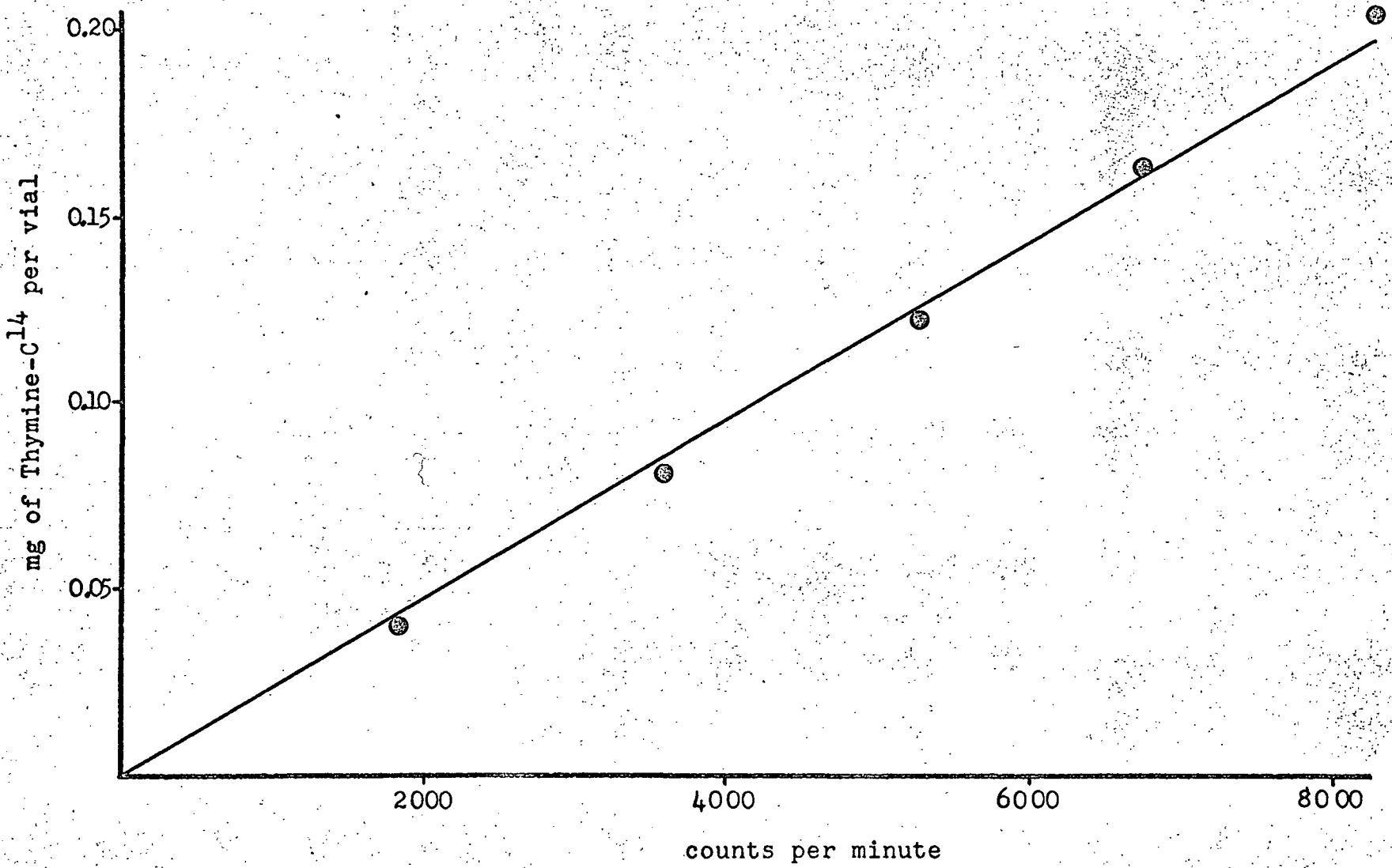

Figure 5. Counts per minute obtained from Thymine-C^{14} dissolved in 1.25 ml of ethylene glycol and 5 ml of Polyether-611.

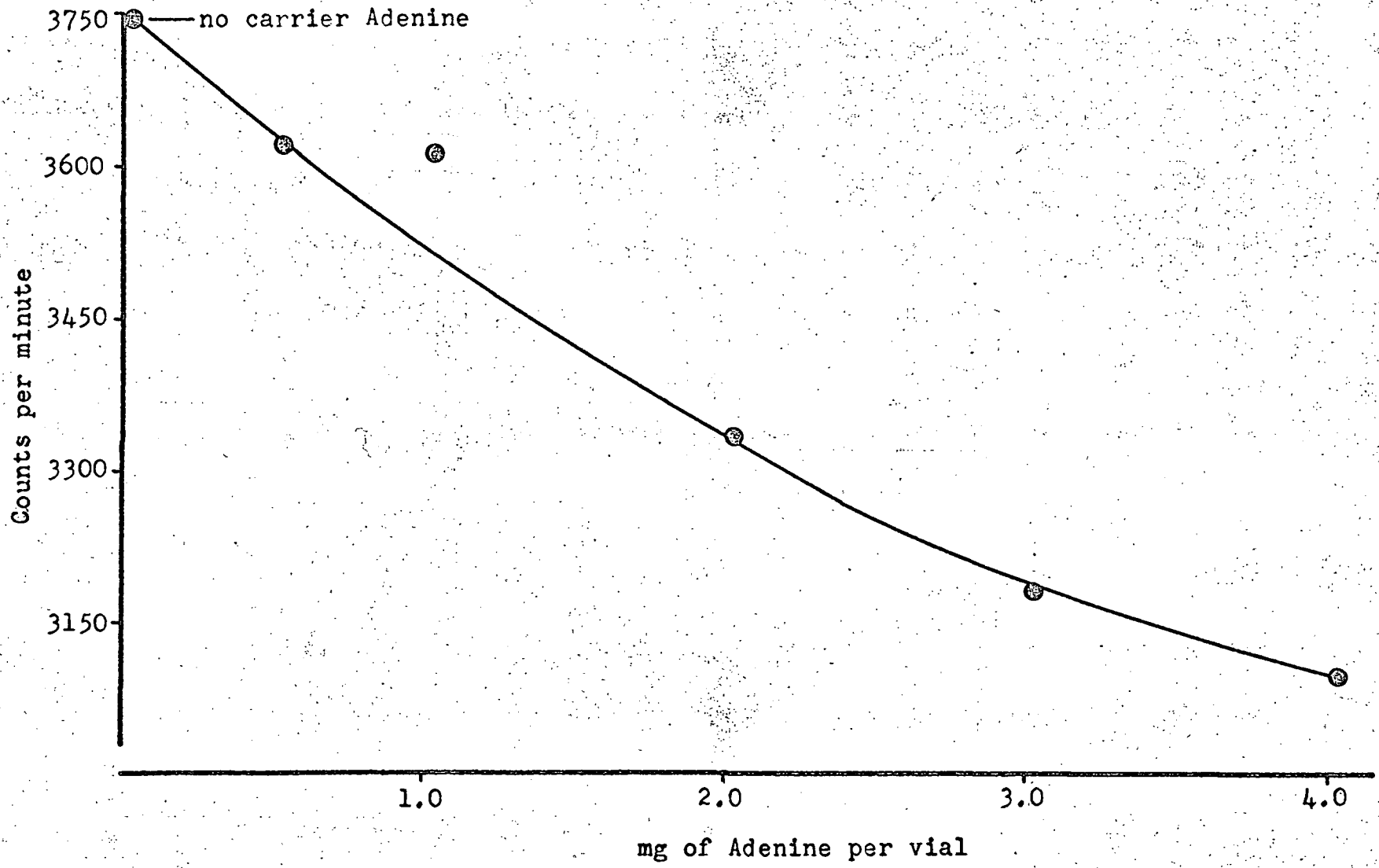

Figure 6. Effect of addition of carrier Adenine to a constant amount of Adenine-C^{14}. Adenine dissolved in 1 ml of ethylene glycol and 4 ml of Polyether-611.

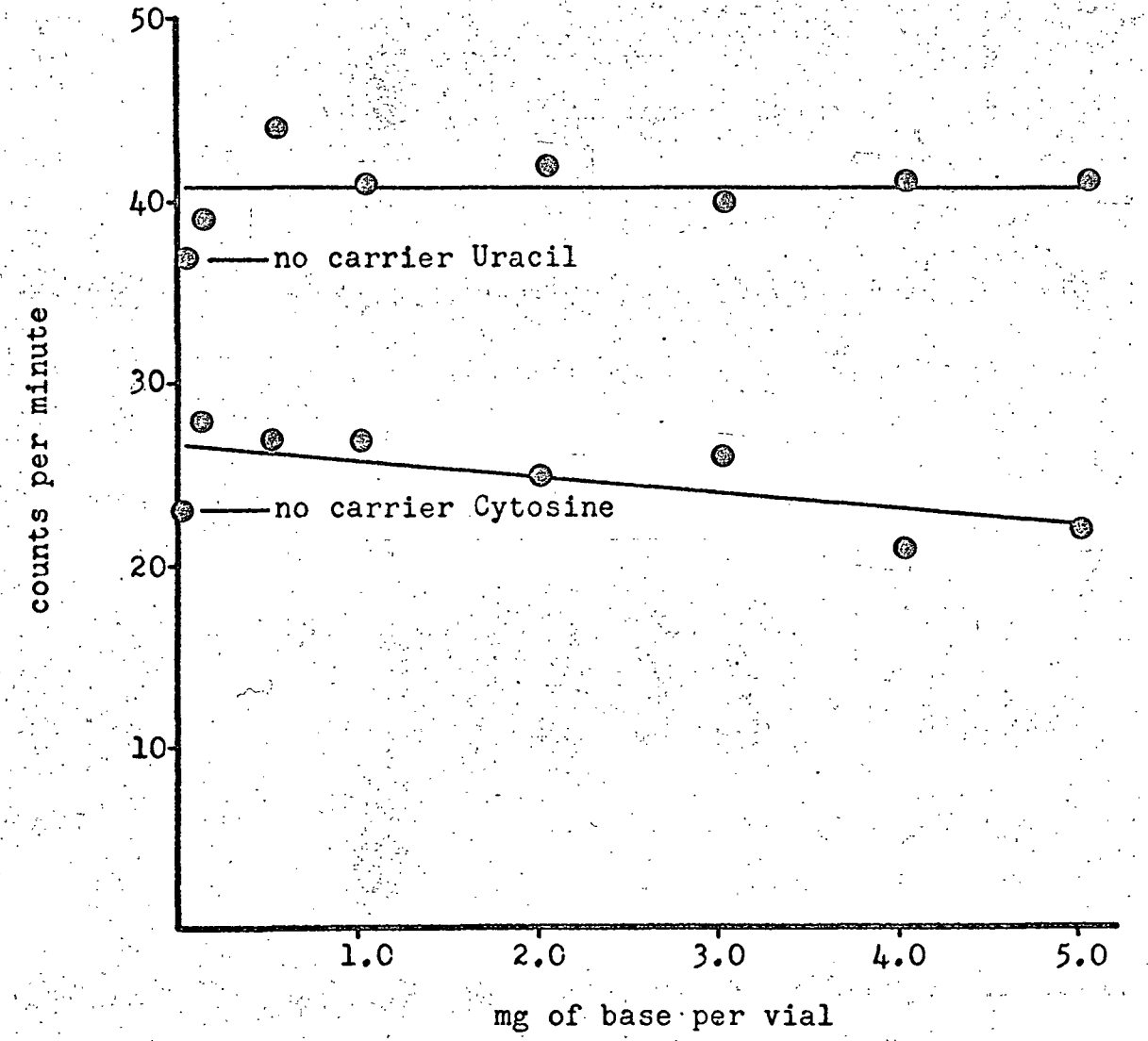

Figure 7. Effect of addition of carrier Uracil and Cytosine to constant amounts of Uracil-C^{14} and Cytosine-C^{14}. Bases dissolved in 1 ml of ethylene glycol and 4 ml of Polyether-611.

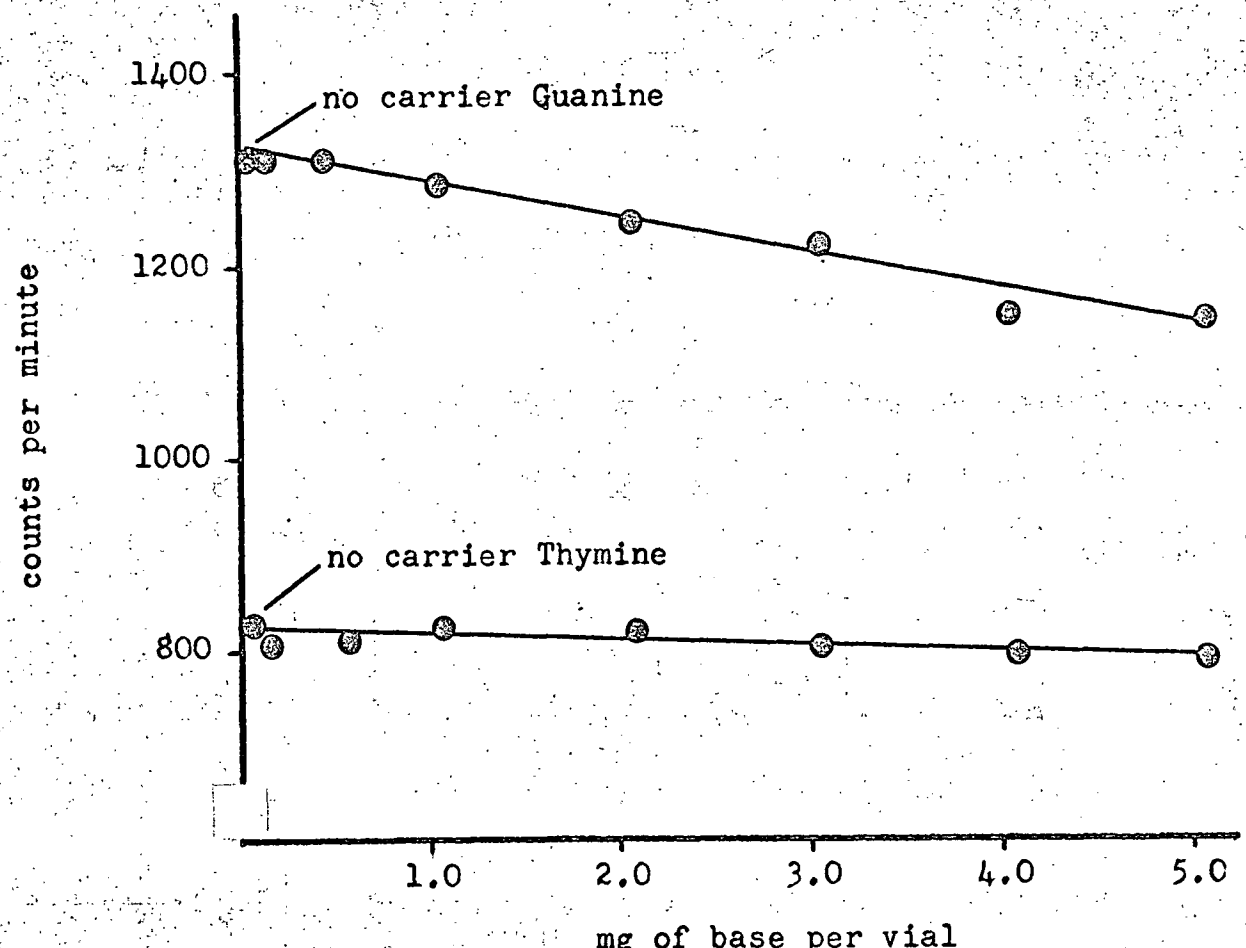

Figure 8. Effect of addition of carrier Guanine and Thymine to constant amounts of Guanine-C^{14} and Thymine-C^{14}. Bases dissolved in 1 ml of ethylene glycol and 4 ml of Polyether-611.

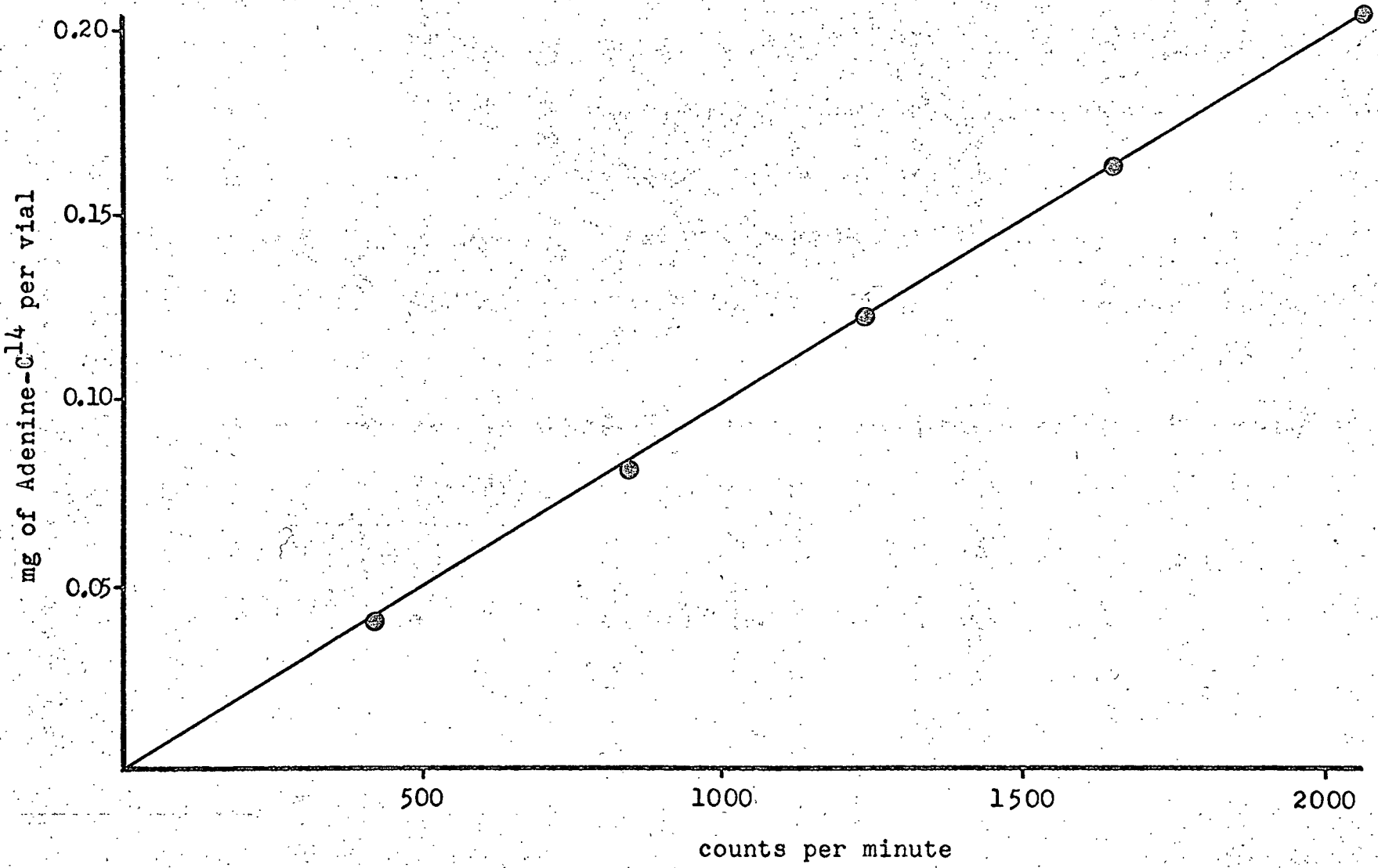

Figure 9. Counts per minute obtained from Adenine-C^{14} dissolved in 1 ml of water and 4 ml of Polyether-611.

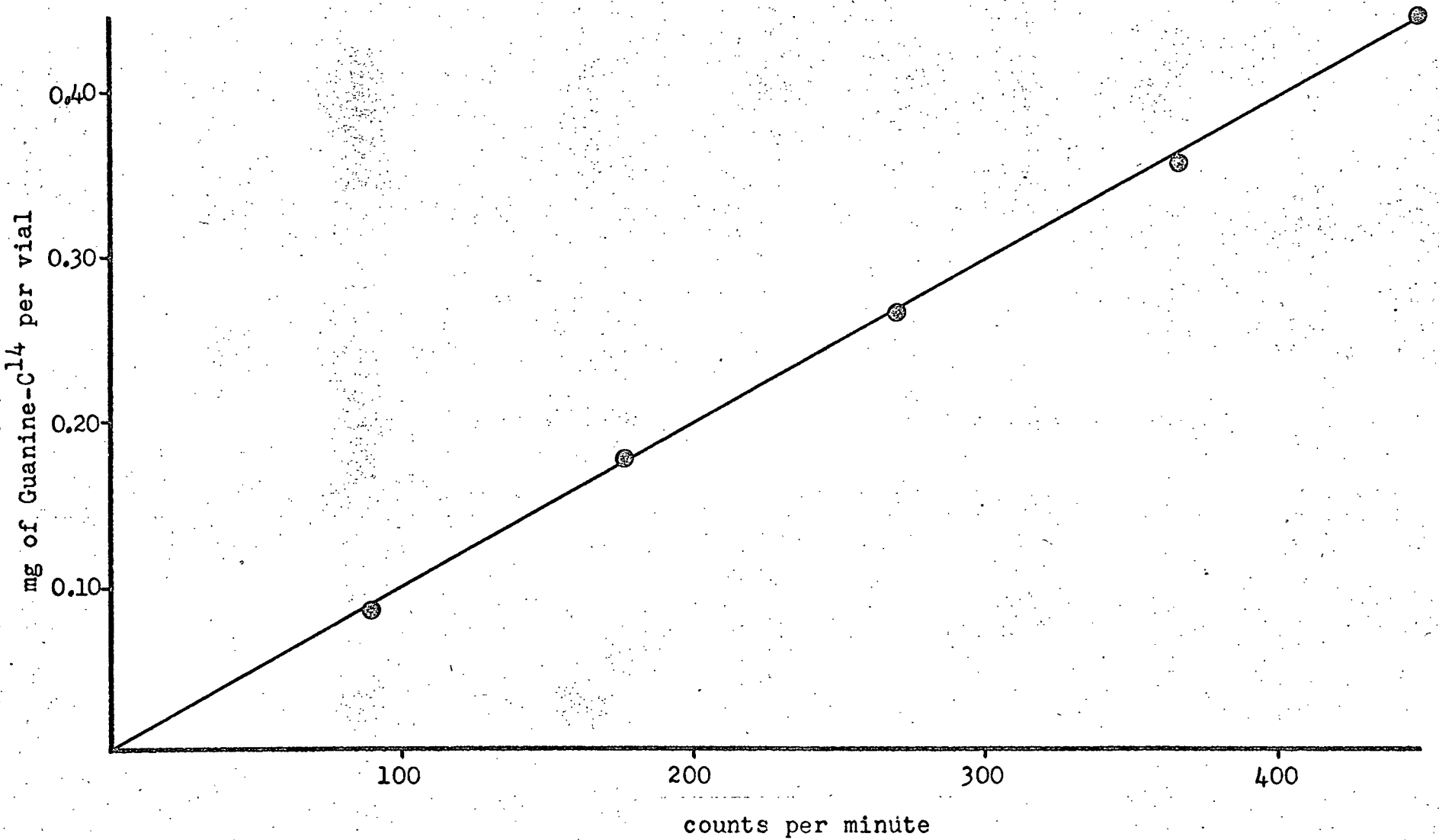

Figure 10. Counts per minute obtained from Guanine-C^{14} dissolved in 1 ml of water and 4 ml of Polyether-611.

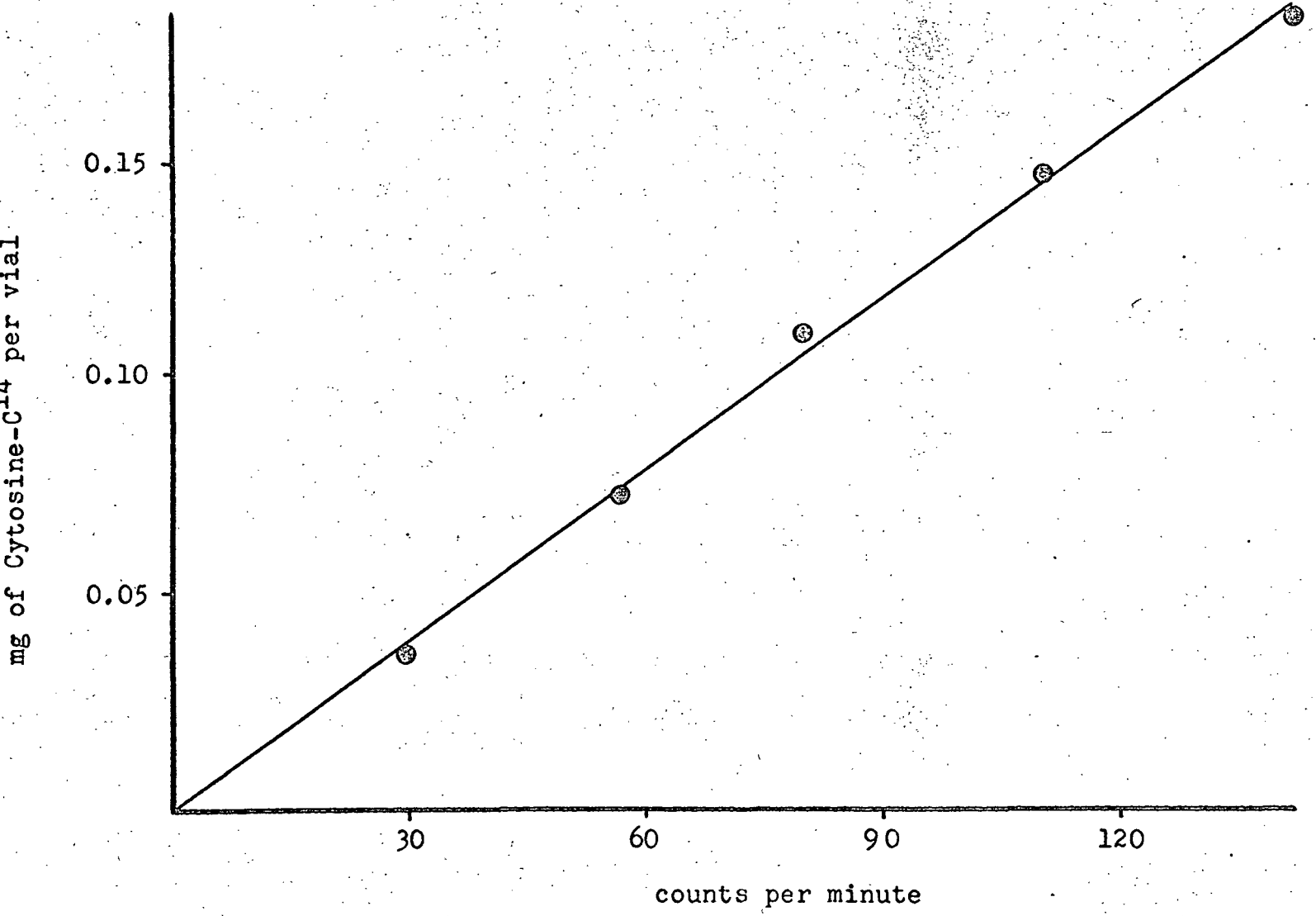

Figure 11. Counts per minute obtained from Cytosine-C^{14} dissolved in 1 ml of water and 4 ml of Polyether-611.

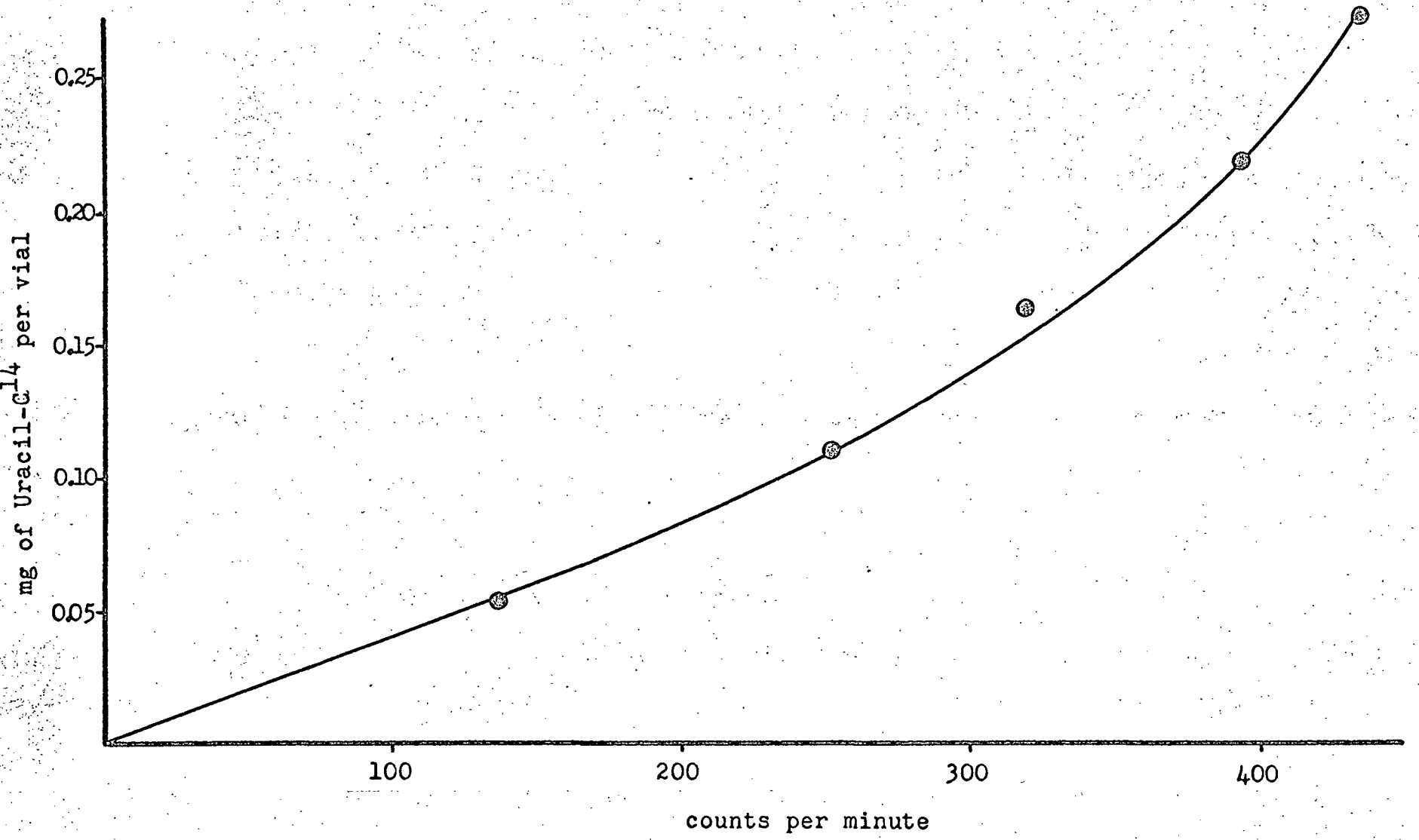

Figure 12. Counts per minute obtained from Uracil-C^{14} dissolved in 1 ml of water and 4 ml of Polyether-611.

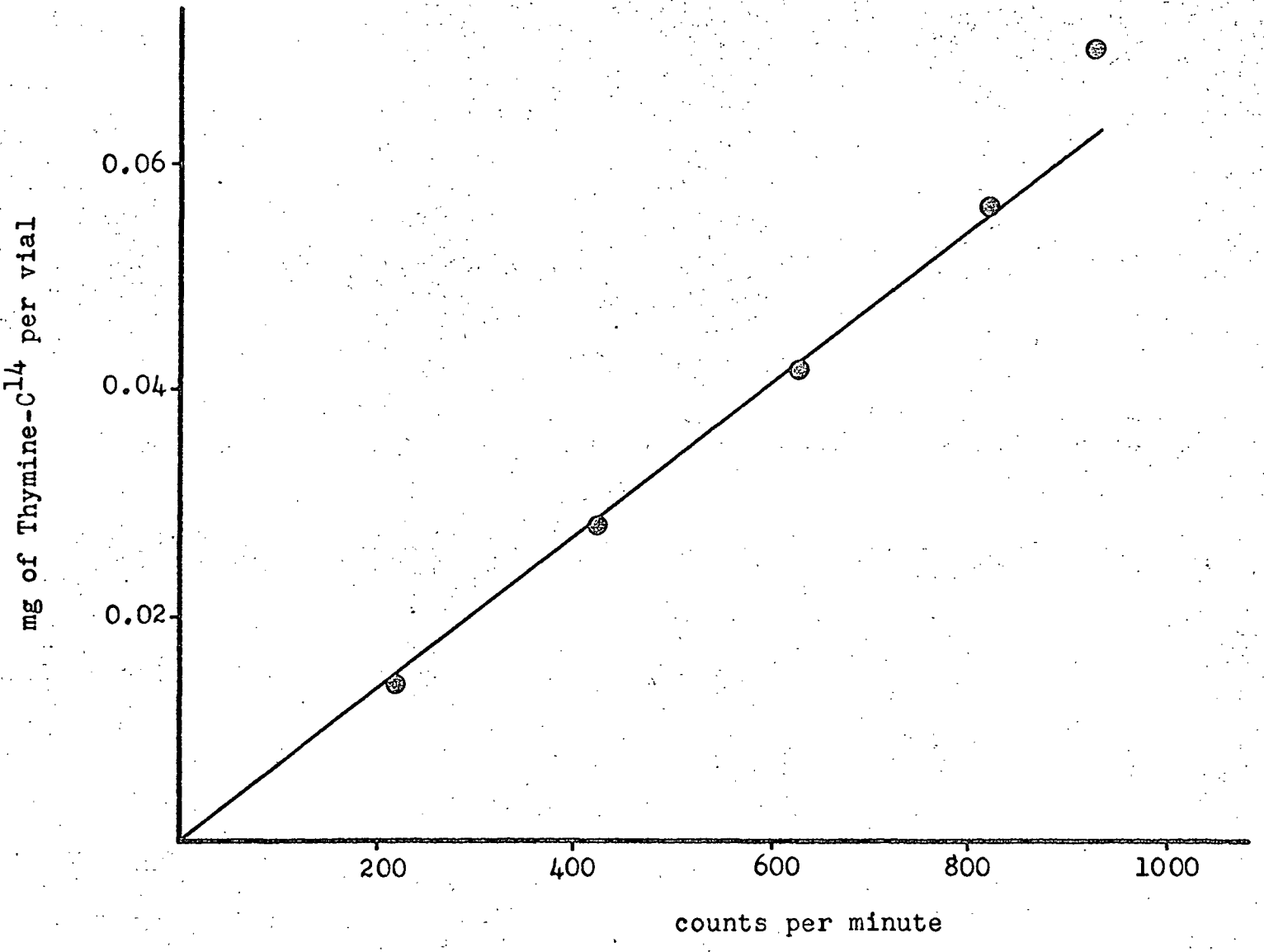

Figure 13. Counts per minute obtained from Thymine-C^{14} dissolved in 1 ml of water and 4 ml of Polyether-611.

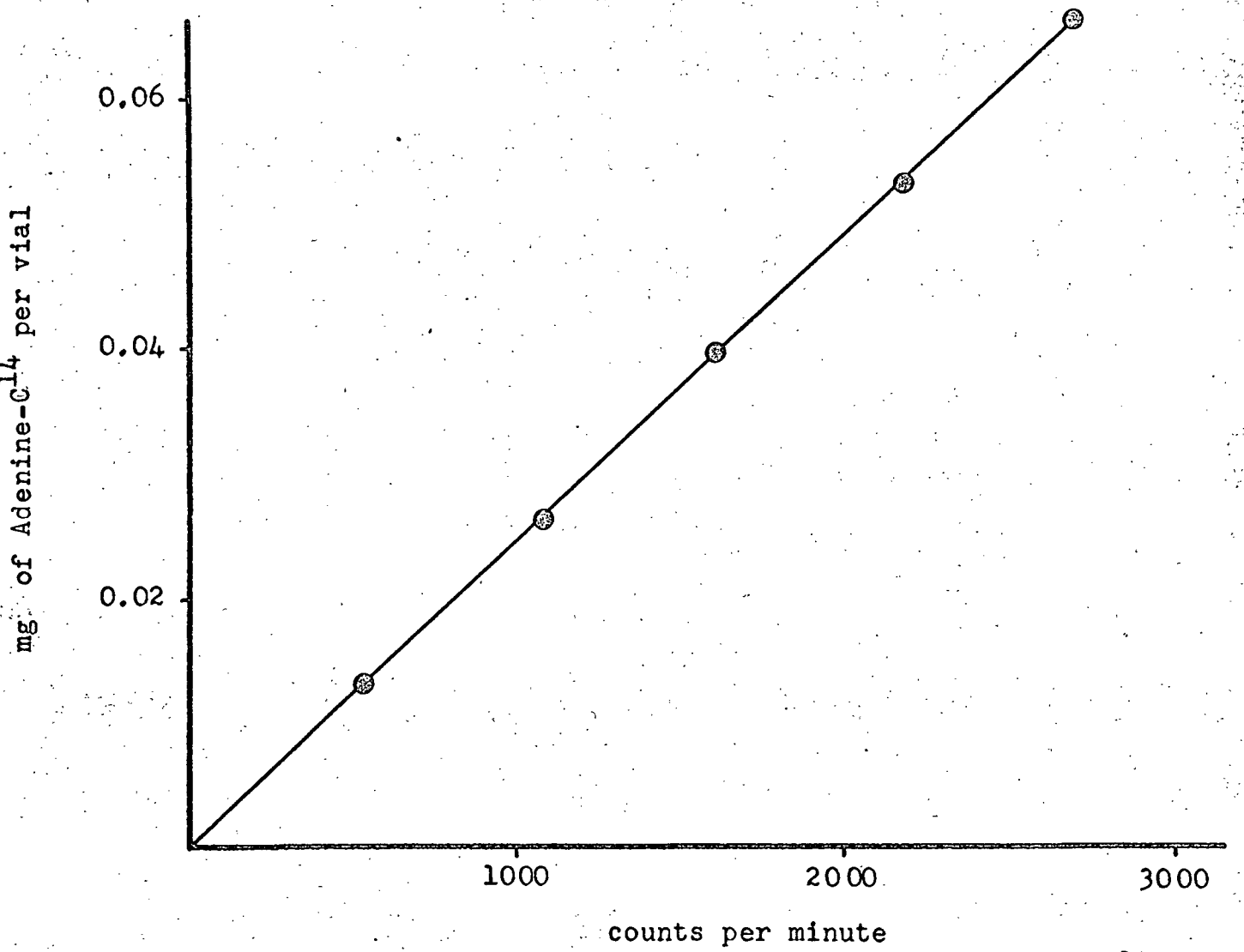

Figure 14. Counts per minute obtained from Adenine-C^{14} dissolved in 1 ml of 0.1N HCl. One-half ml of hyamine hydroxide and 4 ml of Polyether-611 added to each vial.

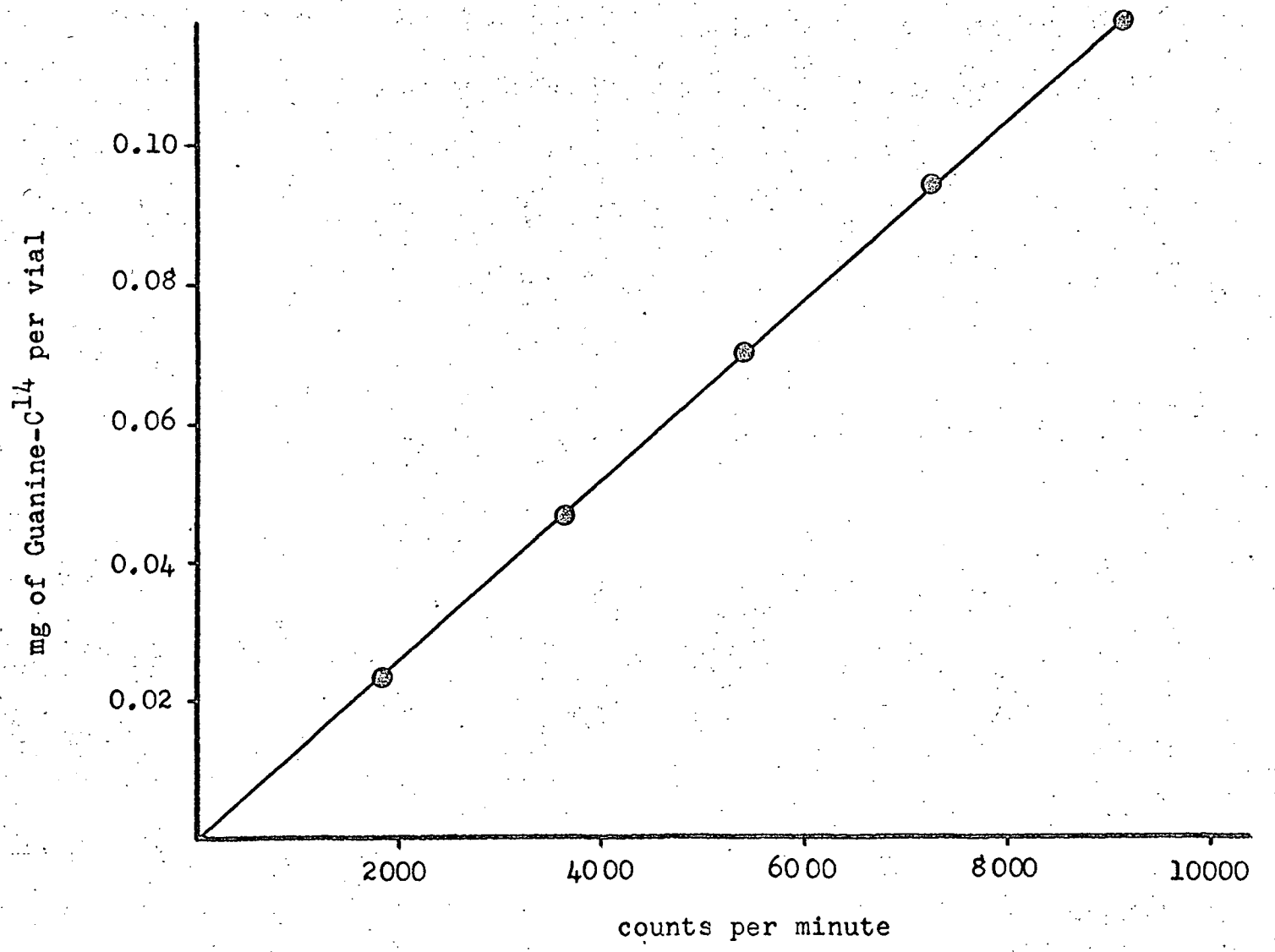

Figure 15. Counts per minute obtained from Guanine-C^{14} dissolved in 1 ml of 0.1N HCl. One-half ml of hyamine hydroxide and 4 ml of Polyether-611 added to each vial.

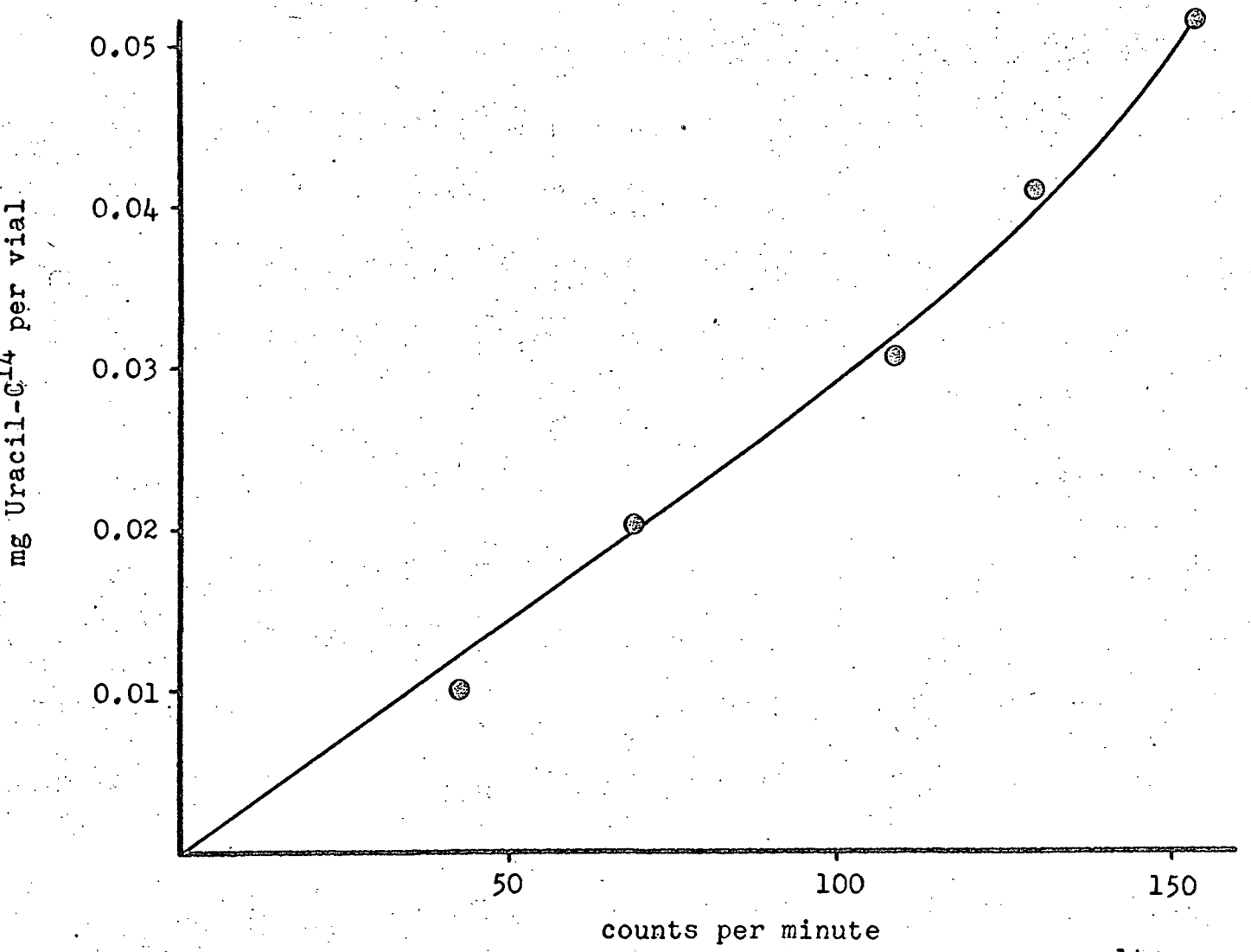

Figure 16. Counts per minute obtained from Uracil-C^{14} dissolved in 1 ml of 0.1N HCl. One-half ml of hyamine hydroxide and 4 ml of Polyether-611 added to each vial.

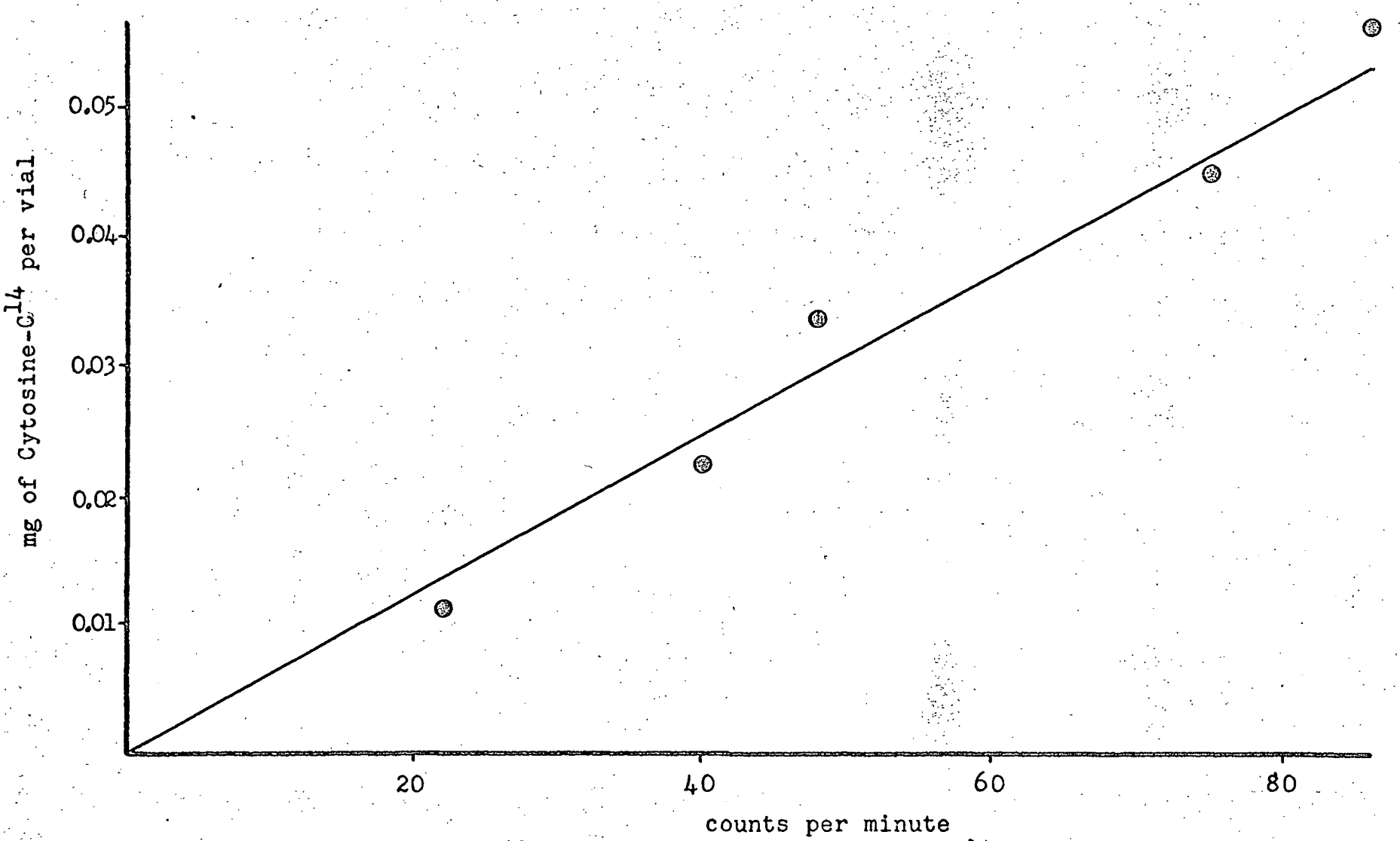

Figure 17. Counts per minute obtained from Cytosine-C^{14} dissolved in 1 ml of 0.1N HCl. One-half ml of hyamine hydroxide and 4 ml of Polyether-611 added to each vial.

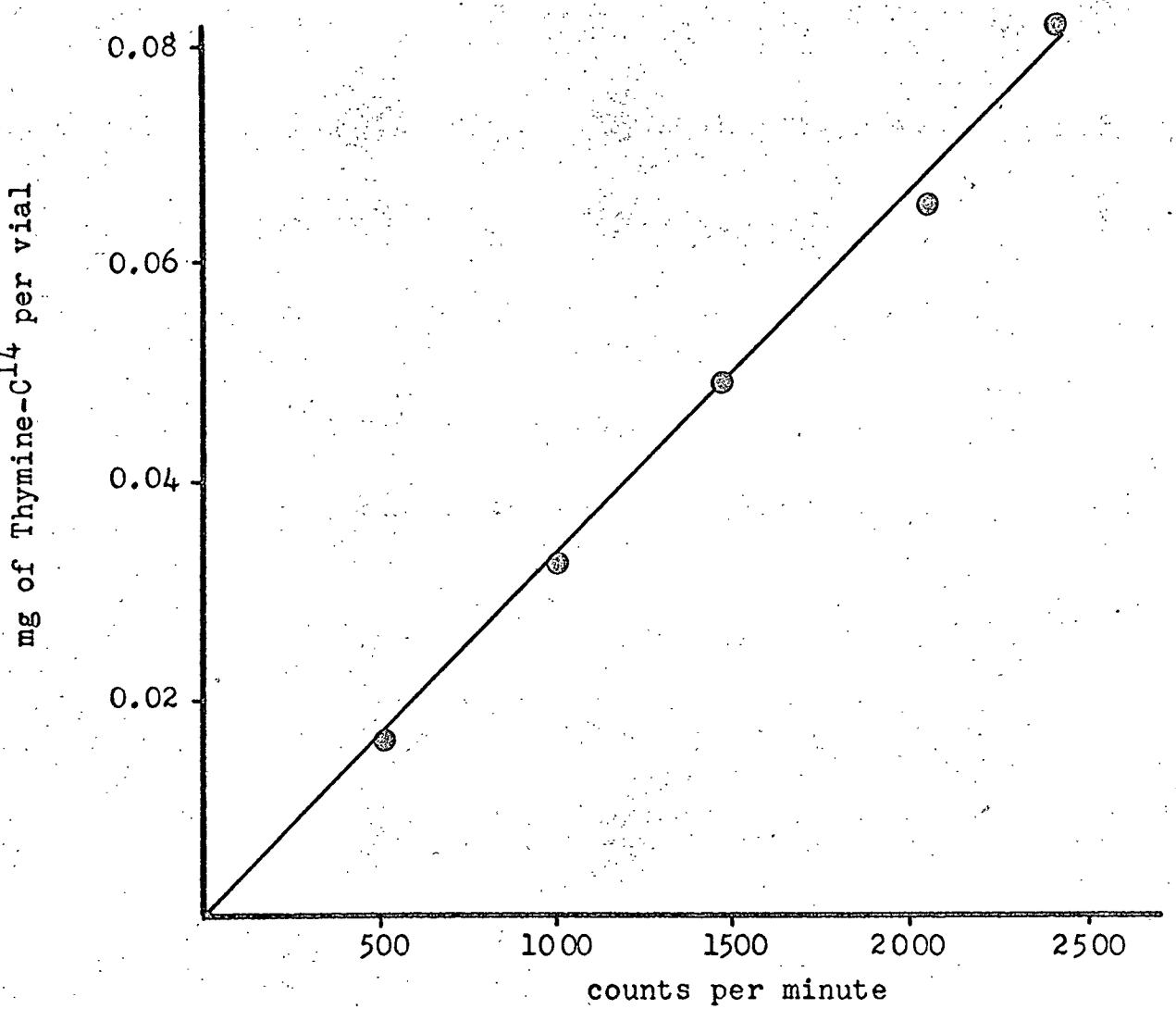

Figure 18. Counts per minute obtained from Thymine-C^{14} dissolved in 1 ml of 0.1N HCl. One-half ml of hyamine hydroxide and 4 ml of Polyether-611 added to each vial.

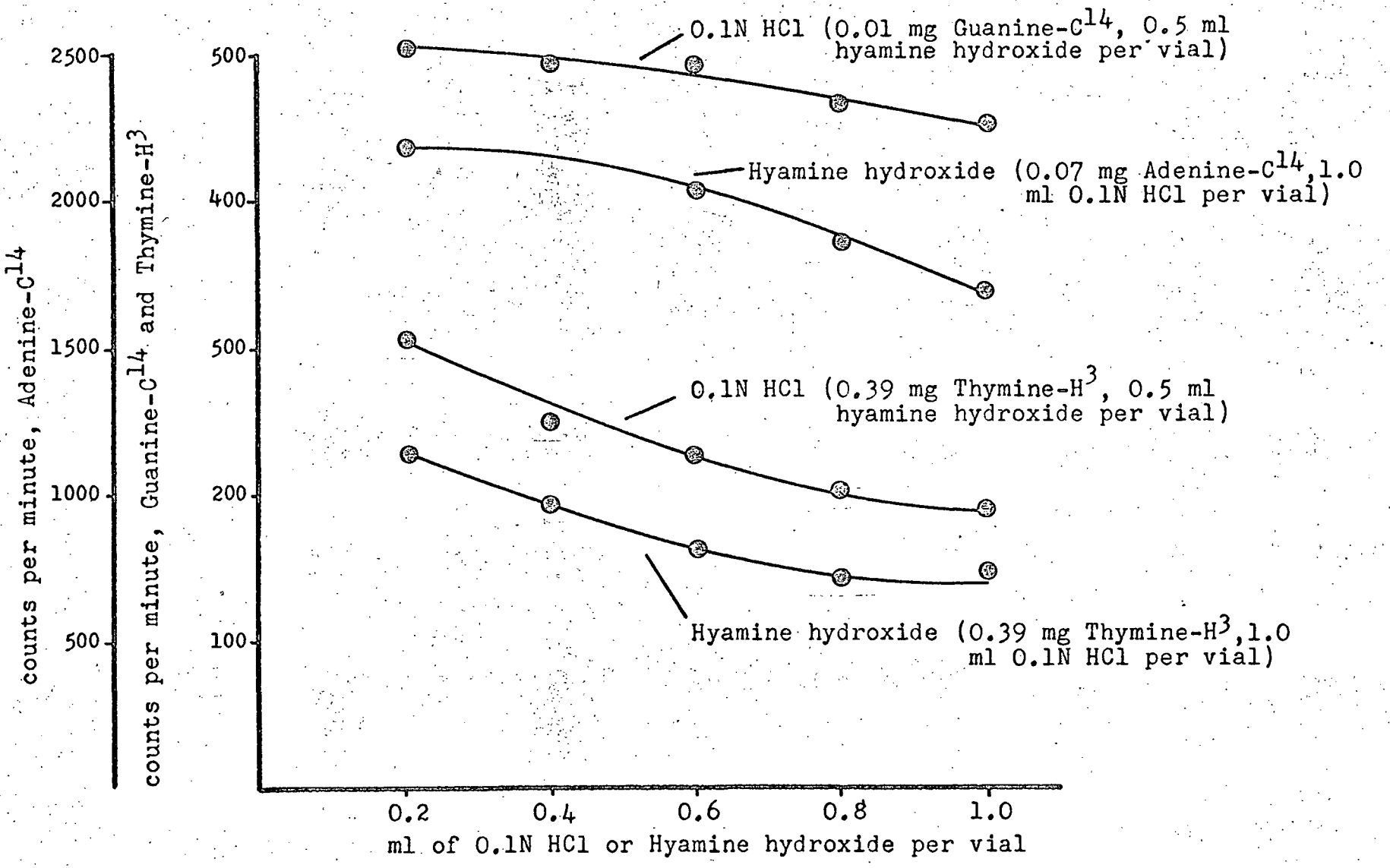

Figure 19. Effect of increasing volume of 0.1N HCl or Hyamine hydroxide. Each vial contained 4 ml of Polyether-611.

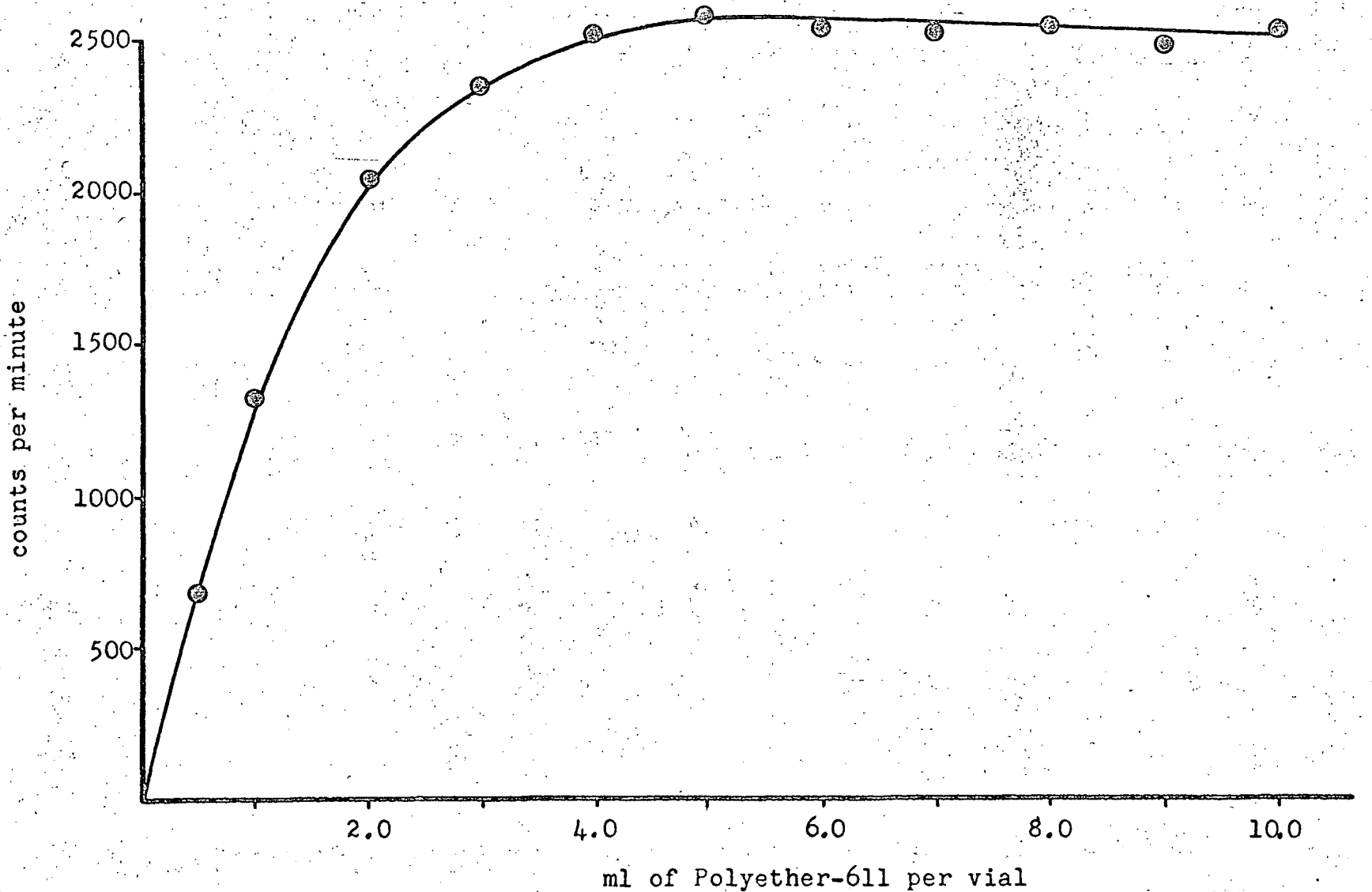

Figure 20. Effect of increasing volume of Polyether-611. Each vial contained 0.03 mg of Adenine-C^{14} in 0.4 ml of 0.1N HCl, and 0.5 ml hyamine hydroxide.

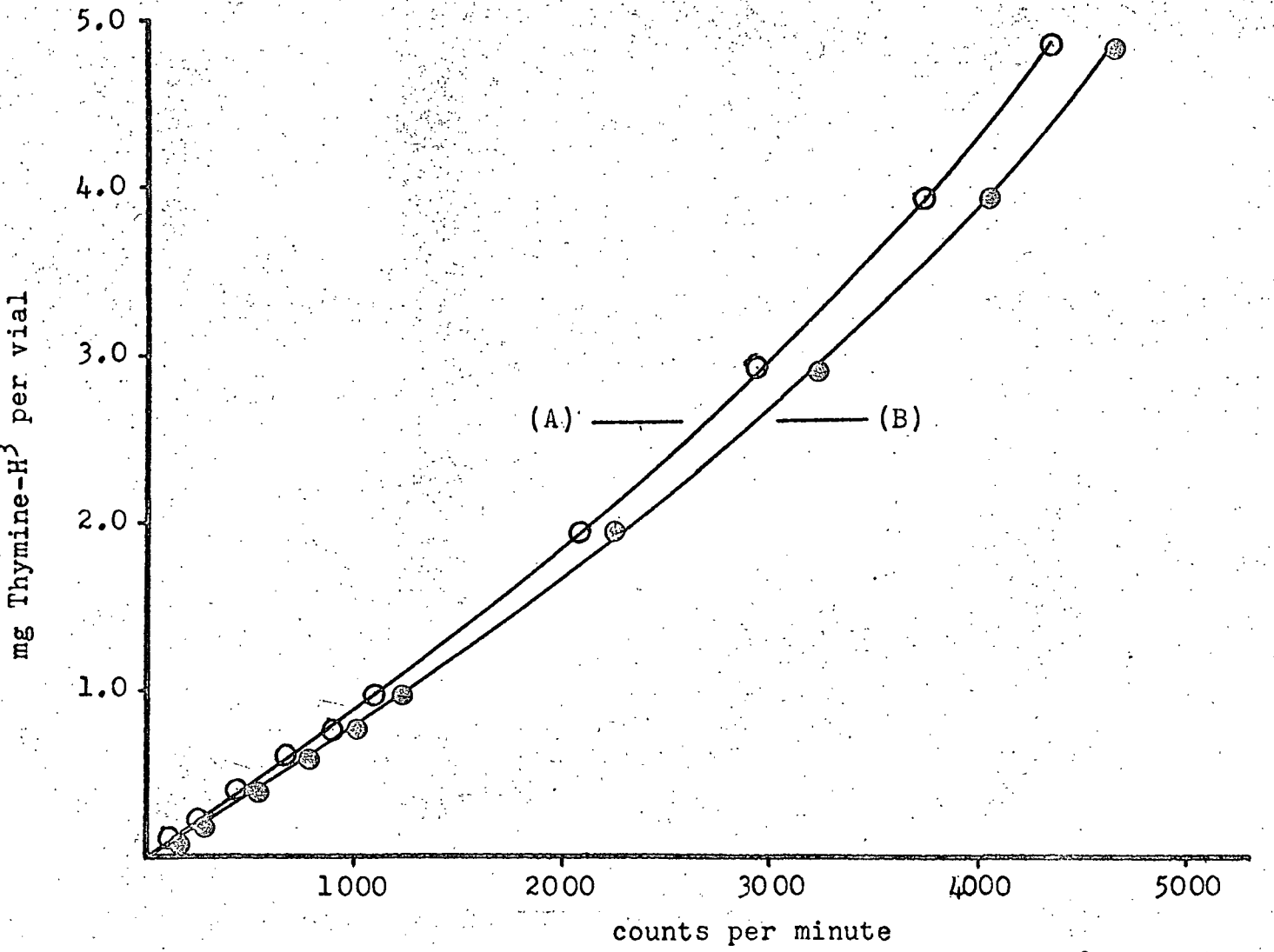

Figure 21. Counts per minute obtained from Thymine-H^3 dissolved in: (A) 1 ml ethylene glycol, (B) 1 ml water; 4 ml Polyether-611 added to each vial.

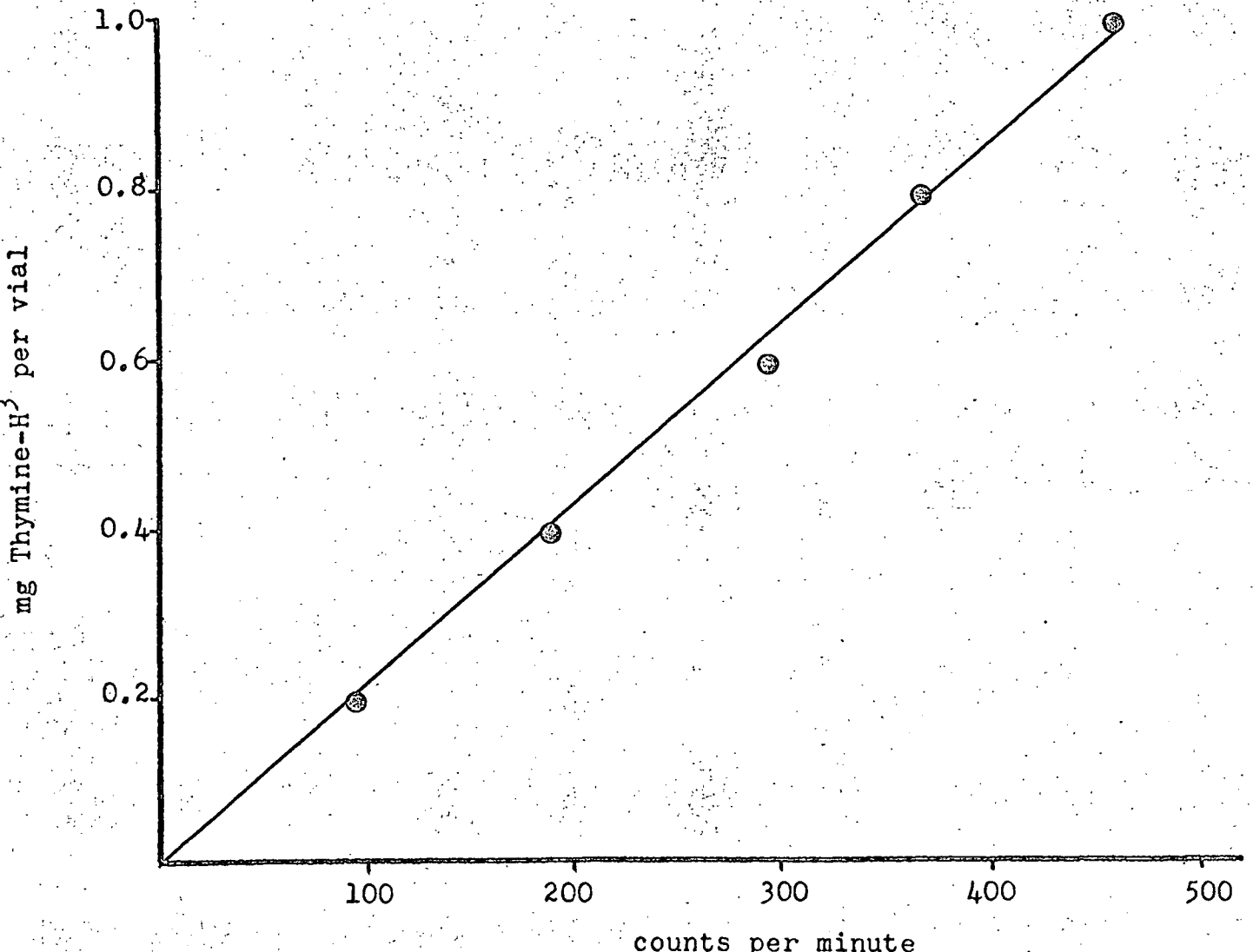

Figure 22. Counts per minute obtained from Thymine-H^3 dissolved in 1 ml of 0.1N HCl. One-half ml of hyamine hydroxide and 4 ml of Polyether-611 added to each vial.

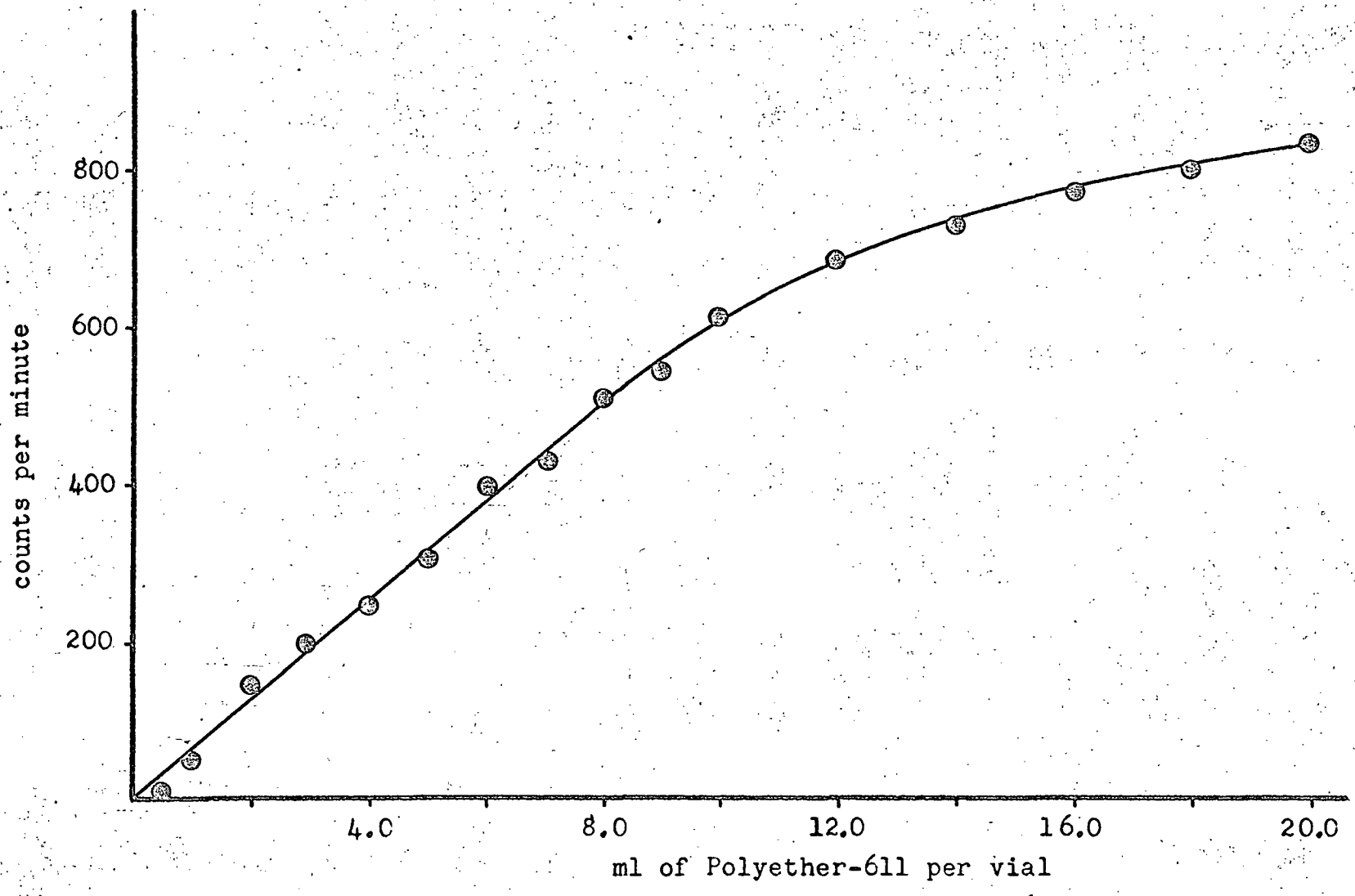

Figure 23. Effect of increasing volume of Polyether-611. Each vial contained 0.39 mg Thymine-H^3 in 0.4 ml of 0.1N HCl, and 0.5 ml hyamine hydroxide.

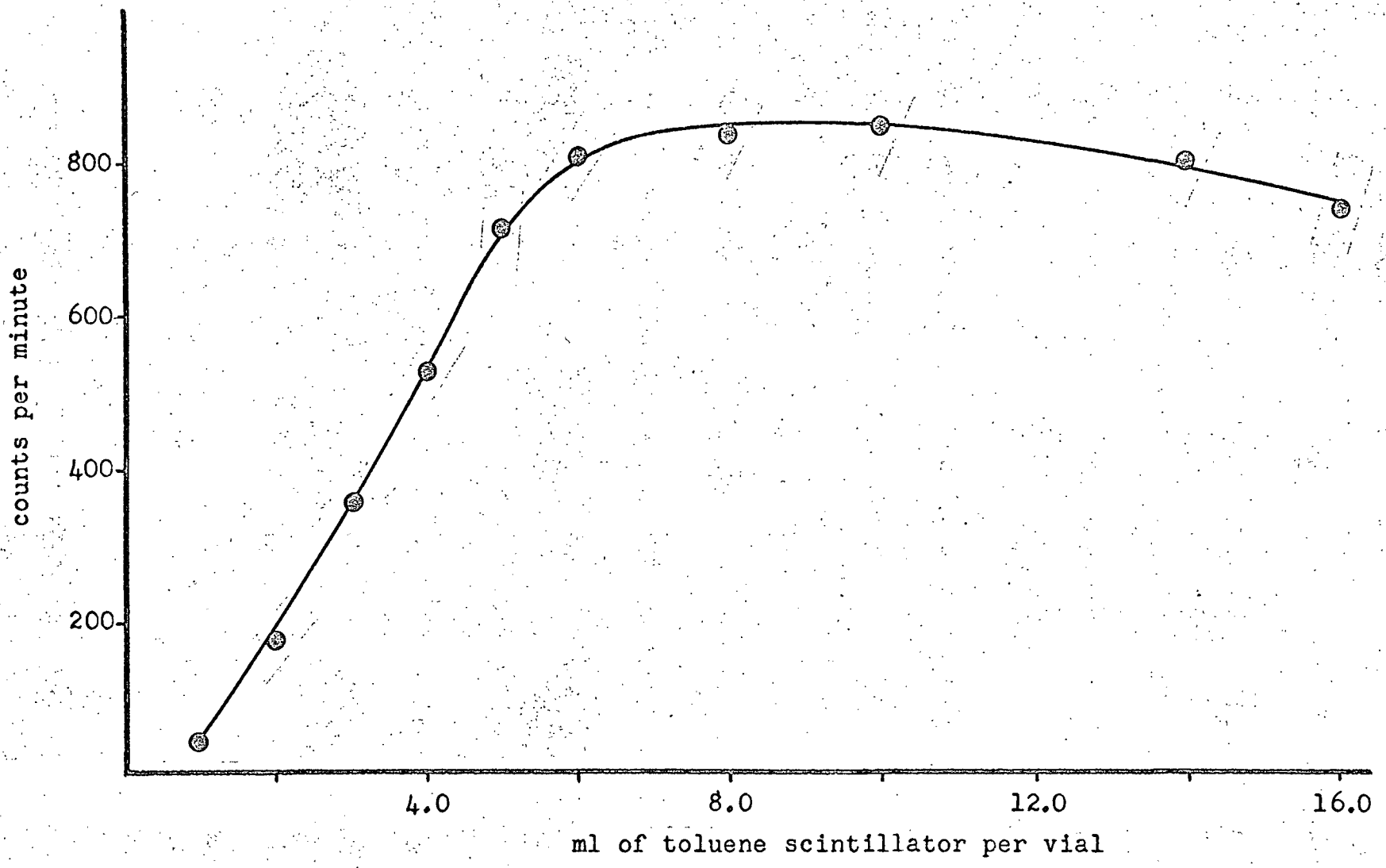

Figure 24. Effect of increasing volume of toluene scintillator system. Each vial contained 0.39 mg Thymine-H^3 in 0.4 ml of 0.1N HCl, 1.0 ml of hyamine hydroxide, and 3.0 ml of absolute ethanol.

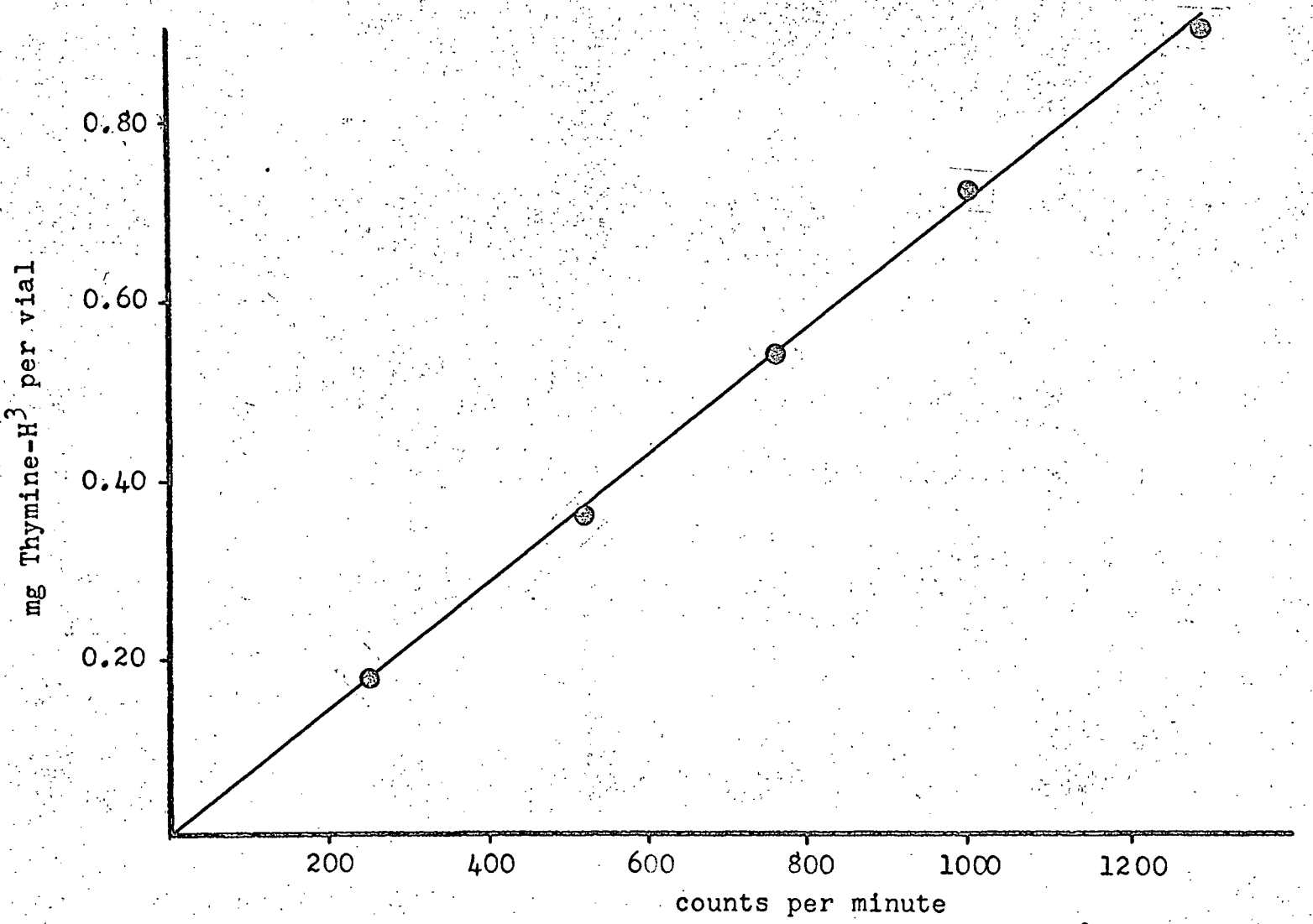

Figure 25. Counts per minute obtained from Thymine-H^3 dissolved in 0.4 ml of 0.1N HCl. One ml hyamine hydroxide, 3.0 ml absolute ethanol, and 5.0 ml toluene scintillator system added to each vial.

II. The Effect of Tumor on the Nucleic Acid Metabolism of Host Tissues

1. Studies using formate-C^{14}:

Groups of female Swiss mice (20-25 grams each) were used in these experiments. The incorporation of formate-C^{14} into the nucleic acid purines and pyrimidines of the host tissues and tumor tissue was determined in tumor-bearing and control animals. Ten mice bearing the solid subcutaneous Ehrlich tumor were injected with formate-C^{14} 11 days after the implantation of the tumor cells. Ten mice bearing the intraperitoneal Ehrlich ascites tumor were injected with formate-C^{14} six days after the implantation of the tumor cells. Two groups of ten mice each and one group of five mice served as controls. Each of the control and tumor-bearing mice was injected subcutaneously in the dorsal region with 0.34 mg of sodium formate-C^{14} containing 1.3×10^7 counts per minute. This amount of sodium formate-C^{14} was contained in 0.1 ml of a solution containing 1 millicurie of sodium formate-C^{14} dissolved in 5 ml of slightly alkaline water. Twenty-four hours after the injection of the formate-C^{14}, the mice were killed by cervical dislocation and the tumor cells removed by draining the peritoneal cavity. The lung, liver, spleen, intestinal mucosa, and kidney were then quickly removed and frozen. The corresponding tissues of each group were pooled and the nucleic acid purines and pyrimidines isolated and determined. The specific activities of the purines and pyrimidines were expressed as counts per minute per micromole (c.p.m./μM).

The specific activities of the nucleic acid purines and pyrimidines obtained from the liver, intestinal mucosa, spleen,

Table II

Specific activities (c.p.m./μM) of nucleic acid purines and pyrimidines of tissues from control and tumor-bearing mice. Each mouse received 0.34 mg of formate-C^{14} containing 1.3×10^7 c.p.m.

	CONTROL			TUMOR-BEARING	
	(10 mice)	(10 mice)	(5 mice)	Ascites (10 mice)	Subcutaneous (10 mice)
Liver					
RNA					
uracil	*	77	238	82	129
cytosine	*	28	45	28	49
guanine	1196	1296	830	1834	980
adenine	*	2580	953	1505	949
DNA					
thymine	235	226	216	350	474
cytosine	71	trace	56	74	53
guanine	1129	1062	596	978	1172
adenine	528	992	733	1410	1215
Mucosa					
RNA					
uracil	*	232	292	340	304
cytosine	*	177	74	210	172
guanine	*	17700	13790	13400	10840
adenine	*	17050	10560	10870	10170
DNA					
thymine	3730	3910	4270	1290	2880
cytosine	390	248	151	125	252
guanine	15820	12350	12810	6910	9420
adenine	15150	14780	11180	6050	9460
Spleen					
RNA					
uracil	*	216	86	304	347
cytosine	*	140	112	104	92
guanine	*	8550	4720	6600	5520
adenine	*	11680	6720	2490	6420
DNA					
thymine	2050	2410	2030	1726	1970
cytosine	176	trace	33	131	119
guanine	5340	7110	5440	4930	4075
adenine	7070	9200	5420	6060	4790

* sample lost

Table II (Cont.)

	CONTROL (10 mice)	CONTROL (10 mice)	CONTROL (5 mice)	TUMOR-BEARING Ascites (10 mice)	TUMOR-BEARING Subcutaneous (10 mice)
Kidney					
RNA					
uracil	*	121	513	141	trace
cytosine	*	60	742	82	trace
guanine	*	5110	3540	3770	2620
adenine	*	7030	5820	5060	4140
DNA					
thymine	141	171	187	207	97
cytosine	38	trace	123	0	0
guanine	204	918	423	847	527
adenine	222	889	229	767	494
Lung					
RNA					
uracil	*	238	1338	960	trace
cytosine	*	215	3070	214	74
guanine	*	*	3080	2570	1280
adenine	*	3780	642	3085	1262
DNA					
thymine	196	427	115	261	481
cytosine	179	0	332	21	37
guanine	483	*	673	991	1558
adenine	438	1319	597	*	1570
Tumor					
RNA					
uracil				886	112
cytosine				134	86
guanine				13390	5140
adenine				13410	4650
DNA					
thymine				3960	1190
cytosine				260	110
guanine				12940	3320
adenine				10910	3045

* sample lost

kidney, lung and tumor of the control and tumor-bearing mice are shown in Table II. In most cases the specific activities of uracil and cytosine were found to be negligible. On comparing the data obtained for the purines and thymine it is evident that most of the values obtained for the tumor-bearing animals fall within the range of the control animals. It is observed that the specific activities of the thymine and purines of the DNA of intestinal mucosa of the tumor-bearing animals are lower than those of the control animals. The difference is most apparent in the data obtained for the ascites tumor-bearing group. The RNA guanine of intestinal mucosa and lung and the RNA adenine of kidney of the subcutaneous tumor-bearing group also have slightly lower observed specific activities than the corresponding purines of the control groups. The results also indicate that the incorporation of formate into the nucleic acids of the Ehrlich ascites tumor is greater than into the solid subcutaneous form of this tumor.

2. Studies using tritiated thymidine:

(a) Ehrlich Ascites Tumor - Groups of female Ha/ICR mice (18-22 grams each) were used in these experiments because this strain of mouse has been shown (156) to be more resistant to infection than the Swiss mouse. Two groups of ten mice each bearing the Ehrlich ascites tumor were injected with thymidine-H^3 six days after the implantation of the tumor. Each mouse was injected subcutaneously in the dorsal region with 0.2 ml of an aqueous solution containing 5 microcuries (μc), 0.014 μM, of thymidine-H^3. Four groups of nine or ten Ha/ICR mice served as controls. Each control was injected with an amount of thymidine-

H^3 equal to that administered to the tumor-bearing counterpart. Twenty-four hours after the injection of the thymidine-H^3 the animals were killed by cervical dislocation and the tumor, lungs, livers, spleens, intestinal mucosa, and kidneys quickly removed and frozen. Because the results of the present work, and also those of others (157) showed that thymidine was not incorporated into RNA nor into DNA adenine, guanine, or cytosine, only the DNA thymine was isolated from the tissues. The specific activities (c.p.m./μM) of the DNA thymine obtained from the tissues of the control and tumor-bearing mice are shown in Table III. It is seen that the specific activities of the DNA thymine of liver from the tumor-bearing mice were higher than those of the control animals. On the other hand, the specific activities of the DNA thymine from the spleen, intestinal mucosa, kidney and lung of the control groups were higher than those of the tumor-bearing animals.

The lower incorporation of thymidine-H^3 into the DNA thymine of spleen, intestinal mucosa, kidney and lung of the tumor-bearing mice might have been the result of a decrease in the "pool" of available thymidine caused by a high degree of incorporation of thymidine into the tumor cells. In order to test this possibility groups of mice were injected with larger quantities of total thymidine although the amount of radioactivity injected per mouse remained unchanged at 5 uc. The results of these experiments are shown in Tables IV and V. When the amount of thymidine injected was increased 10- and 100-fold the specific activities of the DNA thymine from the livers of the tumor-bearing

mice were again considerably higher than those of the control mice. Furthermore, the specific activities of the DNA thymine from the intestinal mucosa of the control mice were again higher than those of the tumor-bearing groups, when 0.14 μM of thymidine-H^3 was injected (Table IV). A similar result was obtained for one of the control groups of mice that received 1.40 μM of thymidine-H^3 per mouse (Table V). However, the DNA thymine obtained from the mucosa of the other control group had a specific activity of the same order as those of the corresponding tumor-bearing groups. In contrast to the results obtained after the injection of 0.014 μM of thymidine-H^3, the specific activities of the DNA thymine from the spleen of the tumor-bearing animals receiving 0.14 uM or 1.40 μM of thymidine-H^3 tended to be slightly greater than those of the control groups. The specific activities of the DNA thymine from kidney of the tumor-bearing mice also tended to be greater than those of the controls after the injection of 0.14 μM of thymidine; however, there was little difference between the two after the injection of 1.40 μM of thymidine. Following the injection of 0.14 μM of the thymidine-H^3 the specific activities of the DNA thymine from the lungs of the tumor-bearing animals were greater than those of the control group. However, when 1.40 μM of thymidine was administered to each mouse, the results obtained for the lung tissue were conflicting. The specific activity of the DNA thymine from lung was higher for one of the control groups as compared to the tumor-bearing groups. On the other hand the other control group had a lower specific activity.

Table III

Specific activities (c.p.m./μM) of DNA thymine from tissues of control mice and mice bearing Ehrlich ascites tumor. Each mouse received 0.014 μM, 5 μc, of thymidine-H^3.

	TUMOR-BEARING			CONTROL		
	10 mice	10 mice	average	10 mice	9 mice	average
Liver	1261	1175	1218	828	975	902
Spleen	2240	2060	2150	3150	3030	3090
Mucosa	4080	2860	3470	lost	9280	9280
Kidney	382	682	532	962	936	949
Lung	558	711	635	1230	1210	1220
Tumor	9540	8650	9095			

Table IV

Specific activities (c.p.m./uM) of DNA thymine from tissues of control mice and mice bearing Ehrlich ascites tumor. Each mouse received 0.14 μM, 5 μc, of thymidine-H^3.

	TUMOR-BEARING			CONTROL		
	10 mice	9 mice	average	10 mice	10 mice	average
Liver	1170	925	1048	240	522	381
Spleen	1600	1855	1728	1198	1620	1409
Mucosa	4425	4420	4423	5760	5690	5725
Kidney	433	330	382	194	330	262
Lung	461	528	495	359	224	292

Table V

Specific activities (c.p.m./μM) of DNA thymine from tissues of control mice and mice bearing Ehrlich ascites tumor. Each mouse received 1.40 μM, 5 μc, of thymidine-H^3.

	TUMOR-BEARING			C O N T R O L		
	10 mice	9 mice	average	10 mice	10 mice	average
Liver	490	536	513	140	171	156
Spleen	1080	1452	1266	719	1042	881
Mucosa	2650	2115	2382	5150	2500	3825
Kidney	113	125	119	120	120	120
Lung	289	282	286	160	613	387

Table VI

Specific activities (c.p.m./uM) of DNA thymine from tissues of control rats and rats bearing Novikoff hepatoma. Each rat received 50 μc, 15 μM, of thymidine-H^3.

	TUMOR-BEARING			C O N T R O L		
	5 rats	3 rats	average	5 rats	5 rats	average
Liver	242	244	243	191	227	209
Spleen	1033	1165	1099	1180	1622	1401
Mucosa	3190	3370	3280	3340	4900	4120
Kidney	107	83	95	125	221	173
Lung	322	546	438	417	492	455

(b) Novikoff hepatoma - Zbarsky et al. (22) have found an increased incorporation of formate into the nucleic acid purines of the host tissues of rats bearing sixth day Novikoff hepatoma. The results of these authors also indicated that the presence of Novikoff hepatoma increased the incorporation of formate into the DNA thymine of the host tissues. In view of these results it was of interest to study the incorporation of thymidine-H^3 into the DNA thymine of the host tissues of rats bearing the Novikoff hepatoma.

Groups of male Sprague-Dawley rats (150-170 grams each) were used in these experiments. One group of 5 rats and one group of 3 rats were injected with thymidine-H^3 four days after the intraperitoneal implantation of the Novikoff hepatoma. Two groups of five rats served as controls. Each of the control and tumor-bearing rats was injected subcutaneously in the dorsal region with 0.5 ml of an aqueous solution containing 50 μc, 15 μM, of thymidine-H^3. This dose of thymidine-H^3 is approximately 10 times that injected into a mouse and was calculated on the basis of the relative weights of the mice and rats used in these experiments. Twenty-four hours after the injection of the tritiated thymidine the animals were killed by decapitation and the liver, lung, kidney, spleen and intestinal mucosa quickly removed and frozen. Corresponding tissues from each group of rats were pooled and the DNA thymine isolated and determined.

The specific activities (c.p.m./μM) of the DNA thymine from the tissues of the control and tumor-bearing rats are shown in Table VI. The degree of incorporation of thymidine-H^3 into

the DNA thymine of the kidney, spleen and intestinal mucosa is on the average greater in the control groups than in the tumor-bearing rats. However on examining the individual data obtained from each group it is seen that certain of the specific activities of the DNA thymine obtained from kidney, spleen, and mucosa of the tumor-bearing animals are of the same order of magnitude as those obtained from corresponding tissues of one of the control groups. The data obtained from the other control group tend to be somewhat higher and therefore have raised the average specific activities obtained for the two control groups. It is felt that more data are required before one can conclude that there are significant differences between the specific activities of the DNA thymine obtained from the kidney, spleen, and mucosa of rats bearing the Novikoff hepatoma as compared to those of the control rats. There is little difference between the average specific activities of the tumor-bearing and control groups in the case of liver and lung. However it is seen that certain of the individual specific activities obtained for the liver and lung of the tumor-bearing groups tend to be higher than those of the control groups.

(c) Walker carcinosarcoma 256 tumor - The results of Kampschmidt et al. (108) showed that the presence of the Walker 256 tumor in an animal depressed the liver catalase and plasma iron to a greater degree than did the Jensen sarcoma, Novikoff hepatoma and Flexner-Jobling carcinoma. Furthermore, the injection of an homogenate of the Walker 256 tumor was more effective in depressing the liver catalase and plasma iron, and increasing the

weights of liver, and spleen, as compared to an homogenate of the Jensen sarcoma. In addition, the injection of a toxohormone preparation from the Walker tumor was found (107) to be very effective in producing anemia, decreased plasma iron, depressed activity of the liver and kidney catalase, increased weight of the liver, spleen, and adrenals, and thymus involution.

These results indicated that the Walker carcinosarcoma 256 tumor produced more profound systemic effects on the host tissues than other tumors. In order to determine whether the larger magnitude of these effects might be attributed to a greater alteration in the DNA metabolism of the host tissues of the animals bearing the Walker tumor, as compared to other tumor-bearing animals, a study was made of the effect of the Walker tumor on the incorporation of tritiated thymidine into the DNA thymine of the host tissues. The effect of the Walker tumor on the plasma iron level was also investigated. Since all of the liver tissue was used for the isolation of liver DNA thymine, it was not possible to determine the effect of the Walker tumor on liver catalase.

Male Sprague-Dawley rats (150-170, or 180-200 grams each) were used in these experiments. Four groups of 2 rats each bearing the intramuscular Walker 256 tumor were injected with thymidine-H^3 11 days after the implantation of the tumor. Similar groups of normal rats served as controls. Each of the control and tumor-bearing rats was injected subcutaneously in the dorsal region with 0.5 ml of aqueous solution containing 50 μc, 15 μM, of tritiated thymidine. Twenty-four hours after the injection of

the thymidine-H^3 the rats were exsanguinated and the tissues quickly removed and frozen. Except for blood, corresponding tissues from each group were pooled and the DNA thymine isolated and determined. The specific activities (c.p.m./μM) of the DNA thymine from the liver, spleen, intestinal mucosa, kidney, and lung of the control and tumor-bearing rats are shown in Table VII. These results indicate that tritiated thymidine tends to be incorporated to a greater degree into the DNA thymine of the lung, and mucosa of the tumor-bearing rats as compared to the control rats. No significant difference in the incorporation of thymidine-H^3 into the DNA of kidney of the tumor-bearing and control groups is observed. However, the most striking results are those obtained for the liver and spleen. It is seen that the specific activities of the DNA thymine of the liver and spleen of the tumor-bearing groups are considerably higher than the corresponding values of the control groups. The degree of difference between the tumor bearing and control groups is about 2- to 3-fold in the case of spleen and up to 5-fold in the case of liver.

The plasma iron values shown in Table VII represent single determinations on the plasma obtained from individual rats. The plasma iron levels of the tumor-bearing rats tend to be lower than those of the controls. However, it should be borne in mind that most of the plasma samples showed evidence of hemolysis, and therefore the values obtained do not represent the true levels of plasma iron in these samples.

Table VII

Specific activities (c.p.m./μM) of DNA thymine from tissues of control rats and rats bearing Walker 256 carcinosarcoma. Each rat received 50 μc, 15 μM, of thymidine-H3.

	TUMOR-BEARING 2 rats	2 rats	average	CONTROL 2 rats	2 rats	average
180-200 g rats						
Liver	843	571	707	232	174	203
Spleen	2790	2815	2803	826	890	858
Mucosa	4240	3100	3670	3190	2420	2805
Kidney	120	191	156	190	124	157
Lung	656	676	666	515	356	436
Plasma iron ug Fe/100 ml	94 102	58 76*	83	152*	140*	146
150-170 g rats						
Liver	727	665	696	266	223	245
Spleen	2670	2010	2340	1260	910	1035
Mucosa	3070	2785	2928	2890	2730	2810
Kidney	355	180	268	248	179	214
Lung	1014	1525	1270	1019	526	773
Plasma iron ug Fe/100 ml	132 84*	72 136*	106	191* 188*	221*132 228*126	181

* Plasma sample slightly hemolysed

III. The Effect of Toxohormone on the Incorporation of Thymine-H^3 into DNA Thymine.

The above experiments have shown that the presence of tumor in an animal increased the incorporation of tritiated thymidine into the DNA thymine of the host's liver, spleen, lung, and intestinal mucosa. This effect was most evident in animals bearing the Walker 256 tumor. The experiments of Kampschmidt et al. (108) suggested that the Walker 256 tumor produced more toxohormone than other tumors studies. In view of these results it was felt that the markedly increased incorporation of tritiated thymidine into the DNA of the host tissues of animals bearing the Walker 256 tumor might be the result of the action of toxohormone released from the tumor. The greatest stimulation of thymidine-H^3 incorporation was found in the case of the liver and spleen of rats bearing the Walker 256 tumor. Because of these findings a study was made of the effect of toxohormone on the incorporation of tritiated thymidine into the DNA thymine of rat liver and spleen.

As yet there is no chemical method for assaying toxohormone, and therefore in the experiments reported herein toxohormone was assayed using biological assay systems. The most commonly used biological assay system for toxohormone is to measure the depression of liver catalase and experiments were performed therefore to determine the effect of tumor tissue on the level of this enzyme. Liver catalase was assayed in one group of eight female Ha/ICR mice bearing seventh day Ehrlich ascites tumor. One group of 3 mice and a second group of 10 mice served as controls. The results shown in Table VIII indicate that the level

Table VIII

Liver catalase activities of control mice and mice bearing Ehrlich ascites tumor. Catalase activity expressed as first order reaction rate constant ($K\ min^{-1}$) for an amount of liver equivalent to 0.01 mg of tyrosine.

	$K \times 10^4$
Tumor-bearing (8 mice)	800
Control (3 mice)	2150
Control (10 mice)	1360

Table IX

Liver catalase activity of control rats and rats bearing Novikoff hepatoma. Catalase activity expressed as first order reaction rate constant ($K\ min^{-1}$) for an amount of liver equivalent to 0.01 mg of tyrosine

	no. of rats per group	weight of rats in grams	$K \times 10^4$
Tumor-bearing	3	170-230	1550
Tumor-bearing	1	250	1810
Tumor-bearing	3	175-188	1250
Control	2	150	1805
Control	3	195-255	2130
Control	3	170-180	2290
Control	2	approx. 150	1235

of liver catalase levels of the control mice. Similar experiments were performed on male Sprague-Dawley rats bearing sixth day intraperitoneal Novikoff hepatoma. However, the data as shown in Table IX reveal that there are no significant differences between the liver catalase levels of groups of tumor-bearing rats as compared to those of the control rats. Considerable variation in the liver catalase levels is noted within both the tumor-bearing and the controlled groups. This variation could be attributed to differences in the weights of the rats in each group.

The liver catalase results indicated that the Ehrlich ascites tumor might have produced more toxohormone than the Novikoff hepatoma. Experiments were undertaken therefore, in an attempt to purify crude toxohormone extracted from Ehrlich ascites tumor cells. The ascitic fluid was removed from eight female Ha/ICR mice 7 days after implantation of the Ehrlich ascites tumor. Approximately 15 grams of packed tumor cells were obtained and yielded 121.4 mg of crude toxohormone extracted by a modification (158) of the method described by Nakagawa et al. (159). The crude toxohormone is obtained as the alcohol precipitate of an aqueous extract of tumor.

Purification of crude toxohormone has been obtained by Ohashi and Ono (110) and Goranson (111) using DEAE-cellulose ion-exchange columns. These authors eluted the toxohormone by a gradient system of phosphate buffers of decreasing pH and increasing molarity and phosphate was removed from the eluted fractions by dialysis. However Yunoki and Griffin (112) have reported loss of toxohormone activity following dialysis and others (6)

have shown that active toxohormone could traverse a dialysis bag. In order to overcome the problem of dialysis, efforts were made to elute toxohormone from DEAE-cellulose columns using the volatile salt ammonium carbonate.

DEAE-cellulose was converted to the carbonate form on a column by washing with 2M ammonium carbonate until the effluent showed no absorption at 258 mμ. The column was then washed with water until the pH of the effluent was equal to that of the eluent. The crude toxohormone was dissolved in 5 ml of water to yield a translucent solution. This was centrifuged at 23,500 xg for twenty minutes and the supernatant placed on a column of DEAE-cellulose measuring 2 x 17 cm. The column was eluted with a gradient system consisting of 1000 ml of water in the mixing vessel and 1000 ml of 1M ammonium carbonate in the reservoir. Fractions of 7 ml were collected at 5 minute intervals using a fraction collector. At tube number 201 elution with 2M ammonium carbonate was begun.

An amino acid analysis of toxohormone by Ono et al. (116) showed that crude toxohormone contained phenylalanine, the λ_{max} of which in 1.0M ammonium carbonate was found to be 258 mμ. Therefore the elution of the fractions from the DEAE-cellulose column was followed by determining the optical density of the individual fractions at 258 mμ. The elution pattern is shown in Figure 26. Five fractions designated I, II, III, IV and V were eluted from the DEAE-cellulose column by the ammonium carbonate eluent, the majority of the ultraviolet-absorbing material being contained in fraction IV. The individual portions of each frac-

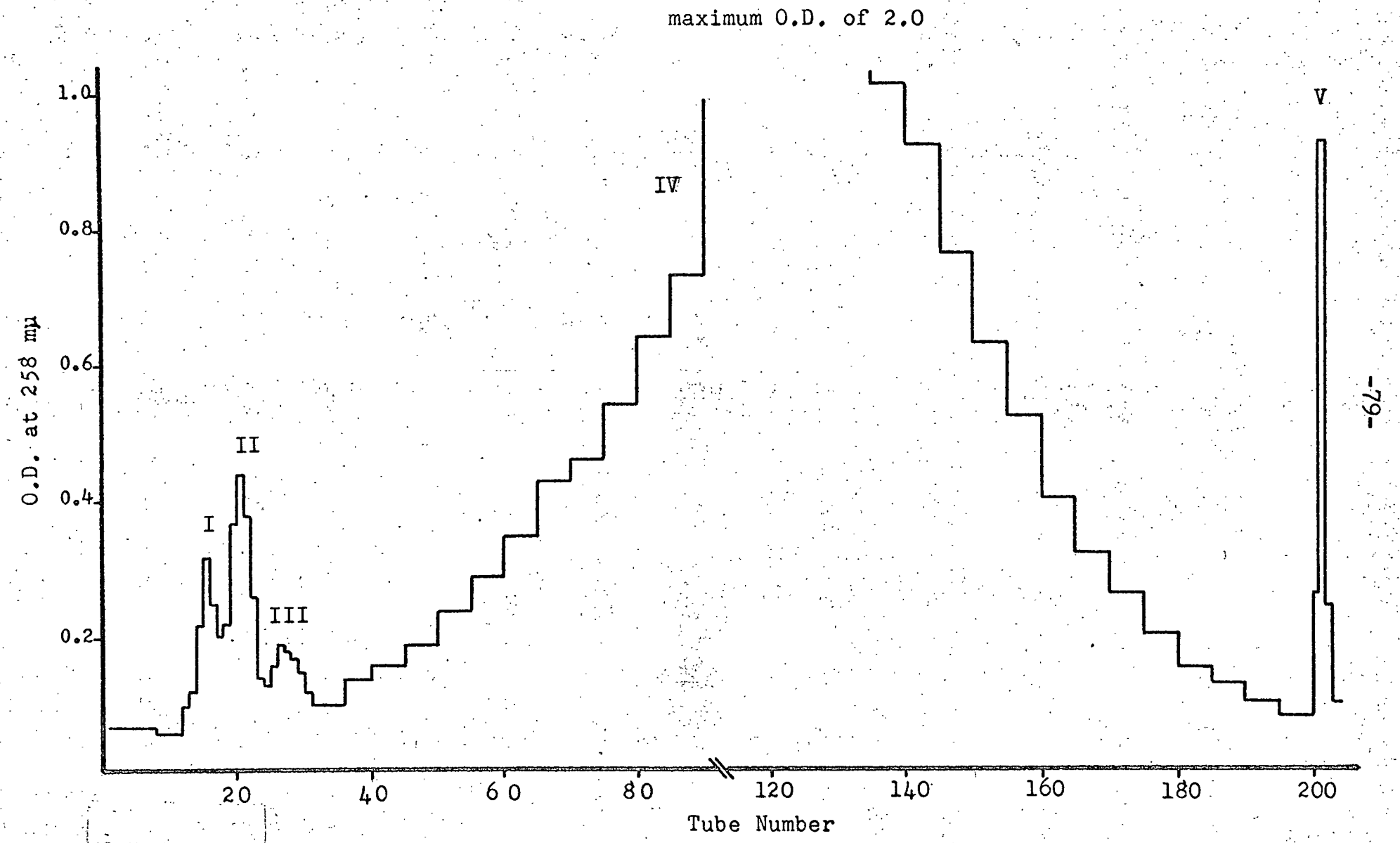

Figure 26. Elution from DEAE-cellulose of toxohormone prepared from Ehrlich ascites tumor by the modified Nakagawa method.

tion were pooled and the ammonium carbonate removed by repeated evaporations *in vacuo* at room temperature using a rotary evaporator. The final residue was first dried at a pressure of 10 mm Hg at 100°C, and then at 0.05 mm Hg at room temperature in order to remove the last traces of ammonium carbonate. Each of the dried residues was taken up in a known volume of water, autoclaved, and the optical density at 258 mμ determined for each fraction.

In order to assay the toxohormone activity of the fractions, aliquots of each fraction were injected intraperitoneally into groups of 3 female Swiss mice (18-22 grams each). One ml of fraction III was arbitrarily selected as a dose of 1 unit since this fraction had the lowest optical density. The dosage of the other fractions were calculated on the basis of their optical densities at 258 mμ relative to that of fraction III at 258 mμ. Two groups of 3 mice each were injected intraperitoneally with 0.3 ml of sterile water and served as controls. Twenty-four hours after the injection of the fractions the animals were killed by cervical dislocation and the livers from the mice of each group removed and pooled. The liver catalase activity of each group was determined (138,139) and the results are shown in Table X. It is seen that fraction IV is the only one which appears to have depressed the liver catalase. The experiment was repeated and larger amounts of fractions II, IV and V were injected. The doses of fractions I and III were not increased since the mice appeared distressed after the intraperitoneal injection of volumes greater than 1 ml. The results of this experiment, shown in Table XI, indicate that none of the fractions have activity towards liver catalase. Increasing the dose of fraction IV to 71

Table X

Liver catalase activities of sham-injected mice, and mice injected with toxohormone fractions eluted from DEAE-cellulose column. Catalase activity expressed as first order reaction rate constant ($K\ min^{-1}$) for an amount of liver equivalent to 0.01 mg of tyrosine.

Fraction	ml of solution injected per mouse	units injected per mouse	K x 10^4
I	0.89	3	1625
II	0.38	1	2005
III	1.00	1	1850
IV	0.35	1	1130
V	0.27	1	1600
sham	0.30	0	1830
sham	0.30	0	1580

Table XI

Liver catalase activities of sham-injected mice, and mice injected with toxohormone fractions eluted from DEAE-cellulose column. Catalase activity expressed as first order reaction rate constant ($K\ min^{-1}$) for an amount of liver equivalent to 0.01 mg of tyrosine.

Fraction	ml of solution injected per mouse	units injected per mouse	$K \times 10^4$
I	0.89	3	1780
II	1.13	3	2100
III	1.00	1	1905
IV	1.05	3	2280
IV	1.00	71	1935
IV	1.00	71	1850
V	0.80	3	1920
sham	1.00	0	1755
sham	1.00	0	2050
sham	1.00	0	2070
sham	1.00	0	1640

units caused no alteration in the liver catalase level. The reason for the apparent activity of fraction IV in the experiments summarized in Table X is unknown.

These experiments indicated that the toxohormone isolated from Ehrlich ascites tumor cells by the modified Nakagawa method (158,159) had no apparent effect on liver catalase when injected into normal mice. It was felt that the failure to demonstrate activity towards liver catalase might be attributed to wide variations in normal levels. Others (160) have observed wide variations in the normal liver catalase values and it was felt that the assay of liver catalase was not adequate as a means of detecting toxohormone activity.

Kampschmidt et al. (107) found that plasma iron was 250 to 500 times as sensitive to toxohormone as liver catalase and therefore the effect on the plasma iron of rats was adopted as the bio-assay for toxohormone. In addition the toxohormone was isolated from the Walker 256 tumor since this tumor appeared to elaborate large amounts of this factor (108). Rats bearing the Walker tumor were sacrificed thirteen days after implantation and the tumors excised by amputating the lower legs. The skin and most of the bone and muscle was removed to yield 126 grams of tissue. The toxohormone was extracted from the tissue using the modified Nakagawa method (158,159) and 248.8 mg of crude toxohormone was obtained. This amount of crude toxohormone was dissolved in 25 ml of water and found to be about 70% soluble at room temperature. Doses of 1, 2, or 4 mg of the soluble material contained in 0.5 ml of water were injected intraperitoneally into

male Sprague-Dawley rats (150-170 grams each). Rats sham-injected intraperitoneally with 0.5 ml of water served as controls. The work of Kampschmidt et al. (107) indicated that toxohormone had a maximal effect on plasma iron 10 hours after injection. Ono et al. (118) determined the effect of toxohormone on plasma iron thirteen hours after injection. Therefore, in the experiments described herein, the animals were sacrificed by exsanguination twelve hours after the injection of the crude toxohormone. The results of the plasma iron determinations are shown in Table XII and represent single determinations on the plasma of individual rats. From Table XII it is seen that a dose of up to 4 mg of crude toxohormone prepared by the modified Nakagawa method (158,159) had no effect on the plasma iron of the rat. In view of these results it was decided to adopt the method which Yunoki and Griffin have recently described (112) for the isolation of toxohormone. In this method the crude toxohormone is precipitated by the addition of ether to an acetic acid extract of the acetone-dried powder of tumor tissue. In a typical experiment 2500 grams of Walker 256 tumor yielded approximately 16 grams of crude toxohormone.

Doses of 10, 20 or 40 mg of the crude toxohormone (prepared by the method of Yunoki and Griffin), dissolved in 0.5 ml of water, were injected intraperitoneally into individual male Sprague-Dawley rats (150-170 grams each). Rats sham-injected intraperitoneally with 0.5 ml of water served as controls. Twelve hours after the injection the animals were exsanguinated and the plasma iron of each rat determined. From Table XIII it is seen that although a dose of 10 mg of crude toxohormone had no effect,

Table XII

Plasma iron levels of sham-injected rats, and rats injected with crude toxohormone prepared by the modified Nakagawa method; 12 hours after injection

	µg Fe/100 ml
crude toxohormone	
1 mg	281, 279
2 mg	224
4 mg	256
sham (15 rats)	181 ± 38

Table XIII

Plasma iron levels of sham-injected rats, and rats injected with crude toxohormone prepared by the method of Yunoki and Griffin; 12 hours after injection

	µg Fe/100 ml
crude toxohormone	
10 mg	155, 244
20 mg	99, 55, 32
40 mg	93, 66, 70
sham (15 rats)	181 ± 38

doses 20 and 40 mg of toxohormone caused a signicant decrease in the plasma iron level as compared to the sham-injected rats. Increasing the dose of toxohormone from 20 to 40 mg did not effect the level of plasma iron to any greater degree. These results indicated that crude toxohormone prepared from Walker tumor by the Yunoki and Griffin method (112) was active in depressing the plasma iron level of a rat.

Therefore this material prepared by the Yunoki and Griffin method (112) was tested for its effect on the incorporation of tritiated thymidine into the DNA thymine of liver and spleen. Groups of 2 male Sprague-Dawley rats (150-170 grams each) were injected intraperitoneally with 40 mg of the crude toxohormone dissolved in 0.5 ml of water. The limit of solubility of the crude toxohormone in water was found to be about 80 mg per ml. Individual rats or groups of 2 rats injected intraperitoneally with 0.5 ml of water served as sham controls. Each of the sham and test animals was injected subcutaneously in the dorsal region with 50 μc, 15 μM, of tritiated thymidine dissolved in 0.5 ml of water. Six, twelve, and twenty-four hours after the injection of the thymine-H^3, the animals were exsanguinated and the tissues removed and frozen. Corresponding tissues from each group were pooled and the DNA thymine isolated and determined. Because the experiments reported herein indicated that the presence of Walker 256 tumor in a rat increased the incorporation of tritiated thymidine into the DNA thymine of the host's liver and spleen to a greater degree than the other tissues studied, the effect of toxohormone on the incorporation of thymidine-H^3 into the DNA thymine of only these tissues was studied. The data in

Table XIV

Specific activities (c.p.m./μM) of DNA thymine from liver and spleen of sham-injected rats, and rats injected with crude toxohormone; 6 hours after injection. Each rat received 50 μc, 15 μM, of thymidine-H^3.

	TOXOHORMONE-INJECTED				SHAM-INJECTED			
	2 rats	2 rats	2 rats	average	2 rats	2 rats	2 rats	average
Liver	169	111	62	114	102	104	111	108
Spleen	1423	1419	1073	1302	2010	1410	1450	1623
Plasma iron μg Fe/100 ml	104	55	40 101	75	109	179	149 156	148

Table XV

Specific activities (c.p.m./μM) of DNA thymine from liver and spleen of sham-injected rats, and rats injected with crude toxohormone; 12 hours after injection. Each rat received 50 μc, 15 μM, of thymidine-H^3.

	TOXOHORMONE-INJECTED				SHAM-INJECTED			
	2 rats	2 rats	2 rats	average	2 rats	2 rats	2 rats	average
Liver	183	151	90	141	133	-	85	109
Spleen	2050	1547	1626	1743	941	681	1370	997
Plasma iron μg Fe/100 ml	24 46	70 61	48 81	66	-	-	167 109	135

Table XVI

Specific activities (c.p.m./μM) of DNA thymine from liver and spleen of sham-injected rats, and rats injected with crude toxohormone; 24 hours after injection. Each rat received 50 μc, 15 μM, of thymidine-H^3.

	TOXOHORMONE-INJECTED				SHAM-INJECTED			
	2 rats	2 rats	2 rats	average	2 rats	2 rats	2 rats	average
Liver	332	332	103	258	221	209	221	217
Spleen	923	860	1212	998	1212	1085	1339	1212
Plasma iron μg Fe/100 ml	85	104 115	63	92	171	176	223 273	211

Tables XIV, XV, XVI show that the plasma iron levels are lowered at 6, 12 and 24 hours after the injection of 40 mg of crude toxohormone. Maximal lowering of the plasma iron appears to occur 12 hours after the injection of the toxohormone, a finding which is in agreement with the results of Kampschmidt et al (107).

Six hours after the injection of the crude toxohormone there was no significant difference between the specific activities of the DNA thymine of liver and spleen from the toxohormone-injected animals as compared to those of the sham-injected controls. However, 12 hours after the injection of 40 mg of crude toxohormone the incorporation of tritiated thymidine into the DNA thymine of spleen of the toxohormone-injected rats tended to be greater than that of the control rats. Toxohormone appears to have no effect upon the incorporation of thymidine-H^3 into the DNA thymine of liver 12 hours after the injection of crude toxohormone (Table XV). On the other hand, the results shown in Table XVI indicate that the specific activities of the DNA thymine from the liver of two of the toxohormone-injected groups are slightly greater than those of the control groups. In contrast, the specific activity of the liver DNA thymine of the third group is lower than the control values. There appeared to be no effect on the incorporation of tritiated thymidine into the spleen DNA thymine 24 hours after the injection of the crude toxohormone.

Since the above results indicated that crude toxohormone might have an effect on the incorporation of tritiated thymidine into the DNA of liver and spleen it was felt that the effect of

purified toxohormone should be studied. Yunoki and Griffin (112) have described a method for the purification of crude toxohormone using Amberlite XE-64 ion-exchange columns. The method described by these authors was employed in the work reported herein.

One gram of crude toxohormone isolated from Walker 256 tumor was added to 20 ml of NaCl-glycine buffer, pH 9.4. The resulting translucent solution was centrifuged at 2000xg for 10 minutes and the supernatant solution placed on a column of Amberlite XE-64 measuring 4.2 x 38 cm and adjusted to pH 9.3 according to the method of Yunoki and Griffin (112). The amount of soluble crude toxohormone placed on the column was calculated to be 731 mg. The column was eluted with a gradient elution system consisting of 1 litre of an NaCl-glycine buffer pH 9.4 (112) in the mixing vessel, and 1 litre of 0.1 NaOH in the reservoir. The effluent was collected in approximately 10 ml portions every 30 minutes using a fraction collector. Aliquots of the fractions were analyzed for protein using the method of Lowry et al. (161). A solution of bovine gamma globulin (400 µg/ml) in glycine buffer pH 9.4 was used as a protein standard. The elution pattern is shown in Figure 27. It is noted that there was a very slow rise in the pH of the effluent from pH 9.06 at tube No. 1 to 9.44 at tube No. 160. Yunoki and Griffin (112) reported a more rapid increase in the pH of the effluent. It was postulated that the crude toxohormone, used in the experiment reported herein, had caused a marked decrease in the pH of the Amberlite XE-64. It was assumed that the crude toxohormone had functioned as an efficient buffer and therefore resulted in a very

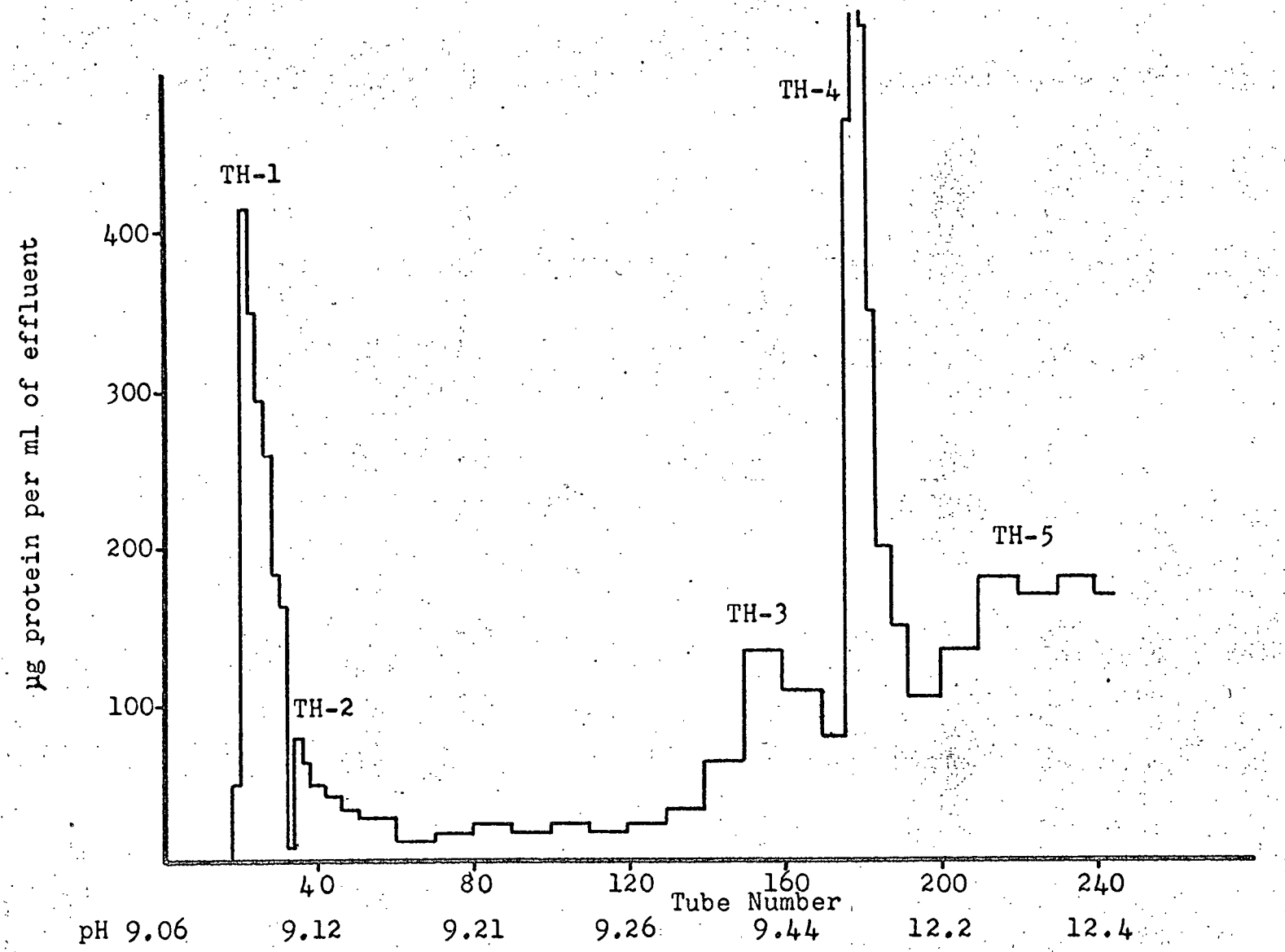

Figure 27. Elution from Amberlite XE-64 of toxohormone prepared from Walker 256 tumor by the Yunoki and Griffin method.

slow rise in the pH of the effluent. At tube No. 150 the pH of the eluant was 12.04, whereas the effluent had a pH of 9.44. In view of these considerations the gradient elution system was changed at tube No. 150. One litre of the NaCl-glycine buffer pH 9.4 was adjusted to pH 12.04 by the addition of 30% NaOH and placed in the mixing flask. The reservoir contained 1 litre of 0.4M NaOH. Gradient elution was continued with the new system. At tube No. 170 the pH of the effluent was approximately 9.4, following by a rise in pH to 10.76 at tube No. 176 with a further rise to pH 12.1 at tube No. 180. Elution was discontinued at tube No. 244 because of the high pH of the effluent. As shown in Figure 27, five fractions were eluted from the Amberlite XE-64 column. These were designated TH-1, TH-2, TH-3, TH-4 and TH-5. The individual portions of each fraction were pooled and the total volume of each was reduced to about 50 ml by evaporating in vacuo at 50°C. using a rotary evaporator. Because Yunoki and Griffin (112) had observed that dialysis of the fractions resulted in a loss of a large portion of each sample, another method was sought which would allow removal of the contaminating NaCl and glycine from the fractions.

The technique of gel filtration has been shown (162-165) to be useful in the separation of low molecular weight compounds such as salts and amino acids from proteins and peptides. A material recently shown to give satisfactory results in gel filtration is Sephadex G 50 (Pharmacia, Uppsala, Sweden) which is a water insoluble substance made by cross-linking the polysaccharide dextran. Grains of this material are highly hydrophilic and when placed in water form gel grains. The water in a column packed with

Sephadex consists of two parts, the internal and the external volume, the former corresponding to the water taken up by the grains of Sephadex and the latter to the volume of water occupying the interstices between the gel grains. Molecules of low molecular weight such as salts can diffuse relatively freely through the network structure of the gel grains. Moderately large molecules will be restricted in their diffusion through the gel grains depending on the porosity of the network structure. Large molecules are completely prevented from entering the gel grains and will be rapidly eluted from the column of Sephadex free of other molecules such as salts and amino acids.

In the experiments described herein Sephadex G50 columns measuring 3.5 x 5 cm were prepared according to the method described by the manufacturer (166). Each of the five fractions obtained from the Amberlite XE-64 column were placed on individual Sephadex columns and eluted with water under 4 pounds per square inch of pressure. An initial flow rate of about 1 ml per minute was obtained; however the flow rate gradually diminished to about 1 ml per 30 minutes. The marked decrease in flow rate was attributed to packing of the gel particles in the Sephadex columns. The fractions eluted from the Sephadex column were collected by means of a fraction collector and aliquots of each fraction analyzed for protein (161), chloride, and glycine. Chloride was determined qualitatively by the silver nitrate method. In order to test for the presence of glycine, portions of each fraction were spotted on chromatography paper. The paper was dried, sprayed with a solution of ninhydrin and dried in an oven at 100°C. The presence of glycine was indicated by an immediate ninhydrin

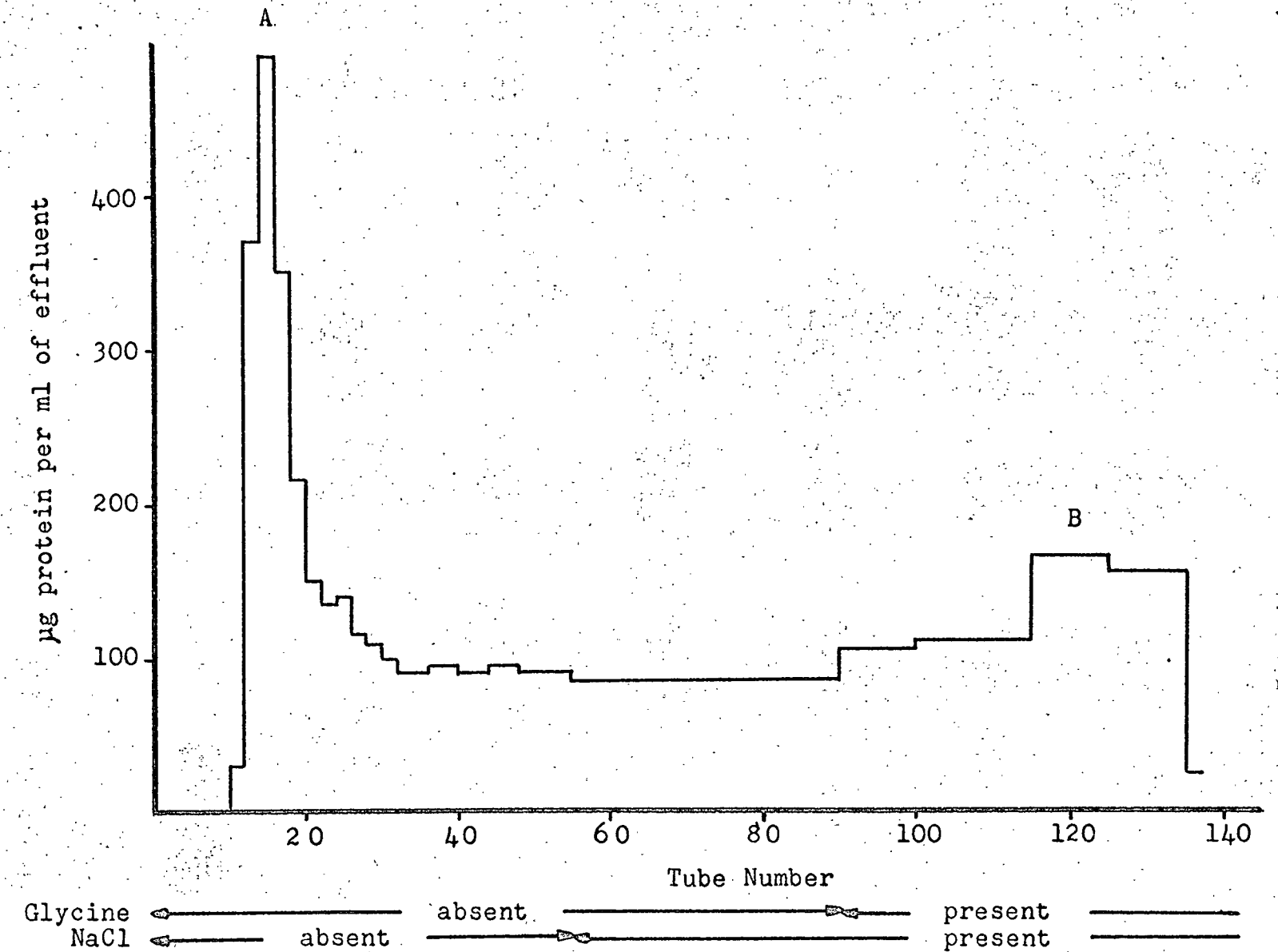

Figure 28. Elution of fraction TH-5 from Sephadex G50.

Table XVII

Total amount of protein in the "A" and "B" toxohormone fractions obtained from the Sephadex column

FRACTION	TOTAL PROTEIN µg
TH-1A	18,200
TH-1B	13,900
TH-2A	558
TH-2B	1,212
TH-3A	25,600
TH-4A	12,780
TH-4B	10,100
TH-5A	13,120
TH-5B	4,920
Total	100,190

reaction.

A typical elution pattern is shown in Figure 28 which illustrates the elution of fraction TH-5 from the Sephadex column. It is seen that an initial peak of protein-containing material is obtained which is free of chloride and glycine. This is followed by a second protein-containing peak which is contaminated with chloride and glycine. These two peaks were designated TH-5A and TH-5B. Similar elution patterns were obtained for fractions TH-1, TH-2, and TH-4. No "B" fraction was obtained in the case of TH-3. Each of the "A" and "B" fractions from the Sephadex columns was evaporated to dryness in vacuo at 50°C. using a rotary evaporator. The residues were dissolved in a known volume of water and the protein concentration of each fraction determined (161). The results of the protein analyses are shown in Table XVII. It is seen that about 100 mg of protein was recovered from the 731 mg of crude toxohormone originally placed on the Amberlite XE-64 column. Fraction TH-3A contained the largest amount of protein and the "A" fractions generally contained more protein than the "B" fractions.

The toxohormone activity of the "A" fractions was assayed by determining the effect on the plasma iron levels of rats. Individual Sprague-Dawley rats (150-170 grams each) were injected intraperitoneally with 200 µg of fractions TH-1A, TH-3A, TH-4A and TH-5A contained in 0.5 ml of water. Because of the limited amount of fraction TH-2A only 93 µg of this fraction were injected into each rat. Twelve hours after the injection of the fractions the animals were sacrificed by exsanguination and the plasma iron levels determined. These were compared with plasma iron

Table XVIII

Plasma iron levels of sham-injected rats, and rats injected with the "A" toxohormone fractions; 12 hours after injection.

PLASMA IRON, µg Fe/100 ml

fraction	µg of protein injected per rat		sham-injected
	200	20	
TH-1A	51,86,80,68	67,153,171	
TH-2A	67,86	110,230,128	
TH-3A	84,67,88,127	87,196,137,173	181 ± 38 (15 rats)
TH-4A	98,47,87,88	100,222,144	
TH-5A	40,91,165	81,101,140	

levels of sham-injected rats. The results in Table XVIII indicate that all of the "A" fractions have toxohormone activity as manifested by the decrease in the plasma iron levels. In order to determine which of the "A" fractions was the most active the experiment was repeated with the injection of 20 μg of each fraction into individual rats. From the results shown in Table XVIII it appeared that fraction TH-5A had the highest plasma iron-depressing activity. Therefore, the effect of fraction TH-5A on the incorporation of tritiated thymidine into the DNA thymine of liver and spleen was studied.

It was also of interest to study the activity of a crude toxohormone preparation from a normal tissue. Toxohormone was extracted (112) from 260 grams of muscle tissue obtained from the front and back limbs and the anterior abdominal wall of male Sprague-Dawley rats (180-200 grams each). The yield of crude toxohormone was 1.04 grams. Groups of two male Sprague-Dawley rats (150-170 grams each) were each injected intraperitoneally with 1.0 mg of fraction TH-5A contained in 0.5 ml of water. Similar groups of two rats were each injected intraperitoneally with a suspension of 40 mg of crude muscle toxohormone contained in 0.5 ml of water. Each toxohormone-injected rat was injected as before with 50 μc, 15 μM, of thymidine-H^3 contained in 0.5 ml of water. The animals were sacrificed by exsanguination twelve hours later and the liver and spleen removed and analyzed for DNA thymine as before. Plasma iron determinations were performed on those samples of plasma which showed no evidence of hemolysis and compared with those of sham-injected rats.

From Table XIX it is seen that the plasma iron levels of

Table XIX

Specific activities (c.p.m./μM) of DNA thymine from liver and spleen of sham-injected rats, rats injected with fraction TH-5A, and rats injected with crude muscle toxohormone; 12 hours after injection. Each rat received 50 μc, 15 μM, of thymidine-H^3.

	FRACTION TH-5A				CRUDE MUSCLE TOXOHORMONE				SHAM-INJECTED			
	2 rats	2 rats	2 rats	avg.	2 rats	2 rats	2 rats	avg.	1 rat	1 rat	2 rats	avg.
Liver	90	66	151	102	84	73	124	94	133	-	85	109
Spleen	706	696	1230	877	830	1058	875	954	941	681	1370	997
Plasma iron μg Fe/100 ml	57 112	60 59	77 66	72	136	116	91 142	121			167 109	135

those rats which were injected with 1.0 mg of fraction TH-5A are generally lower than those of the sham-injected controls whereas the injection of 40 mg of crude muscle toxohormone did not depress the plasma iron levels. From Table XIX it is also seen that the specific activities of the DNA thymine of liver and spleen from the animals injected with fraction TH-5A are of the same order of magnitude as those of the sham-injected animals. Crude muscle toxohormone similarly had no effect on the incorporation of thymidine-H^3 into the DNA thymine of rat liver and spleen.

IV. Chemical Studies of Toxohormone.

Because of the small amount of fraction TH-5A remaining only preliminary studies of the chemical nature of this fraction were undertaken. One mg of fraction TH-5A was hydrolysed in 1 ml of 6N HCl at 100°C. for 48 hours. The excess HCl was removed by repeated evaporation in vacuo and the residue dissolved in approximately 1 ml of water. A portion of this solution and a series of standard amino acids were chromatographed on Whatman No. 1 paper using a butanol-acetic acid solvent system (167). The hydrolysate was found to yield several ninhydrin-reacting components which corresponded in Rf values to histidine, lysine, aspartic acid, hydroxyproline, glutamic acid, proline, tyrosine, methionine and valine. These results are partially in agreement with the findings of Yunoki and Griffin (113) who found the above amino acids, except for histidine and hydroxyproline, in a hydrolysate of a purified fraction of toxohormone. These authors observed in addition the presence of threonine, serine, glycine, alanine, cystine, isoleucine, leucine, phenylalanine, and argi-

nine. In the experiments reported herein, the amino acids were not adequately resolved by the paper chromatography and therefore it was difficult, on the basis of these results, to accurately determine the amino acids contained in a hydrolysate of purified toxohormone.

DISCUSSION

The experiments described in this thesis were undertaken in order to study the effect of tumor tissue on the nucleic acid metabolism of the host's liver, spleen, intestinal mucosa, kidney, and lung. In particular the DNA metabolism of the host tissues has been investigated by studying the incorporation of tritiated thymidine into the DNA thymine of the host tissues. Considering the important role of DNA in the cell (131), it was of interest to investigate the metabolism of this material in the host tissues, since alterations in its metabolism in the host might explain in whole or in part the observed systemic effects of tumor tissues. The results of other investigators (16-19, 21, 22, 24-27), which have been outlined in the introduction of this thesis, indicated that the presence of tumor in an animal caused an increased incorporation of several precursors into the nucleic acids of the host tissues. Furthermore an increased concentration of the nucleic acids in the host tissues has been demonstrated (8-15).

The results of the formate-C^{14} incorporation studies reported herein are not in agreement with several of the findings of others. For example neither the solid subcutaneous form nor the ascites form of the Ehrlich tumor in mice appeared to have an effect on the incorporation of formate into the nucleic acids of the host's spleen, liver, kidney, or lung. However, the presence of the Ehrlich ascites tumor has caused a decrease in formate incorporation into the DNA purines and thymine of the host intestinal mucosa. These results are in partial agreement with

other experiments reported (23) in which the presence of Novikoff hepatoma in rats was observed to cause a decrease in the formate uptake by the nucleic acid purines and thymine of the host spleen and intestinal mucosa, but had little effect on the host liver. In agreement are the findings of LePage and Heidelberger (20) who found no difference in glycine incorporation into the nucleic acids of normal livers as compared to livers of rats bearing Flexner-Jobling carcinoma. In contrast, others have reported (16-19, 21, 22, 24-27) that several nucleic acid precursors were utilized to a greater degree by the nucleic acids of host tissues of tumor-bearing animals than by those of tissues of normal animals.

At this stage in the experiments it was felt that formate-C^{14} was not well suited for studies of the effect of tumor on the nucleic acid metabolism of host tissues. It was observed (22, 23, 133) that formate was rapidly excreted as expired CO_2 following subcutaneous injection and, therefore, had a transitory existence in the animal. Furthermore formate can be incorporated into many metabolites and therefore is not a specific precursor of nucleic acid. In this regard it is important to note that the methods employed by other investigators were not designed to specifically study the nucleic acid metabolism of the host tissues. For instance, the work of Cerecedo et al. (8-15) was limited to the determination of the nucleic acid concentration in the host tissues. Other investigators (16-18, 21-23) studied the incorporation of a number of non-specific precursors into the nucleic acids of the host tissues. In contrast, thymidine has been

shown (134) to be a specific precursor of DNA thymine, and is therefore admirably suited for a study of alterations in DNA metabolism of the host tissues of tumor-bearing animals. The possible relationship of the DNA metabolism of the host tissues to the systemic effects of tumor has already been mentioned. With these considerations in mind, a study was made of the incorporation of tritiated thymidine into the DNA of the host tissues of animals bearing the Ehrlich ascites tumor, Novikoff hepatoma, and the Walker carcinosarcoma 256.

The results of the experiments reported herein indicated that the presence of Ehrlich ascites tumor caused an increased incorporation of thymidine into the DNA of the host liver when 0.014, 0.14 or 1.40 μM of thymidine-H^3 was injected per mouse. With the lowest dose level the presence of the Ehrlich ascites tumor caused a decreased incorporation of thymidine into the DNA thymine of the other tissues examined. However, when the amount of thymidine-H^3 injected was increased to 0.14 μM the specific activities of the DNA thymine of spleen and kidney tended to be higher in the tumor-bearing animals as compared to the controls. When the amount of thymidine-H^3 administered was increased to 1.40 uM the specific activity of the DNA thymine of spleen was the same or higher in the tumor-bearing animals. However, there was little difference between the two groups in the case of kidney and for lung the results were conflicting. The degree of incorporation of thymidine into the DNA of intestinal mucosa was generally lower in the tumor-bearing groups of animals receiving 0.14 μM or 1.40 μM of thymidine-H^3. This result is in accord

with the finding of a lower incorporation of formate-C^{14} into the DNA purines and thymines of the host intestinal mucosa of mice bearing the Ehrlich ascites tumor.

The results of these experiments indicate that the presence of Ehrlich ascites tumor in a mouse caused an increased incorporation of thymidine into the DNA of the host's liver, spleen, kidney and lung providing the pool of thymidine was large enough. Since the tumor cells would be expected to have a high degree of incorporation of thymidine, the pool of thymidine available to the other tissues could be decreased in the tumor-bearing animals. It is possible that this decrease in the pool of thymidine would be manifested by a decreased incorporation of radioactivity into the DNA of the host tissues. Intestinal mucosa is a rapidly dividing tissue (168) and, therefore, has a high demand for thymidine. The lower degree of incorporation of thymidine into the intestinal mucosa DNA in the tumor-bearing animal might be the result of an insufficient supply of thymidine to meet the demands of the intestinal mucosa even though the amount of thymidine injected was increased 100-fold. Therefore, in order to demonstrate a stimulatory effect of the Ehrlich ascites on DNA synthesis in intestinal mucosa it may be necessary to further increase the amount of injected thymidine.

The results of the experiments involving rats bearing the Novikoff hepatoma indicated that the incorporation of thymidine-H^3 into the DNA of the kidney, spleen and intestinal mucosa was greater in the control groups than in the tumor-bearing rats. In contrast, the specific activities of the liver DNA thymine of the tumor-bearing groups were slightly higher than those of the con-

trols. A similar result was found in one instance for lung. However an assessment of the significance of these findings is difficult since the differences are not consistent for all of the groups.

The most striking effect of tumor on the incorporation of tritiated thymidine into the DNA of the host tissues was observed in the experiments with rats bearing the Walker carcinosarcoma 256. The specific activities of the DNA thymine of lung and intestinal mucosa tended to be higher in the rats bearing the Walker tumor as compared to the control rats. No significant difference was found in the case of the kidney tissues. However there was a marked increase in the incorporation of thymidine-H^3 into the DNA thymine of liver and spleen of rats bearing the Walker tumor. Examination of the data shows an increased uptake of thymidine-H^3 by the liver and spleen of each group of tumor-bearing animals. In the case of spleen the increase was two- to three-fold and up to five-fold in the case of liver. These results are in agreement with those of Kelly and associates (16-19) who have observed an increased incorporation of phosphorous, glycine, and formate into the nucleic acids of the tissues of tumor-bearing animals and with those of others (24-27) who have found an increased incorporation of adenine, hypoxanthine and guanine.

The findings of the present work indicated that the presence of the Walker 256 tumor in an animal had a more profound effect on the DNA metabolism of the host tissues than did the Ehrlich ascites tumor or the Novikoff hepatoma. Of interest in this connection are the experiments of Kampschmidt et al (108)

who have shown that the rate of increase of spleen weight was greater in animals bearing the Walker 256 or Flexner-Jobling tumors than in animals bearing Jensen sarcoma or the Novikoff hepatoma. Furthermore, liver and kidney catalase activity showed the greatest decrease in rats bearing the Walker tumor, followed closely by those bearing the Flexner-Jobling carcinoma. Animals bearing the Novikoff hepatoma or Jensen sarcoma showed a smaller effect on the liver and kidney catalase levels. In addition alterations in organ weights and catalase activity after the injection of tumor toxohormone extracts from different tumors, corresponded well with those of the rat bearing that same tumor. The greatest changes were effected by extracts from the Walker tumor, as compared to extracts of the Flexner-Jobling carcinoma, Jensen sarcoma, or Novikoff hepatoma. Furthermore, the injection of toxohormone from the Walker tumor caused a more profound decrease in the plasma iron level than did toxohormone preparations from the three other tumors. These results indicated that the Walker 256 tumor produced either more toxohormone or a more active type of toxohormone, as compared to the Flexner-Jobling carcinoma, Jensen sarcoma, or Novikoff hepatoma. It was felt, therefore, that there might be a correlation between the findings reported herein and the findings of Kampschmidt et al. (108). It was postulated that the greater effect of the Walker tumor on the DNA metabolism of the host tissues might be related to the high toxohormone activity observed in animals bearing the Walker tumor. This postulate suggested that the effect of the tumor on the DNA metabolism of the host could be attributed to the action of toxohormone. Therefore, it

was of interest to study the effect of toxohormone preparations on the incorporation of tritiated thymidine into DNA thymine.

The method used most commonly to assay toxohormone has been to measure the depression of liver catalase in an animal injected with toxohormone. However, the results of the experiments reported herein indicated that the determination of liver catalase depression was not satisfactory because of variations in the normal liver catalase levels. These variations were confirmed by others (160) and therefore the liver catalase method was abandoned as a means of measuring toxohormone activity. Kampschmidt et al. (107) have observed that the plasma iron level was 250-500 times as sensitive to toxohormone as liver catalase and therefore the depression of plasma iron was considered to be the method of choice for assaying toxohormone activity. Crude toxohormone was extracted from Walker 256 tumor by a modification (158) of the method of Nakagawa et al. (159) and also by the method of Yunoki and Griffin (112). A dose of 4 mg per rat of crude toxohormone, prepared by the modified Nakagawa method (158,159), had no effect on the plasma iron, whereas doses of 20 or 40 mg per rat of crude toxohormone, prepared by the method of Yunoki and Griffin (112), was found to significantly lower the plasma iron level. It should be noted that these results do not indicate a difference in activity of the two preparations since the effect of larger doses of toxohormone prepared by the Nakagawa method (158,159) was not determined. However, the method of Yunoki and Griffin (112) was found to be the more facile procedure and yielded about three times the amount of crude toxohormone as compared to the modified

Nakagawa method (158,159). Because of these advantages toxohormone prepared by the Yunoki and Griffin method (112) was used in these experiments.

It was evident, from the work reported herein, that the presence of Walker 256 tumor in a rat caused a striking increase in the incorporation of thymidine-H^3 into the DNA thymine of the host tissues, particularly in the case of liver and spleen. In view of these findings a study was made of the effect of toxohormone preparations from Walker 256 tumor on the incorporation of thymidine-H^3 into the DNA thymine of rat liver and spleen. An increase in the uptake of thymidine-H^3 into the DNA thymine of spleen was observed twelve hours after 40 mg of crude toxohormone was injected into groups of normal rats. No effect was found at six and twenty-four hours after the injection of crude toxohormone. Therefore, these results indicated that crude toxohormone had a maximal effect on the incorporation of thymidine-H^3 into the DNA thymine of spleen approximately twelve hours after injection. Others (107) have found a maximal depression of plasma iron levels ten hours after the injection of toxohormone. The results of the experiments reported herein are in agreement since the plasma iron levels, twelve hours after the injection of toxohormone, were on the average lower than those at six and twenty-four hours. Therefore, the maximal effect of toxohormone on both the DNA metabolism of spleen and the plasma iron level occurred within the same time interval after injection. This finding is of interest in view of the role of the spleen in the hematopoietic system.

The reason for the transient effect of crude toxohormone

on the DNA metabolism of spleen is not apparent. However, it is unlikely that a single dose of crude toxohormone would cause a permanent increase in the incorporation of thymidine-H^3 into the DNA thymine of spleen. It would be of interest to study the effect of repeated doses of toxohormone on the DNA metabolism of the spleen. In this regard, Kelly and Jones (28) observed that repeated injections of tumor homogenates caused an increased incorporation of P^{32} into the DNA of tissues of normal animals. On the other hand Kampschmidt et al. (107) reported that repeated injections of toxohormone failed to maintain the depression of liver and kidney catalase, and plasma iron.

In contrast to the results obtained for spleen, the injection of crude toxohormone was found to have no effect on the incorporation of thymidine-H^3 into the DNA of liver after an interval of twelve hours. Furthermore, no effect on liver DNA metabolism was observed six hours after the injection of toxohormone. Results obtained for liver twenty-four hours after the injection of the toxohormone were equivocal. The specific activities of the liver DNA thymine from two of the toxohormone-injected groups were higher than those of the sham-injected groups. However, the specific activity of the third group of toxohormone-injected rats was lower than the specific activities of the sham-injected groups. Therefore, it is difficult to determine, on the basis of these results whether crude toxohormone has an effect on the incorporation of thymidine-H^3 into the DNA of liver. The effect of toxohormone on the metabolism of liver DNA may be delayed, and therefore may require a longer interval than twenty-four hours to manifest itself.

The results reported herein indicated that the crude toxohormone had an effect on the DNA metabolism of spleen and might have an effect on the DNA metabolism of liver. In view of these results a study was made of the effect of a purified preparation of toxohormone on the DNA metabolism of liver and spleen. A crude toxohormone preparation was fractionated by ion-exchange chromatography on Amberlite XE-64 as described by Yunoki and Griffin (112). The elution pattern obtained in the experiments described herein which indicated five fractions did not correspond to that of Yunoki and Griffin (112) who obtained only three fractions. In later experiments Yunoki and Griffin (113) observed that one of their fractions (TH_2) could be resolved into three sub-fractions. The five fractions obtained in the present work possessed toxohormone activity at a dose level of 200 µg per rat, whereas when the dose was reduced to 20 µg per rat only fraction TH-5A was active. This fraction was considered to be the most active fraction. Compared to the crude toxohormone preparation this represents about a 1000-fold increase in activity. Yunoki and Griffin (112) found that their second fraction (TH_2) eluted from the Amberlite XE-64 column was the most active of the three fractions obtained, and represented a 10,000-fold increase in activity over the crude preparation. On the other hand the results of the present work indicated that the fifth fraction (TH-5A) was the most active toxohormone fraction. The discrepancy in these results could be explained on the basis of the difference in elution patterns. Yunoki and Griffin (112) reported that fraction (TH_2) was eluted at pH 9.5 and the final fraction around pH 9.6. In the present work the first three fractions were

eluted by the time the pH of the effluent had risen to 9.4. Fractions TH-4 and TH-5 were eluted when the pH of the effluent rapidly increased to values greater than 10. Therefore it is very likely that fractions TH-4 and TH-5 were not adequately resolved on the ion-exchange column due to the rapid increase in pH. These two fractions probably contain a number of molecular species one of which may correspond to the TH_2 fraction of Yunoki and Griffin (112).

The use of gel-filtration as a substitute for dialysis has been described in the present work. Gel-filtration was found to be a satisfactory method for the removal of chloride and glycine from the toxohormone fractions obtained from the Amberlite XE-64 column. Since preparations of toxohormone lose activity on dialysis (112), the technique of gel-filtration offers an alternate method for the removal of low molecular weight species from preparations of toxohormones. It was found that four of the fractions obtained from the Amberlite column were each resolved into two fractions by gel-filtration on Sephadex. In each case the first fraction eluted from the Sephadex column was free of chloride and glycine, and was designated the "A" fraction. Each of the second fractions ("B" fractions) was contaminated with chloride and glycine. The toxohormone activity of the "B" fractions was not determined. It is possible that the "B" fractions could contain polypeptides, with toxohormone activity, which could not be separated from the chloride and glycine by gel-filtration. It would therefore be of interest to fractionate each of the "B" fractions on columns of DEAE-Sephadex. Such columns have been reported (169) to be highly satisfactory for the fractionation of polypeptides.

The effect of fraction TH-5A on the DNA metabolism of liver and spleen has been studied in the present work and the results of these experiments indicated that this purified fraction of toxohormone had no effect on the incorporation of thymidine-H^3 into the DNA thymine of rat liver and spleen. In contrast, a crude preparation of toxohormone produced an increased incorporation of thymidine-H^3 into the spleen and possibly liver. It would appear therefore that a highly purified toxohormone preparation has no effect on DNA metabolism whereas the crude preparation does have an effect. Several explanations are possible for these findings.

The crude toxohormone preparation could contain a DNA-active factor which had little or no plasma iron-depressing activity, but was isolated from the tumor tissue by the process used for the extraction of toxohormone. This factor could have been eliminated at several stages during the purification of the crude material. Since only the soluble portion of the crude toxohormone was placed on the Amberlite XE-64 column, the DNA-active factor could have remained in the insoluble portion. Furthermore it is possible that the DNA-factor was tightly bound to the Amberlite XE-64 column and was not eluted by the conditions described. The possibility should also be considered that the DNA-active factor could be contained in fractions TH-1A to TH-4A or in the "B" fractions obtained from the Sephadex columns, none of which were studied.

As has been pointed out, the increased incorporation of thymidine into the DNA of spleen following the administration of crude toxohormone was not striking, and the results obtained for

liver were equivocal. If it is to be assumed that in an animal bearing a tumor the increase in thymidine incorporation into the DNA of the host tissues is the result of a humoral factor continuously produced by the tumor, then it is also valid to assume that the humoral factor would be continuously present in the tissues and fluids of the tumor-bearing animals. In order then to demonstrate a marked stimulation of thymidine incorporation into the DNA of the host tissues it may be necessary to administer repeated doses of the humoral factor in an attempt to simulate the conditions of the tumor-bearing animals.

It is possible that an increased incorporation of thymidine into the DNA of a tissue is a manifestation of an increased synthesis of DNA in this tissue, which in turn may be considered a stimulation of the growth of that tissue. A number of growth-promoting factors have been isolated from several tissues including tumors. It has been repeatedly shown (170-172) that implantation of fragments of organs from adult chicks or from older chicks embryos to the chorioallantoic membrane of chick embryos, stimulated the growth of homologous embryonic organs. It was suggested that organ-specific parts of the donor cell proteins stimulated the specific receptor organs either by serving as building blocks or as templates. However, Simonsen (173) has postulated that the enlargement of the embryonal organs was not the result of growth and multiplication of the cells, but rather due to invasion of the embryonal organs by the donor cells.

More conclusive evidence for the existence of a growth-promoting factor has been obtained from studies of regenerating liver. When a partial hepatectomy was performed on one partner

of parabiotic rats, the liver of the intact parabiont showed stimulation of mitosis (174-176). Furthermore the presence of regenerating liver caused an increase in the mitotic rate of the cornea and an increase in the epiphyseal width. Others (176,177) have shown a stimulation of mitosis in rat liver following the injection of serum from partially hepatectomized rats. Recently, Van Lancker and Sempoux (178) have studied the effect of regenerating liver on the incorporation of orotic acid-C^{14} into liver DNA. Orotic acid-C^{14} was injected into parabiotic pairs, one of which was partially hepatectomized. The specific activity of the liver DNA of the non-hepatectomized partner was shown to be six times greater than that of a non-parabiotic, non-hepatectomized control.

The work of Menkin (179) indicated that inflammatory exudates contained a factor which on repeated local injection induced hyperplastic changes in the breast tissue of non-pregnant rats. Menkin (180,181) has also shown similar effects in cartilaginous structures and skin of rabbits repeatedly injected with the growth-promoting factor of exudates. Furthermore, Nowell (182) found that the serum of rats with an acute infection produced a two- to three-fold increase in the mitotic rate of rat marrow cells in vitro. On the other hand, Stitch and Florian (177) have reported that the serum of adult rats inhibited liver cell mitosis.

Evidence for the production of a growth-promoting factor by tumor tissue has been reviewed by Paschkis (130). Heat-stable preparations from tumors have been found (120-122) to stimulate tumor growth and enhancement of tumor growth has also been found (123,124) following the injection of fresh or lyophilized tumor

homogenates. Haven et al (125) observed an increase in body weight when phospholipids prepared from tumor were fed to normal rats. The work of Kelly and Jones (28) showed that daily injections of tumor homogenates into mice caused an increased incorporation of p^{32} into the DNA of liver and spleen and Ito and coworkers (37-39) have isolated a mitosis-promoting substance from tumor tissue. The chemical nature of the growth-promoting factors isolated from malignant and non-malignant tissues has not been extensively studied. Although there was no evidence to indicate that the promotion of growth by these factors could be attributed to a single factor such as toxohormone, nevertheless the isolation procedures used to obtain the several growth-promoting factors did not preclude the presence of toxohormone in each of the preparations.

Cantarow et al. (183) have suggested that tumor tissue may produce growth hormone, and Haven et al. (125) have stated that the changes occurring in the normal rat following the administration of growth hormone are in many respects similar to those evoked by the growth of Walker 256 tumor. Growth hormone is a protein with a molecular weight of 45,000 (184), and has been shown (183) to stimulate the incorporation of uracil-2-C^{14} into the liver RNA of normal rats. The growth-promoting factor obtained by Menkin (185) from inflammatory exudates was reported to contain peptides and nucleotide structures, and Kutsky (186) has shown that a nucleoprotein fraction obtained from extracts of embryo possessed growth-promoting activity towards tissue cultures. On separating the nucleic acid and protein components it was observed (187) that the growth-promoting activity resided in the protein component.

Lieberman and Ove (188) have purified a protein growth factor from human and animal serum.

On the basis of the present available knowledge it is not possible to draw any conclusions regarding the structural similarity of toxohormone, growth hormone and the other growth-promoting factors that have been discussed. Nevertheless, it is interesting to note that toxohormone has certain characteristics which are similar to some of the other growth-promoting factors. Toxohormone, when originally isolated from tumor tissue by Nakahara and Fukuoka (45), was associated with nucleic acid. However, later studies showed that the activity of toxohormone resided in the non-nucleic acid component. This latter component is probably a polypeptide with a molecular weight of 4000 to 6000. The findings of Yunoki and Griffin (113) indicated that there was also a lipid component in toxohormone, although it was not known whether the lipid component was necessary for activity. In this regard Kutsky (187) has shown that the removal of lipid did not decrease the activity of a growth-promoting factor which he studied.

The site of action of the various growth-promoting factors is as yet unknown. Van Lancker and Sempoux (178) postulated that the most probable site of action of a humoral factor stimulating DNA synthesis would be the conversion of the ribotide into the deoxyribotide. These authors stated that stimulation at this step has the advantage of offering a uniform explanation of the stimulation of the incorporation of all the nucleotides into DNA when synthesis occurs. However the results of the experiments reported herein indicated that the stimulation of DNA synthesis could occur at one or more of the steps involved in the incorporation of thy-

midine into DNA. This finding does not substantiate the hypothesis of Van Lancker and Sempoux (178) and tends to indicate that the stimulation of DNA synthesis occurs after the formation of the deoxyribotides.

The studies of Furlong et al. (29) indicated that tumor tissues elaborated a factor which increased the incorporation in vitro of adenine into the DNA of normal tissue. The factor was reported to have the following properties:

> "1. It was completely or at least partially inactivated by heat.
>
> 2. It was inactivated or solubilized in solutions of low pH.
>
> 3. It was rapidly inactivated in tumor homogenates left at room temperature.
>
> 4. It was either inactivated or not precipitated by acetone."

More recent experiments by Furlong (31) and Furlong and Griffin (32) showed that the soluble fraction of a Novikoff hepatoma homogenate possessed DNA polymerase-like activity. The properties listed above by Furlong et al. (29) are compatible with an enzyme such as DNA polymerase. It is possible therefore, that the increased incorporation of thymidine into the DNA of the host tissues in tumor-bearing animals could be the result of an increased level of DNA polymerase in the host tissues. Such an increase in the DNA polymerase level could result from a continual secretion of the enzyme into the circulatory system at the site of the tumor. The production of excess amounts of DNA polymerase by tumor tissues is in accord with their rapid rate of growth. It is unlikely however that DNA polymerase would survive the con-

ditions used to isolate crude toxohormone. One could speculate therefore that tumor tissue may produce another factor which increases the incorporation of thymidine-H^3 into the DNA thymine of liver and spleen.

The research reported herein indicated that the presence of tumor in an animal causes an alteration in the DNA metabolism of certain of the host tissues. This alteration was possibly mediated through the action of a toxohormone-like fac tor produced by the tumor. In view of the important role of DNA in the cell (131), one could postulate that the other observed systemic effects of tumor occur as a result of the alteration of DNA metabolism in the host tissues. Studies of the mechanism by which the DNA metabolism of host tissues is altered are important in the field of tumor-host relations and such studies might indicate an approach to the therapy of cancer. In addition, studies of the factor(s) produced by tumors are potentially important in the field of cancer diagnosis. The detection of such a factor(s) in the body fluids might serve as a universal test for cancer.

SUMMARY

1. Liquid scintillation counting methods have been developed for the determination of radioactivity in C^{14}-labelled purines, pyrimidines and tritium-labelled thymine. Scintillator systems containing hyamine hydroxide and Polyether-611, or hyamine hydroxide, ethanol and toluene were found to be equally satisfactory for the counting of C^{14}-labelled purines and pyrimidines. The latter system yielded the highest counting efficiency for tritiated thymine.

2. A study has been made of the effect of Ehrlich tumor, Novikoff hepatoma, and Walker carcinosarcoma 256 tumor on the incorporation *in vivo* of formate-C^{14} and tritiated thymidine into the nucleic acids of the host tissues of animals bearing these tumors.

3. Neither the subcutaneous Ehrlich tumor nor the Ehrlich ascites tumor had an effect on the incorporation *in vivo* of formate-C^{14} into the nucleic acids of the host spleen, liver, kidney or lung. In contrast the presence of the Ehrlich ascites tumor caused an increased incorporation of tritiated thymidine into the DNA thymine of the host's liver, spleen and kidney.

4. Rats bearing the Novikoff hepatoma showed little or no increase in the uptake of tritiated thymidine by the DNA thymine of the host tissues.

SUMMARY - 2

5. The presence of the Walker 256 tumor in a rat caused an increased incorporation of thymidine-H^3 into the DNA thymine of the host tissues. This effect was particularly striking in the case of liver and spleen.

6. The effect of toxohormone on the DNA metabolism of rat liver and spleen has also been investigated. Crude toxohormone, isolated from Walker 256 tumor, produced a slight increase in the incorporation *in vivo* of the thymidine-H^3 into the DNA of rat spleen, but the results for liver were equivocal. In contrast a highly purified preparation of toxohormone, prepared by ion-exchange chromatography and gel filtration had no effect on the uptake of thymidine-H^3 by the DNA thymine of rat liver or spleen.

7. These results have been discussed in relation to the systemic effects of malignant disease.

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