EFFECT OF ERYTHROBLASTOSIS INFECTION ON RNA POLYMERASE ACTIVITY OF ERYTHROBLASTS FROM AVIAN BONE MARROW

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A THESIS

PRESENTED TO

THE FACULTY OF GRADUATE STUDIES AND RESEARCH

THE UNIVERSITY OF MANITOBA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

BY

ROBERT LLOYD STAINES

JANUARY 1968

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TO MY WIFE

ABBREVIATIONS

The following abbreviations are used in this thesis:

RNA	deoxyribonucleic acid
DNA	ribonucleic acid
RNase	ribonuclease
DNase	deoxyribonuclease
ATP	adenosine 5' - triphosphate
GTP	guanosine 5' - triphosphate
UTP	uridine 5' - triphosphate
CTP	cytidine 5' - triphosphate
A	adenine
G	guanine
U	uracil
C	cytosine
Pi	inorganic phosphate
PPi	inorganic pyrophosphate
Mn^{++}	manganese ions
Mg^{++}	magnesium ions
AmS	ammonium sulphate
Act.D.	actinomycin D

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INTRODUCTION

I Polynucleotide Synthesis

Polyribonucleotides may be synthesized by any one of four types of reactions (1):

(1) by the action of polynucleotide phosphorylase (polyase)

(2) by the action of DNA-dependent RNA polymerase

(3) by homopolymer synthesis from ribonucleoside triphosphates

(4) by the action of RNA-dependent RNA polymerase (RNA replicase).

1. Polynucleotide Phosphorylase

The first enzyme found to catalyze the synthesis of polynucleotides was isolated in 1955 by Ochoa, Ortiz, and Grunberg-Manago (2, 3, 4, 5). This enzyme, polynucleotide phosphorylase (polyase), was found to catalyze the synthesis of high molecular weight polyribonucleotides from nucleoside 5'-diphosphates in the presence of Mg^{++} with the elimination of inorganic orthophosphate. However, it later became apparent that polyase could not be the enzyme responsible for

RNA synthesis in the living cell since the base composition of the polynucleotide formed reflected substrate concentration rather than primer composition (2, 6, 7) suggesting that substrate concentration rather than the primary genetic material, DNA, determined the base composition of the product RNA. High substrate concentrations were required for RNA synthesis; the Km's for the nucleoside diphosphates were of the order of 0.02 M (8,9). In addition, the high Pi levels in living cells favour phosphorolysis rather than polymerization (9). It has been suggested that the physiological role of polyase is in the breakdown of messenger RNA (8, 9).

2. DNA-Dependent RNA Polymerase

The first evidence for the existence of a DNA-dependent RNA polymerase was provided by Weiss and Gladstone (10) in 1959, who studied the incorporation of cytidylate from CTP-α-P^{32}, into RNA, by a cell-free preparation of rat liver. This enzyme was soon found to be present in cell-free preparations from bacterial, plant, and mammalian cells by many workers including Stevens (11), Hurwitz, Bresler, and Diringer (12), Burdon and Smellie (13, 14), Ochoa, Burma, Kröger, and Weill (15), Huang, Maheshwari, and Bonner (16), and

Chamberlin and Berg (17). In each of these studies the product formed was acid-insoluble, non-dialyzable, and DNase-resistant but sensitive to RNase and alkaline hydrolysis. For maximal activity, the enzyme required the presence of all four of the nucleoside triphosphates, ATP, CTP, GTP, and UTP (10-17), which were incorporated internally into RNA with the release of pyrophosphate (18). That the incorporation was into the internal linkages of RNA and not just at the termini of pre-existing molecules of RNA was indicated by the finding that the label from ATP-α-P^{32} or from any one of the other three nucleoside triphosphates, similarly labeled, was recovered, after alkaline hydrolysis, in 3' (2') - AMP, 3' (2') UMP, 3' (2') - CMP and 3' (2') - GMP indicating that the labeled substrate was joined by phosphodiester linkages to each of the other nucleotide components of RNA (9, 10, 15, 19, 20). In addition, when labeled RNA prepared with ATP-8-C^{14} was subjected to alkaline hydrolysis and the resulting nucleotides separated by electrophoresis, eighty-five per cent of the label was recovered as 3'(2') - AMP indicating that AMP-C^{14} was incorporated into non-terminal positions since AMP-C^{14} incorporated into terminal positions would have been recovered mainly as labeled

adenosine (15). With partially purified enzyme preparations from each of the sources listed above the apparent Km for each ribonucleoside triphosphate was approximately 10^{-5} M (21).

The rate of RNA synthesis was maximal in the presence of a divalent cation such as magnesium (11, 17, 20, 22-24) or manganese (17, 20, 25) and a sulfhydryl compound such as mercaptoethanol (17, 20, 26). The reaction was found to be inhibited by PPi but not by Pi (1, 10).

In vitro studies indicated that DNA played an extremely important role in the reaction, for RNA synthesis depended on the presence of an intact native DNA molecule (25, 27-29) and was greatly inhibited by DNase (10-12). Furthermore, Hurwitz, Furth, Goldman, Anders, and Evans (29, 30), Stevens (28), and Weiss and Nakamoto (19, 31, 32) showed that ribonucleotides were incorporated into RNA by a base-pairing mechanism where the over-all base composition and base sequence of the RNA formed was dictated by the primer. Hurwitz et al (29, 30) utilized a number of DNA preparations including DNA from T2 bacteriophage, Micrococcus lysodeikticus, Bacillus subtilis, calf thymus, E.coli, and human bone marrow to obtain this correlation. The relationship

between the base sequence of the RNA product and that of the DNA template was derived from analyses of nearest neighbour base frequencies, (30, 32) of both the product RNA and the DNA template. In this analysis, RNA, synthesized in the presence of RNA polymerase, DNA, and the four nucleoside triphosphates, one of which was labeled with α-P^{32}, was degraded to 3' (2') - ribonucleoside monophosphates with alkali so that the P^{32} of the phosphodiester bond remained attached to the neighbouring nucleoside. The labeled monophosphates were separated and their radioactivities determined so as to determine the relative frequency with which the labeled nucleotide occurred next to a given neighbour in the RNA chain. The process was repeated with each of the other nucleotides similarly labeled and the nearest neighbour frequencies determined. These frequencies were then shown to correspond to the base frequencies of the DNA primer indicating that both strands of the DNA were transcribed in vitro. Hurwitz et al (29, 30) also found native DNA to be a more efficient primer than partially degraded DNA; similar results were obtained by Weiss with T2 DNA (27) and by Chamberlin and Berg with T6 DNA and salmon DNA (17).

More evidence that the RNA synthesized was a replica of the DNA template was provided by Hall and Spiegelman (33, 34) and by Geiduschek, Natamoto, Moohr, and Weiss (35, 36) utilizing a hybridization technique. In this technique, P^{32} - labeled NTP and RNA polymerase was mixed with its DNA primer, the mixture heated to 100° C for 10 minutes to cause separation of the two DNA strands, cooled slowly, and equilibrated at 40° for 12 hours. A hybrid between the RNA and long complementary sequences on the DNA formed during the annealing process. These studies showed that all of the strands of DNA primer are capable of hybrid formation with product RNA (35) and, further, that both strands of primer DNA function as a template *in vitro*. Chamberlin and Berg (17), using double stranded ØX174 DNA (synthesized with DNA polymerase and single-stranded ØX174 DNA), also showed that the base composition of product RNA was complementary to both strands of the DNA template being copied *in vitro*.

However, Hayashi, Hayashi, and Spiegelman (37) found that, with *E. coli* infected with ØX174 phage, hybridization tests and base composition studies revealed that only one strand of the double-stranded replicating form of the DNA was transcribed *in vivo*. Furthermore,

Tocchini-Valentini, Stodolsky, Aurisicchio, Sarnot, Graziosi, Weiss, and Geiduschek (38) using Bacillus megatherium infected with phage α found that the phage specific RNA, purified after a pulse of carrier-free P^{32} was administered during viral development, hybridized with only one strand of the viral DNA, yet RNA synthesized in vitro hybridized with both strands of the viral DNA. Later Hayashi et al, using partially purified RNA polymerase, found that with carefully prepared intact native double-stranded circular DNA of ϕX174 phage, only one strand of the DNA was copied in vitro (39), and that this same strand was copied in vivo (39); however, both strands were copied in vitro when the circles were disrupted (39). Geiduschek, Tocchini-Valentini, and Sarnot (40) then found that crude extracts of Bacillus megatherium utilized only one strand of carefully prepared α-phage DNA in its native form as template for RNA synthesis in vitro. These studies indicated that only one strand of DNA was copied when the DNA was in its native state as in the intact cell but that both strands were copied if the secondary structure of the DNA was disrupted as might occur in in vitro studies during the purification of template DNA. Presumably the latter treatment may

result in destruction of the sites which control initiation of transcription.

Two forms of RNA polymerase have been isolated from animal sources: one is the free enzyme or "soluble" RNA polymerase (41-44) which utilizes exogenous DNA as template for RNA synthesis in vitro and has the same characteristics as purified bacterial RNA polymerase; the other is the "aggregate" enzyme (16, 27, 45-57) originally described by Weiss (19), which is a deoxyribonucleoprotein complex. The"soluble" enzyme has been shown to be strongly inhibited by ammonium sulphate at high ionic strengths of 0.8 to 1.0 (41-44) but at least in the case of testicular RNA polymerase (58) and E. coli RNA polymerase (59) lower ionic strengths of 0.1 to 0.3 have been shown to stimulate enzyme activity. On the other hand, high ionic strengths of 0.8 to 1.0 have been shown to maximally stimulate "aggregate" enzyme activity (50, 60) probably by removal of histones. Ammonium sulphate (AmS) was shown to stimulate RNA synthesis by "aggregate" enzymes prepared in vitro by mixing either deoxyribonucleoprotein or purified DNA, histones, and purified bacterial RNA polymerase (60-63).

Although "soluble" RNA polymerase synthesizes RNA which resembles its DNA template in base composition and nearest neighbour base frequencies, the "aggregate" form synthesizes RNA having a high guanosine-cytosine (G-C) content which is characteristic of ribosomal RNA. However, in the presence of high ionic strengths of AmS the RNA synthesized is more DNA-like in its base composition (64) suggesting that AmS may convert the "aggregate" form of the enzyme to the "soluble" form. This possibility was strengthened by studies on DNA-RNA polymerase-histone complexes which synthesized ribosomal-like RNA except in the presence of AmS (60) or after pretreatment with trypsin (61) to remove histones and other soluble proteins when the RNA synthesized resembled the composition of the template DNA. Furthermore, addition of histones to a medium containing DNA and the "soluble" enzyme inhibited the synthesis of RNA greatly and the composition of the RNA synthesized was shifted toward a high G-C content (60, 64).

Thus "soluble" enzyme probably utilizes a euchromatin template for RNA synthesis, whereas, "aggregate" enzyme most likely utilizes a heterochromatin template in which the DNA is repressed, partially at least, by histones (65). The template may be de-repressed by hormones as

indicated in the work of Dahmus and Bonner (62) who found that "chromatin isolated from the liver of hydrocortisone-treated adrenalectomized rats possesses a greater template activity for DNA-dependent RNA synthesis than does chromatin isolated from the livers of rats not treated with hydrocortisone. This difference in template activity is abolished by the removal of proteins associated with the DNA".

Tata (66), on the other hand, believes that there is no good evidence that the metabolism of histones or the DNA-histone interaction is under hormonal control. In a review article (66) he has referred to several reports (66-68) which provided evidence that testosterone (67), estrogen (68), and growth hormone (66), do not affect the histone/DNA ratio although there is an increase in the template function of the chromatin and thus the rate of synthesis of RNA. Tata is considering then only the histone/DNA ratio but is not considering changes in the interaction of DNA and histone.

Addition of detergents such as sodium dodecylsulphate or sodium deoxycholate to the incubation medium has been found to stimulate "aggregate" enzyme activity <u>in vitro</u> but to a lesser extent than high ionic strength (19, 50, 69). Whether this stimulation is due to removal of

histones from the template DNA has not been shown, although Goldberg stated that detergents " may act to stimulate RNA formation by changing the physical state of the enzymic nucleoprotein complex to a form more suitable for reaction and/or by stabilization of this system or the product of the reaction by inhibition of degradative enzymes. "

Generally Mn^{++} has been found to be a more effective activator of "soluble" and bacterial RNA polymerase than Mg^{++} (17, 44, 58), whereas Mg^{++} is more effective in the stimulation of " aggregate " enzyme at low ionic strengths (19, 50). Studies of the " soluble " and " aggregate " forms of RNA polymerase activity of intact nuclei have not been as definitive. Widnell and Tata (70, 71) reported that the RNA polymerase of rat liver nuclei showed a preference for Mg^{++} at low ionic strengths but for Mn^{++} at high ionic strengths which would indicate a conversion of the " aggregate " form of the enzyme to the " soluble " form. However, MacGregor and Mahler (72) reported no preference for Mn^{++} over Mg^{++} in the activation of RNA polymerase of rat liver nuclei at high ionic strengths. These workers attributed this discrepancy in their studies and those of Widnell and Tata (70, 71) to the fact that the latter isolated

liver nuclei in a sucrose solution containing Mg^{++} so that Mg^{++} was already present in reaction medium used for the studies of Mn^{++} requirements.

Recently Maul and Hamilton (73) investigated the intranuclear localization of those RNA polymerase activities of isolated nuclei described by Widnell and Tata (70, 71) by the method of high resolution autoradiography. They found the Mg^{++}-activated RNA polymerase activity that synthesized ribosomal-like RNA (70, 71) in the absence of AmS to be located mainly in the nucleolus with only very little activity being apparent in the extranucleolar chromatin. This finding agreed with other evidence that ribosomal RNA was synthesized in the nucleolar area (61, 74-79) and indicated the presence of " aggregate-like " RNA polymerase in nucleoli. However, these workers found the Mn^{++} AmS-activated RNA polymerase that synthesizes DNA-like RNA (70, 71) was located primarily on the chromosomes in the extranucleolar area and that there was little of this activity in the nucleolar region. However, they were unable to explain why RNA polymerase in the nucleolar area was not activated by AmS as was the extranucleolar RNA polymerase but offered the suggestion that RNA polymerases differing in terms of activation requirements as well as

intranuclear positions may exist. Although Maul and Hamilton (73) noted that the long incubation times used in the presence or absence of AmS resulted either in disintegration or aggregation of the chromatin, and further that incubation in the presence of AmS produced a fragmented nucleolus they did not consider the effects of these changes in nuclear organization on the rates of RNA synthesis. Pogo, Littau, Allfrey, and Mirsky (78), on the other hand, did similar studies in more detail being careful to maintain native intactness of organization of the nuclei by careful isolation and short-term incubations of four to five minutes as compared to the fifteen to sixty minute incubations of Maul and Hamilton (73). In essential agreement with Maul and Hamilton (73), Pogo et al (78) found that in the presence of Mg^{++} and absence of AmS the RNA synthesized had a high G-C content characteristic of ribosomal RNA and autoradiographic studies revealed that this RNA synthesis occurred predominantly in the nucleolar area but with some extranucleolar synthesis occurring as well. At low salt concentrations (0.04 M) and in the presence of manganese RNA synthesis was enhanced and the base composition of the newly-synthesized RNA was changed to a higher A+U/G+C ratio, that is, more DNA-like in nature,

indicating that different genetic loci other than those associated with the synthesis of ribosomal RNA had been activated by manganese plus a low concentration of AmS. Autoradiographic studies revealed that this RNA synthesis had been initiated in areas of the chromatin which were previously inactive or less active, although concomitantly synthesis of RNA continued in the nucleolus but not at a stimulated rate. At low salt concentrations the nucleolus was still intact and the chromatin had not condensed. At high salt concentrations (0.4 M) with Mn^{++} RNA synthesis increased greatly and the RNA synthesized was DNA-like in base composition. Autoradiographic studies revealed that even greater activation of previously inactive areas of the chromatin had been achieved. It was not, until this high salt concentration (0.4 M) was used that the structure of the nucleus was disrupted. Although these studies are by no means definitive they indicate that the nucleolar area contains mainly " aggregate " RNA polymerase which is not stimulated by AmS and extranucleolar areas contain the two forms ("soluble" and "aggregate") of RNA polymerase both of which appear to be stimulated at different levels of AmS concentration.

Pogo et al (78, 79) have recently shown that the

RNA synthetic capacity of regenerating rat liver nuclei greatly exceeds that of normal liver nuclei, this change being largely due to an increase in the number of DNA sites available for transcription in the RNA polymerase reaction. In agreement with this they found that the RNA synthetic capacity of the regenerating system is much less stimulated (one third) by added AmS than is the normal nuclear system. Similar findings have been reported for rat liver nuclei stimulated by hormones (79, 80).

In 1966, Anthony, Zeszotek, and Goldthwait (81) using highly-purified " soluble " enzyme from E. coli provided good evidence that RNA synthesis occurs in three steps:

(1) ASSOCIATION: DNA + Enzyme $\rightleftharpoons$ DNA - enzyme

(2) INITIATION: DNA-enzyme + purine nucleotide $\longrightarrow$ (DNA-enzyme-purine nucleotide)

(3) POLYMERIZATION: (DNA-enzyme-purine nucleotide) + NTP $\longrightarrow$ DNA-enzyme-oligoribonucleotide + PPi

They showed that the first step could be inhibited by high ionic strength but that the DNA-enzyme-purine nucleotide complex formed in the initiation step was not easily dissociated by high ionic strength although there is a partial inhibition of RNA synthesis. This work

agreed with the earlier work of Chambon, Ramuz, and Doly (82).

Several workers have shown that RNA's are effective inhibitors of DNA-directed RNA synthesis (83 - 86) and that the inhibition may be competitive when RNA and DNA compete for the same site on the enzyme (83, 84, 87). However, the binding of DNA to RNA polymerase was found to be irreversible (85, 88, 89) rather than reversible as had been assumed earlier (83, 87), and therefore this inhibition could not be viewed as strictly competitive inhibition but rather as "pseudo-competitive" inhibition.

3. Homopolymer Synthesis

RNA polymerase has been found to catalyze the synthesis of homopolymers in vitro (17, 84, 90 - 92). Poly A formation occurs to a much higher extent than that of poly U or poly C, and poly G formation is barely detectable (91). Homopolymer formation occurs by the reiterative copying of a short sequence of a single base in single-stranded template DNA (92, 93). The homopolymer formed may exceed the length of the template sequence as much as 10 to 20 times (17, 92, 93). Homoplymer formation is inhibited by addition of low concentrations, less than 10^{-6} M, of the other ribonucleoside triphosphates (92). There is no known

function for homopolymer synthesis *in vivo* although it has been suggested that poly A may play some part in the control of protein synthesis (94, 95).

4. RNA-dependent RNA Polymerase

In addition to a DNA-dependent RNA polymerase, an RNA-dependent RNA polymerase which forms RNA complementary to an RNA template, has been described in extracts from bacterial and mammalian sources (83 - 85, 94, 96 - 100). However, the bacterial activities have been shown to be due to DNA-dependent RNA polymerase (83, 98, 99) since the RNA-dependent activity occurs only at very low concentrations of DNA and increasing concentrations of DNA inhibit transcription from the RNA templates (83, 85, 98). Furthermore, the presence of a second RNA primer has been shown to inhibit RNA-directed RNA synthesis (81). In the case of mammalian cells it is not quite clear whether a single enzyme is involved or whether there are two separate enzymes since Burdon, Smellie, and co-workers have claimed to have found a separate RNA-dependent RNA polymerase in both Krebs II ascites cells (97, 101) and Landschutz ascites carcinoma (102a). They claim that this enzyme has different pH optima and ionic requirements from that of DNA-dependent RNA polymerase. However, they may be studying the

" soluble " and " aggregate " forms of DNA-dependent polymerase.

Evidence has clearly shown that there exists a separate RNA-dependent RNA polymerase, or RNA replicase (102b) in mammalian (103 - 107), bacterial (102b, 108 - 112) and plant cells (113, 114) infected with RNA-containing viruses. This RNA replicase controls the replication of viral RNA which codes for the formation of viral coat proteins. The replication of the single-stranded parental viral RNA of bacterial MS2 phage (115, 116) and ME virus (117) has been shown to occur in two stages:

(1) Viral RNA that has entered the host cell combines with pre-existing ribosomes to form polysomes and then codes for the synthesis of RNA replicase and viral coat proteins. The RNA replicase utilizes the parental " plus " RNA strand as a template to synthesize a complementary " minus " strand, forming the double-stranded replicative form of RNA.

(2) The " minus " strand of the double-stranded replicative RNA becomes the template for further synthesis of " plus " strands which displace their counterparts from the template. A similar process occurs in plants infected with TMV virus (113, 114) and ascites cells infected

with encephalomyocarditis virus (118) and may be a process common to the production of all RNA viruses.

II Erythroblastosis

The oncogenic virus, erythroblastosis, is one of two principal forms of avian leukemias (the other form being myeloblastosis) and is characterized by the appearance of immature red blood cells, the erythroblasts, in the peripheral blood of infected chickens, by anemia, and by a reduced number of platelets in the blood stream (119).

Onset of the disease is marked by focal leukemic changes in some of the sinusoids of the bone marrow (120) resulting in rapid proliferation of erythroblasts (121). This is followed by the appearance in the peripheral blood of polychrome erythrocytes having abnormalities in their size, shape, and nuclear structure (120, 121) and then, finally, by the appearance of large erythroblasts which terminally may reach a concentration of 600,000 per mm^3. Death following injection of viral particles in filtered blood plasma normally occurs in 8 to 10 days (121, 122).

Erythroblast virus particles of the Engelbreth-Holm strain " R " (123) have been isolated (124 - 126) from filtered blood plasma and some of their properties described. The virus particles have been shown in electron microscope

studies to be spheroidal in shape and approximately 75 - 80 mμ in diameter (125 - 127). These particles contain a firm, relatively opaque, inner structure, 35 - 40 mμ in diameter, surrounded by a soft deformable lipid or phospholipid peripheral layer (125 - 128) which, common to avian tumor viruses, is sensitive to ether or chloroform (129). In percent dry weight, the virus particles contain approximately 2.5% ribonucleic acids, 35% lipids, 10% nitrogen, up to 1.3% phosphorus (probably due to phospholipid), but no DNA's (130).

Electron microscope studies have revealed that the viral particles are present in vacuoles in the cytoplasm of erythroblasts of infected chickens (125, 131) as well as in the plasma. In fact it has been suggested that the outerstructure of the virus particle originates from the host cell membrane and contiguous cell cytoplasm (125, 131, 132). Other evidence indicates that some of the virus-containing bodies are remnants of mitochondria which have undergone morphological changes after the invasion by infectious virus (125, 133). It has further been suggested that the virus may make use of the energy-producing metabolic processes of the mitochondria for its own reproduction leaving the structural fragments

behind but also inducing a stimulation of the cellular growth mechanism (130, 133, 134). Other evidence indicates that the mature virus particle may be expelled through the cell membrane into the plasma (125).

RNA has been isolated from the virus particles and its base ratios determined (135). These tentative studies indicated that the RNA is similar to that of the myeloblastosis virus (135) but has a higher guanylic acid content. Very high levels of RNA have been found in erythroblastotic chicken bone marrow in comparison with maximally regenerating bone marrow (130). In addition, increased nuclease activities and over-all increased rate of growth and cell division were found in erythroblastosis-infected avian bone marrow except in the final stages of the disease when nuclease activities and mitotic rates were lowered (130). When cellular DNA was destroyed with DNase, RNA synthesis was found to still occur in the cytoplasm of infected cells but not in the nucleus, indicating the presence of an RNA replicase in the cytoplasm only (136). This finding agreed with observations that there were no virus particles present in the nuclei of infected erythroblasts as determined by electron microscope studies (125, 131) and that most, if not all, of the active virus was localized

in the cytoplasm as determined by fractionation of the infected cells with subsequent titration of the virus activities in the fractions (130).

At 2° C, the pH range optimal for stability of the virus was shown to be in the region of pH 5 to 8.7, with the maximum at pH 7.0 (124). Virus infectivity remained constant for months at - 78° C, but inactivation progressed as a reaction of the first order at each increasing temperature, with the rate of inactivation being linear with the reciprocals of the absolute temperatures between 46° and 56° C (6).

The non-tumorigenic RNA viruses, mengovirus (137, 138) and poliovirus (138 - 142) have been shown to inhibit host cell protein synthesis and normal (DNA-dependent) cellular RNA synthesis but to allow viral RNA synthesis catalyzed by a newly synthesized RNA replicase and viral coat protein synthesis to continue. The mechanism of this inactivation of host cell RNA polymerase appears to be related to the synthesis of a repressor protein induced by the viral genome (138, 141, 142).

In this thesis studies of the effect of erythroblastosis infection on the activity of RNA polymerase of avian erythroblasts infected with erythroblastosis

are described. Erythroblast-rich fractions were obtained from erythroblastosis-infected and regenerating avian bone marrow after centrifugation through dextran. Nuclei were isolated after the homogenization of whole cells in the presence of saponin and optimal conditions were established for the RNA polymerase activities from the two types of bone marrow and the activities were compared. These studies reveal that erythroblastosis infection results in increases in both " soluble " and " aggregate " forms of RNA polymerase. The " aggregate " form appears to be converted by AmS not to a completely de-repressed enzyme ("soluble "),but,rather to a partially de-repressed form. Such a partially de-repressed " aggregate " enzyme was found to be predominant in erythroblast nuclei isolated from regenerating bone marrow and was not susceptible to stimulation by AmS. Unlike mengovirus (137, 138) or poliovirus (138 - 142) infection, erythroblast infection does not bring about an inhibition of host cell RNA polymerase activity.

MATERIALS AND METHODS

Materials

ATP - H^3 and ATP - 8 - C^{14} were purchased from Schwarz Bioresearch. Unlabeled nucleoside triphosphates were purchased from the Sigma Chemical Co. Highly polymerized calf thymus DNA, DNase I (RNase free), and RNase I - A were obtained from Mann Research Laboratories. Actinomycin D was a gift from Merck, Sharp, and Dohme Co. Phosphoenol pyruvic acid and pyruvate kinase were obtained from Calbiochem. Dextran T - 80 was purchased from Pharmacia of Canada. BBOT (2,5 - bis - (2 - (5 butylbenzoxazolyl) - thiophene was purchased from either the Packard Instrument Co. or from Nuclear Enterprises (Winnipeg) Ltd. Millipore filters, white, plain, 25 mm of 0.45 μ pore size were obtained from the Millipore Co. Filtered chicken serum was obtained from Colorado Serum Co.

Isolation of Nuclei

Regenerating bone marrow was obtained from white Leghorn chickens (line 15, East Lansing) which had been treated with phenylhydrazine as described by Bristow and Yamada (143).

Erythroblastosis-infected bone marrow was obtained from white Leghorn chickens (line 15, East Lansing) that had been injected intravenously with a 20 % homogenate (w/v) of chicken liver infected with erythroblastosis strain " R " (obtained from Prof. B. Thorell, Stockholm) suspended in 0.9% saline at a dose of 1 ml. per kg. body weight. The course of the disease in the infected chicken was determined by examination of blood smears from the wing; the presence of erythroblasts in the peripheral blood denoted a diseased chicken. Normally eight to nine days lapsed between injection of the viral liver preparation and sacrifice of a viral infected chicken just before morbidity.

The chickens were sacrificed by decapitation and the leg bones were removed and were immediately placed in crushed ice. In a cold room, at 2 - 4° C., the ends of the leg bones were cut off and the bone marrow was blown into a preweighed beaker by applying air pressure at one end of the bone. The bone marrow was suspended in four volumes of calcium-free Hank's balanced salt solution plus ten percent chicken serum and was dispersed by forcing the suspension through a syringe, then through a stainless wire gauze (no. 60 mesh), and was finally filtered through four layers of cotton gauze.

Four ml of this suspension was layered on top of each of several tubes containing 25 ml of 5 % (w/w) dextran T - 80 in Hank's. Originally discontinuous gradients containing 10 ml of 17.5 % (w/w) dextran, 10 ml of 15 % (w/w) dextran, and 20 ml of 5 % (w/w) dextran in Hank's were used as described by M. Leroux (144). Leroux layered 4 ml of the suspension on top of discontinuous gradients which were then spun in a swing-out bucket rotor (Sorvall HB4) at 750 r.p.m. (120 x g) for five minutes, and then for ten minutes at 3000 r.p.m. (1465 x g) to obtain three layers of cells. The top layer contained cell debris, erythrocytes, few erythoblasts, myeloblasts and white blood cells and was discarded; the middle and bottom layers containing mainly erythroblasts and red blood cells were pooled. No cells were seen to penetrate the 17.5 % dextran layer. However, since the middle layer of cells was located just below the interface of the 5% and 15% dextran layers and the bottom layer at the interface of the 15% and 17.5% dextran layers, and since these two layers were pooled anyway, it became apparent that centrifugation of cell suspension through 5% dextran in Hank's would achieve the same separation as that of the discontinuous gradients described by Leroux. Therefore, in most of the

studies described here, the cell suspensions were centrifuged through 5 % dextran in Hank's in an HB4 Sorvall rotor at 750 r.p.m. (120 x g) for five minutes and 3000 r.p.m. (1465 x g) for ten minutes. The pellet obtained was washed three times with Hank's and during the last wash, before final centrifugation at 1500 x g for ten minutes, a sample of the suspension was taken for differential counts of the cells. The final pellet was suspended in 1 % saponin in a 0.01 M Tris buffer, pH 7.4 containing 0.0015 M $MgCl_2$, 0.01 M KCl and 0.005 M β-mercaptoethanol (this buffer solution, minus the saponin, was called TKM solution), placed in a glass homogenizing vessel of the Potter-Elvehjem type fitted with a loose-fitting feflon pestle, and subjected to eighteen strokes of the pestle. The presence of 1 % saponin greatly aided in breaking the cell membranes and very clean nuclei free of cytoplasmic tags as seen under the light microscope were obtained. There was no loss of enzyme activity during saponin treatment since the nuclei were fully as active as those obtained when saponin was omitted from the homogenizing solution, albeit the latter nuclei were much less pure. The homogenate was then diluted with three to four volumes of TKM solution to dilute the saponin and was spun at

900 x g for eight minutes. The nuclear pellet was washed twice with TKM solution and finally suspended in 0.05 M Tris buffer, pH 7.0 containing 0.005 M β- mercaptoethanol. This nuclear fraction was used as the enzyme source in all assays. The nuclei, as seen under the light microscope, appeared to be undistorted and free of cytoplasmic tags. The nuclei were stored frozen at - 20° C until use with no loss of activity and no change in appearance of the nuclei even after twelve days.

In the studies reported in this thesis the nuclei isolated from the bone marrow of erythroblastosis-infected chickens will be referred to as " viral " nuclei; nuclei from regenerating bone marrow will be referred to as " regen " nuclei.

Protein Analysis

Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (145).

Assay of RNA Polymerase Activity

Enzyme activity was assayed by determining the rate of incorporation of AMP-H^3 or AMP - 8 - C^{14} into acid-insoluble RNA. Unless otherwise indicated the reaction mixture contained the following in a final volume of

0.25 ml: Tris buffer, pH 9.0, 25 µmoles; magnesium acetate, 1.75 µmoles; phosphoenol pyruvate, 2.5 µmoles; β-mercaptoethanol, 1.25 µmoles; DNA, 25 µg; nuclear fraction, 0.75 - 1.0 mg of protein, pyruvate kinase, 2.5 µg; UTP, GTP, CTP, 30 mµmoles of each; ATP - 8 - C^{14} (2.22×10^5 dpm, 70 % efficiency), 25 mµmoles or ATP - H^3 (1.10×10^6 dpm, 17 % efficiency), 14.3 mµmoles. The final pH of the incubation medium was 8.6 and the temperature 37°. The reaction was started by the addition of the nucleoside triphosphates and was terminated by the addition of 1 ml of 10 % trichloroacetic acid and 1 mg of carrier albumin. The precipitate was collected on Millipore filters (0.45 µ pore size) and washed seven times with 5 % trichloroacetic acid containing 0.02 M tetrasodium pyrophosphate. The filters were dried at 90 - 100° for ten minutes in a drying oven and placed in glass scintillator vials. Fifteen ml of BBOT scintillation consisting of 4 g BBOT, 80 g naphthalene, 600 ml xylene, and 400 ml methylcellosolve, and freed of dissolved oxygen by bubbling with nitrogen, was added to each vial and the radioactivity measured in a Packard Tri-carb liquid scintillation spectrometer which counts C^{14} disintegrations with 70 % efficiency and tritium with 17 % efficiency in the systems used in these studies.

All tests were performed in duplicate along with zero time controls in which trichloroacetic acid was added immediately after the addition of radioactive ATP. The counts of radioactivity taken from the Tri-carb were converted to μμmoles AMP - C^{14} incorporated using the factor 1 μμmole AMP - C^{14} incorporated equals 6.2160 counts per minute. For the few tests in which tritiated ATP was used the counts taken from the Tri-carb were converted to μμmoles AMP - H^3 incorporated using the factor 1 μμmole AMP - H^3 incorporated equals 48.335 counts per minute.

RESULTS

Activity and Enzyme Concentration

The relationship between the activity of intact nuclei and protein concentration is shown in Figure 1. Earlier studies from this laboratory (144) indicated that linear rates of RNA synthesis in intact nuclei obtained up to approximately 5 min of incubation time. The rate then fell off and reached maximal values after 15 min. These incubation times were used in the present studies. It is apparent from Figure 1 that there is a linear relationship between activity of " viral " nuclei and protein concentration up to 1.5 mg and 1.2 mg of protein for incubation periods of 5 and 15 min, respectively, in the absence of AmS. In the presence of AmS a linear relationship obtained only up to 1.0 mg of protein for both periods of incubation.

That the activity of " regen " nuclei is proportional to protein concentration up to 1.8 mg and 1.1 mg of protein for incubation periods of 5 and 15 min, respectively, in the absence of AmS, is shown in Figure 2. As was the case for " viral " nuclei a linear relationship obtained at lower protein concentrations in the presence

of AmS than in its absence. These results with " regen " nuclei agree with those reported by Leroux (144).

These results could not be compared to those of workers other than Leroux (144) since a search of the literature did not reveal similar studies. However, Weiss (19), in studies of isolated rat liver nuclei, used 6 mg of nuclear protein per ml of incubation medium for a 15 min incubation period in the absence of AmS, which corresponds to the protein concentration at the limit of linearity in the studies described in Figures 1 and 2.

pH Optimum

In these studies the pH values were determined in reaction mixtures which contained the constuents of the standard assay but in which nuclei were replaced by the buffer in which they were suspended routinely. These reaction mixtures were incubated for the same length of time as the test solutions and the pH readings were taken immediately after the incubation period.

Figure 3 shows that the activity of " viral " nuclei in the absence of AmS is maximal at pH values of 8.6 - 9.0 when incubation was continued for 5 min (5-min activity); in the presence of AmS, after incubation for 15 min (15-min activity), it approaches maximal values

at slightly lower pH's (8.4 - 8.6). Thus, although there is a slight shift of pH optimum in the presence of AmS, it is much less than the change in pH optimum of the 15-min activity of rat liver nuclei from pH values of 8.0-9.5, in the absence of AmS (70, 71, 72, 146), to 7.5 in the presence of AmS reported by Widnell and Tata (70, 71).

These workers pointed out the similarity in pH optimum of the activity of rat liver nuclei in the presence of AmS to that of " soluble " RNA polymerase isolated from bacterial and mammalian sources (17, 42 - 44, 58, 84). The latter pH's range from 7.2 for preparations from rat liver (43), 7.7 for rat testes (42) to 7.8 - 8.2 for preparations from E. coli (17). On the other hand, the " aggregate " form of RNA polymerase isolated from rat liver (10) and calf thymus nuclei (51) had maximal activity in the pH range of 8.0 - 9.5 at low ionic strength; no pH optimum studies have been reported for isolated " aggregate " enzyme activity at high ionic strength.

The pH optimum of the 5-min activity of " viral " nuclei in the absence of AmS is more similar to that of isolated " aggregate " enzyme preparations than to that of isolated " soluble " enzyme preparations. The same is true for the 15-min activity in the presence of AmS. However, our studies were done with intact nuclei and not isolated enzyme preparations and so do not preclude the presence of both forms of the enzyme.

Figure 4 shows that the 5-min activity of " regen " nuclei is maximal over a pH range of 7.8 - 8.6 and no shift in the pH optimum was observed with AmS for the

15-min activity. Since these pH optima are distinctively characteristic of neither the " aggregate " or " soluble " forms they may indicate that both forms are present.

Substrates and Inhibitors

Figure 5 reveals that addition of highly polymerized calf thymus DNA had little effect on the 5 or 15-min activities of " regen " nuclei. Similarly DNA stimulated the activity of " viral " nuclei very little. Although the nuclei contain much endogenous DNA (DNA analysis of one " viral " nuclear preparation showed that it contained approximately 0.26 mg DNA/mg protein), 25 µg of highly polymerized calf thymus DNA was added to the reaction mixture of all subsequent assays in case the endogenous DNA was bound in " aggregate " enzyme and was not available as a template for " soluble " enzyme that might be present.

That the incorporation of AMP-C^{14} was DNA-dependent was shown by the effect of DNase and actinomycin D (Table I). The incorporation of AMP-C^{14} into RNA was inhibited up to 71.6 % in the presence of DNase and up to 91.5 % in the presence of actinomycin D. Pogo, Alfrey, and Mirsky (79) required higher concentrations of these inhibitors to show that the RNA polymerase activity of rat liver nuclei was DNA dependent. When RNase was

included in the incubation mixture (Table I) there was a drastic reduction in the incorporation of AMP-C^{14} into acid-insoluble product indicating that the acid-insoluble product formed with both " viral " and " regen " preparations was indeed RNA. Furthermore, the amount of RNase used was 1/10 that used by Pogo et al (79) to achieve the same level of inhibition in rat liver nuclei.

Figure 6 shows that at a fixed concentration of ATP-C^{14}, increasing concentrations of the other nucleoside triphosphates in equimolar proportions stimulated the activity of both " viral " and " regen " nuclear preparations. Stimulation was greatest for both the 5 and 15-min activities for both preparations when 30 mμ moles of each of the NTP's was added to the reaction mixture.

Divalent Cations

Generally Mg^{++} has been found to be the better activator of the " aggregate " form of RNA polymerase at low ionic strength (19), whereas, Mn^{++} is the better activator of the " soluble " enzyme (17, 43, 44, 58). Widnell and Tata (70, 71) found a change in the divalent cation requirements of the 15-min RNA polymerase activity of isolated nuclei when AmS was added to the reaction

mixture. In the absence of AmS, Mg^{++} was the better activator, whereas, in the presence of AmS, Mn^{++} was the better activator; this change was accompanied by a shift in the pH optimum from 8.5 to 7.5, suggesting that AmS may have caused some conversion of the " aggregate " to the " soluble " form. However, these findings were disputed by MacGregor and Mahler (72) who showed no difference in Mg^{++} or Mn^{++} activation of rat liver nuclei, in the presence of high AmS concentrations, when nuclei were suspended in a Mg^{++}-free medium such as that used in our studies.

Both Mg^{++} and Mn^{++} were found to stimulate RNA polymerase activity in " viral " nuclear preparations (Figures 7 and 8). For the 5-min activity in the absence of AmS (Figure 7) Mn^{++} proved to be the better activator, suggesting a predominance of the " soluble " enzyme, which was not apparent from the observed pH optimum (8.6). However, for the 15-min activity in the absence of AmS, Mg^{++} proved to be the better activator which may indicate that the " aggregate " enzyme activity predominates after longer incubation periods.

With " viral " nuclei in the presence of AmS, Mg^{++} proved to be the better activator of the 5-min activity

(Figure 9) which would suggest that the " soluble " activity observed without AmS has been inhibited and also that the activity of some " aggregate " enzyme is measurable after short periods of incubation. For the 15-min activity in the presence of AmS (Figure 10) Mg^{++} and Mn^{++} activated to approximately the same extent. These results are in agreement with the findings of MacGregor and Mahler (72) for the AmS-stimulated activity of rat liver nuclei. The relative rise in Mn^{++} activation may indicate that AmS has brought about a partial conversion of the " aggregate " to the " soluble " form which is accompanied by a slight shift in the pH optimum to lower pH's as noted earlier. This change in pH optimum and divalent cation requirements in the presence of 0.35 M AmS is much less than that observed by Widnell and Tata (70, 71) with rat liver nuclei and would seem to indicate that the conversion of " aggregate " enzyme to the " soluble " form is not complete in " viral " nuclei.

Both Mg^{++} and Mn^{++} were also found to stimulate the RNA polymerase activity of " regen " nuclei, although Mg^{++} proved to be the better activator of both the 5 and 15-min activities in the absence (Figures 11 and 12) or presence (Figures 13 and 14) of AmS. This would suggest

that the " aggregate " enzyme activity is predominant and that AmS has brought about little conversion of the " aggregate " to the " soluble " form which is in agreement with the finding that AmS does not cause a shift in the pH optimum. These results suggest that " regen " nuclei contain an " aggregate - like " enzyme that is similar to that found in rat liver nucleoli which synthesizes a G-C type of RNA and is not stimulated by AmS (78, 79).

Ionic Strength and Polyamines

Figure 15 shows that over a 2-fold stimulation of the 15-min RNA polymerase activity of " viral " nuclei was obtained with 0.35 M AmS (ionic strength approximately 0.7) which is similar to the stimulation observed with rat liver nuclei (70, 71) and indicates the presence of " aggregate " enzyme (50). There is also a slight stimulation of the 5-min activity with AmS.

Increasing concentrations of AmS inhibit the 5-min RNA polymerase activity of " regen " nuclei (Figure 16). The 15-min activity is inhibited at low AmS concentrations but with higher concentrations the activity reaches the level found when AmS is omitted from the reaction mixture.

KCl concentrations up to 0.6 M were found to have little effect on the RNA polymerase activity of either " viral " or " regen " nuclei (Figure 17). At optimal concentrations of AmS (0.35 M) increasing KCl concentrations only inhibited the 15-min activity of " viral " nuclei (Figure 18). KCl has been shown to have some effect on isolated systems (50, 59) but none have been found so far for nuclei.

Figure 19 shows that spermine had little effect on the RNA polymerase activity of either " viral " or " regen " nuclei; neither did increasing concentrations of spermidine significantly alter the activity (Figure 20). No further stimulation of the activity of " viral " nuclei was brought about by increasing concentrations of spermine in the presence of 0.35 M AmS (Figure 21). Although spermine has been shown to stimulate the activities of isolated " soluble " enzyme systems (84), there are no reports of stimulation on whole nuclei or " aggregate " enzyme.

Time Relationships

Figure 22 illustrates the time course of the reaction with " viral " nuclei in the presence or absence of AmS. In the absence of AmS the reaction was linear for the first 3 min (in some preparations linearity was obtained up

to 5 min), reached a maximum at 25 min, and then declined slightly. These results are comparable with those obtained by many workers using rat liver nuclei (70-72, 79, 146) and isolated " aggregate " enzyme (19) at low ionic strengths. In the presence of AmS the reaction was again linear for the first 3 min (in some preparations linearity was obtained up to 5 min), activity then declined slightly and a new linear rate was obtained from 3 to 15 min, with a maximum being reached at 25 min, after which the activity declined very slightly. Goldberg (50) found the activity of " aggregate " enzyme, isolated from HeLa cells, in the presence of high AmS concentrations to be linear with time for 20 - 30 min; it fell off gradually during the next 50 - 60 min. Widnell and Tata (70, 71), on the other hand, using rat liver nuclei in the presence of high AmS concentrations, found the linear rates to obtain for 15 min after the start of incubation and continue up to 60 min. Unfortunately, they did not investigate the possibility that linear rates may exist during the first 5 or 10 min of the reaction as the shortest incubation time used in their studies was 5 min. Pogo et al (79) found the activity of rat liver nuclei to be linear with time during the first

8 min of incubation at low ionic strengths. Figure 22 also shows the marked activation of RNA polymerase activity in " viral " nuclei by AmS especially at the longer incubation times.

Figure 23 shows the relationship between the activity of " regen " nuclei and time both in the presence and absence of AmS. In both cases the activity was proportional to time for at least the first 3 min of incubation and reached maximum levels between 15 and 25 min. In some preparations, incubated in the presence of AmS, further increases in activity after 25 min of incubation were noted. However, these increases were small and were found to be insignificant (see "Comparison" section of Results). The activities of " regen " nuclei in the presence or absence of AmS are thus very similar.

Shorter incubation times were studied and the results are given in Figure 24. That linear rates obtained for at least 3 min for both " viral " and " regen " nuclei in the presence or absence of AmS is apparent from this figure. The stimulation of " viral " nuclei by AmS is apparent even at these short incubation times, as is the inhibition of " regen " nuclei by AmS.

Comparison of " Viral " and " Regen " Activities

The activities of " viral " and " regen " nuclei were

compared. In this comparison 12 samples were used. For each sample the bone marrow of 3 or more chickens was pooled. A layer rich in erythroblasts was obtained by centrifugation in 5 % dextran. The cell components of each of the erythroblast-rich fractions from which nuclei were isolated are given in Table II. It is apparent from this table that the erythrocytes contribute the major contaminant of the erythroblast suspensions. However, an additional sample (sample 13) containing only erythrocytes was prepared, and, in agreement with others (147, 148), negligible " soluble " or " aggregate " activity was found to be present in this sample.

Table II also shows that the percentage of erythroblasts varies from preparation to preparation. This is particularly evident in the " viral " samples and is due to the fact that the bone marrow of several chickens had to be pooled in order to obtain sufficient material for enzyme assays and these chickens showed slight variations in rate of susceptibility to the virus and in rate of regeneration of bone marrow after the injection of phenylhydrazine. As is shown later these variations did not interfere with the assays of enzyme activity.

Subsequently activity-time curves were studied for

nuclei from each of the 12 preparations described in Table II. For each, the activity-time curves were similar to those described in Figures 22 and 23. Since initial rates obtained up to 3 min of incubation time for all the preparations tested, these initial rates were considered to be valid as one basis of comparison between the " viral " and " regen " samples. The total incorporation after 25 min of incubation was considered to be valid as a second basis of comparison since at this time, in all samples tested, the incorporation of labeled precursor into RNA had reached maximal levels. The results are summarized in Tables III and IV. Regression lines were calculated from these data by the " method of least squares." Sample 13, a suspension of nuclei of erythrocytes which contained no erythroblasts and no activity provided control values. Correlation coefficients (r) were also calculated. High degrees of correlation between initial rates and percent erythroblasts both in the presence and absence of AmS and between total activity (after 25 min) and percent erythroblasts in the presence or absence of AmS were found (P values, 0.01 to 0.001).

Plots of the regression lines for the initial rates and percent erythroblasts are given in Figures 25 and 26.

It is apparent from Figure 25 that " viral " nuclei contain significantly more 3-min RNA polymerase activity than do " regen " nuclei (2 times). The addition of 0.35 M AmS to the reaction mixture resulted in a slight stimulation of the activity of the " viral " nuclei but a slight inhibition of the activity of " regen " nuclei. Although neither of these AmS effects were statistically significant this may be attributable to the small number of samples studied.

Figure 26 shows the regression lines when total activity is plotted against percent erythroblasts. The total activity of " viral " nuclei is about 2-fold greater than that of " regen " nuclei in the presence of AmS. The presence of AmS did not cause any significant stimulation of the activity of " regen " nuclei.

The fact that there is a linear relationship between maximum incorporation (15 to 25 min) and enzyme concentration indicates the maximum incorporation is a measure of enzyme concentration. Presumably this is because there is the inhibition of enzyme activity by product RNA (151) and by nucleases (14) which is proportional to enzyme concentration.

DISCUSSION AND CONCLUSIONS

Our results indicate that " viral " nuclei contain both " soluble " and " aggregate " forms of RNA polymerase. The " soluble " enzyme activity is apparent after short incubation periods (3 - 5 min) since this activity, like that of the " soluble " enzyme preparations shows a preference for Mn^{++} over Mg^{++} (11, 12, 15, 24, 25, 31, 43, 58) and is not stimulated by high concentrations of AmS (41 - 44). It differs from the isolated soluble enzyme in its pH optimum (17, 43, 58) and lack of stimulation by 0.1 - 0.3 M KCl (58, 59) and spermine (85, 149), but the latter effect has not been found for intact nuclei but only for the isolated systems. AmS of 0.08 ionic strength has been reported to stimulate Mn^{++}-activated RNA polymerase of chromatin of rat liver (78, 79) which may indicate that the enzyme is loosely bound with protein which is removed even at low concentrations of AmS. Such an effect was not apparent in " viral " nuclei.

The " aggregate " enzyme activity of " viral " nuclei

is apparent after longer incubation periods and it resembles that of isolated " aggregate " enzyme preparations in that it shows a preferential activation by Mg^{++} in the absence of AmS (19), a pH optimum characteristic of the " aggregate " form (10, 51), and most importantly a two-fold stimulation of activity by high concentrations of AmS (50). Our results indicate that the " aggregate " enzyme is not completely converted to the " soluble " form by AmS since there is only a slight shift in the pH optimum from 8.6 to 8.4 and Mg^{++} activates to the same extent as Mn^{++} in the presence of the salt. In comparison, Widnell and Tata (70, 71) reported that the " aggregate " enzyme of rat liver nuclei, exhibits a shift in pH optimum from 8.5 to 7.5 and a preferential activation by Mn^{++} at high AmS concentrations indicating a conversion by AmS of a large amount of " aggregate " to the " soluble " form. MacGregor's and Mahler's findings (72), however, are more in keeping with those reported here.

Our results indicate that " regen " nuclei contain RNA polymerase in a form that is not distinctly characteristic of either the " soluble " or the " aggregate " form. The pH optimum of 7.8 - 8.6 is intermediate between that of isolated " soluble " preparations (17, 43, 58) and that of isolated

" aggregate " preparations (100) at low ionic strengths. The preferential activation by Mg^{++} under all conditions of ionic strength and the lack of stimulation by high AmS concentrations would suggest that this enzyme differs from the " aggregate " enzyme of rat liver nuclei (70, 71) and " viral " nuclei. It is known that the response of " aggregate " enzyme to high salt concentrations is minimal when the genome has already been activated by hormones (62, 66) in vivo or by partial hepatectomy (78, 79). Similarly, Ramuz, Mandel, and Chambon (150) observed that the " aggregate " enzyme from regenerating liver was less stimulated by added salts than was the corresponding preparation from normal liver. This decrease in response was verified for the isolated liver nucleus by Bogo et al (78, 79). However, in the latter preparations in the presence of high AmS concentrations the presence of Mn^{++} was reported to be obligatory for obtaining maximal enzyme activity. Moreover, it is reported that only in the presence of Mn^{++} and AmS is there a shift in base composition of product RNA from the high G-C type to the high A-U type. Nevertheless, no clear cut evidence has been presented in any of the above-cited papers that there is a preference for Mn^{++} over Mg^{++} in the presence

of AmS, nor has the base composition of RNA in the presence of Mg^{++} alone in genome-activated systems been reported. However, Liau et al (61) found the base composition of RNA synthesized in the presence of Mg^{++} by nucleoli treated with trypsin to remove nucleolar proteins (mainly histones) changed from that of high G+C to low G+C. In addition MacGregor and Mahler (72) showed that at high AmS concentrations at pH 8.5 the " aggregate " enzyme activity of isolated rat liver nuclei was not noticeably more activated by Mn^{++} than Mg^{++}.

Admittedly, the base composition of the product RNA synthesized by RNA polymerase of " regen " or " viral " nuclei was not studied in the work reported in this thesis. This does not preclude the tentative conclusion that " aggregate " enzyme may exist in nuclei either in a completely repressed form or in a partially de-repressed form. Complete removal of repressors would result in the conversion of " aggregate " to the " soluble " form. However, such complete removal is not always apparent, but, rather partial removal resulting in a " partially-de-repressed aggregate " can occur. Such a partially de-repressed " aggregate " which is no longer susceptible to stimulation by AmS is present in " regen " nuclei.

It is formed in " viral " nuclei by the action of AmS on the " aggregate " enzyme.

What then is the effect of virus infection on the synthesis of RNA by erythroblast nuclei? The first notable difference between " viral " and " regen " nuclei is in the forms of RNA polymerase present: " regen " nuclei appear to contain mainly a partially de-repressed " aggregate " form, which is little further de-repressed by AmS, and possibly some " soluble " enzyme; " viral " nuclei contain much " soluble " enzyme and an " aggregate " form, which is missing in " regen " nuclei, which can be converted by AmS to a form resembling the partially de-repressed form of the " regen " nuclei. The presence of the repressed " aggregate " in " viral " nuclei raises the question as to whether this repression is due to the virogenic nature of the erythroblastosis virus. Unfortunately, Baltimore and Franklin (137, 140) did not investigate the types of RNA polymerase present in L cell or <u>HeLa</u> cell nuclei when they reported repression of RNA polymerase of host cells after infection by a non-tumorigenic virus such as poliovirus or mengovirus and so conclusions are not possible at this time.

The higher initial rates of the activity of " viral "

nuclei (2 x " regen ") in the absence of AmS is probably a reflection of the presence of the more active " soluble " form of the enzyme which is in low concentrations or lacking in " regen " nuclei. Moreover, the higher total activity (25-min activity) of " viral " nuclei in the presence of AmS indicates that erythroblastosis infection also results in an increase in the " aggregate " form of the enzyme. Since there is an increase in the " soluble " form of the enzyme and also an increase in the amount of de-repressible " aggregate ", as revealed by AmS de-repression, this would seem to be a reflection of the oncogenic (tumor-producing) nature of the virus. In agreement with this view is the finding by Brody and Thorell (121) that the mitotic index of erythroblasts of infected bone marrow is greater than that of erythroblasts of regenerating bone marrow. Of pertinence to our studies is the report of Furth and Ho (44) that " soluble " RNA polymerase activity is greater in lymphosarcoma cells than in cells of normal lymph nodes. In this case, however, the higher activity of " soluble " RNA polymerase is probably a reflection, at least in part, of the composition of the two tissues. The lymphosarcoma contains a relatively homogeneous population of proliferating immature cells, whereas, the normal lymph nodes contain a

heterogeneous population of large and small lymphocytes as well as a greater amount of stromal elements. It is apparent, then, that the effect observed after erythroblastosis infection is even more striking since this comparison is with erythroblasts from erythroblastosis-infected bone marrow and those from rapidly regenerating bone marrow.

It is interesting to note that the total activities of both " viral " and " regen " nuclei were similar in the absence of AmS. This points out the inherent danger of comparing only total activities of the two sample types under one incubation condition. Whether the " aggregate " form of RNA polymerase in nuclei from virus-infected erythroblasts can be de-repressed under certain physiological conditions is uncertain.

SUMMARY

Fractions rich in erythroblasts were obtained from erythroblastosis - infected and regenerating avian bone marrow by centrifugation through 5 % dextran. Nuclei were isolated after treatment with saponin and the optimal conditions for the RNA polymerase activities of these nuclei were determined. The activities of the two types of nuclear preparations were compared. Linear relationship between the activities of " viral " or " regen " nuclei and protein concentrations obtained up to 1.8 mg protein in the absence of AmS, and up to 1.0 mg protein in the presence of AmS. For " viral " or " regen " nuclei the 5-min activities in the absence of AmS were maximal at pH values of 8.6 - 9.0 and 7.8 - 8.6 respectively; the 15-min activities in the presence of AmS were maximal at pH values of 8.4 - 8.6 and 8.5 - 8.6 respectively. Although DNA stimulated the activity of " viral " and " regen " nuclei very little, because of the presence of endogenous DNA, inhibition by DNase and actinomycin D showed the DNA-dependency of the reaction.

There was little incorporation of AMP - C^{14} into acid insoluble product when RNase was included in the incubation mixture. At a fixed concentration of ATP - C^{14} (25 mμ moles) maximum stimulation by the other nucleoside triphosphates occurred when 30 mμ moles of each was included in the reaction mixture. For the 5-min activity of " viral " nuclei Mn^{++} was the divalent cation of choice in the absence of AmS, whereas Mg^{++} was the better activator in the presence of AmS; for the 15 min activity, Mg^{++} proved to be the better activator in the absence of AmS, whereas both Mg^{++} and Mn^{++} activated to the same extent in the presence of AmS. For " regen " nuclei Mg^{++} was the better activator in the presence or absence of AmS and with both incubation times. Increasing concentrations of AmS stimulated both the 5 and 15-min activities of " viral " nuclei, maximal stimulation (over 2 fold) occurring with the 15-min activity in the presence of 0.35 M AmS. Although AmS slightly inhibited the 5-min activity of " regen " nuclei, there was no inhibition or stimulation of the 15-min activity with 0.35 M AmS. KCl concentrations up to 0.6 M had little effect on the RNA polymerase activities from both sources, but increasing KCl concentrations in the presence of 0.35 M AmS inhibited the 15-min activity

of " viral " nuclei. The polyamines, spermine and spermidine, did not effect the activity of either " viral " or " regen " preparations. For both " viral " and " regen " nuclei incorporation of AMP - C^{14} into acid insoluble RNA was linear at least for the first 3 minutes of incubation in the presence or absence of 0.35 M AmS and reached maximal levels between 15 and 25 minutes.

Twelve samples plus an erythroblast-free preparation of red blood cell nuclei were used in the comparison of the activity of " viral " and " regen " nuclei. The erythroblast-free sample was shown to have no " aggregate " or " soluble " enzyme activities. The activity-time curves of each of the nuclear preparations were studied and the initial rates (which obtain during the first 3 min of incubation) were used as one basis of comparison. The total incorporation at 25 min was used as a second basis of comparison. A high degree of correlation was found between initial rates and percent erythroblasts both with and without AmS and also between the total activity and percent erythroblasts. From plots of the regression lines for the initial rates it was apparent that " viral " nuclei were 2 times as active as " regen " nuclei. The regression lines for total activity plotted against percent erythroblasts revealed that " viral " and

" regen " nuclei had the same level of activity in the absence of AmS but the total activity of " viral " nuclei was twice that of " regen " nuclei in the presence of AmS.

Our results indicate that erythroblastosis infection results in an increase in both the " soluble " and " aggregate " forms of RNA polymerase and that the " aggregate " enzyme present in the " viral " nuclei can be converted to a form resembling the partially de-repressed " aggregate " form we find to dominate the activity of " regen " nuclei. These increases in activity as a result of viral infection are probably due to the oncogenic nature of the virus though the " aggregate " enzyme produced as a result of a virus infection may be a reflection of the virogenic nature of the virus.

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FIGURES

Figure 1: The relationship between protein concentration and enzyme activity of " viral " nuclei. Activity was measured under the standard conditions of assay. Incubations were carried out for 5 min in the absence of AmS (open circles), 5 min in the presence of 0.35 M AmS (closed circles), 15 min in the absence of AmS (open squares), and 15 min in the presence of 0.35 M AmS (closed squares).

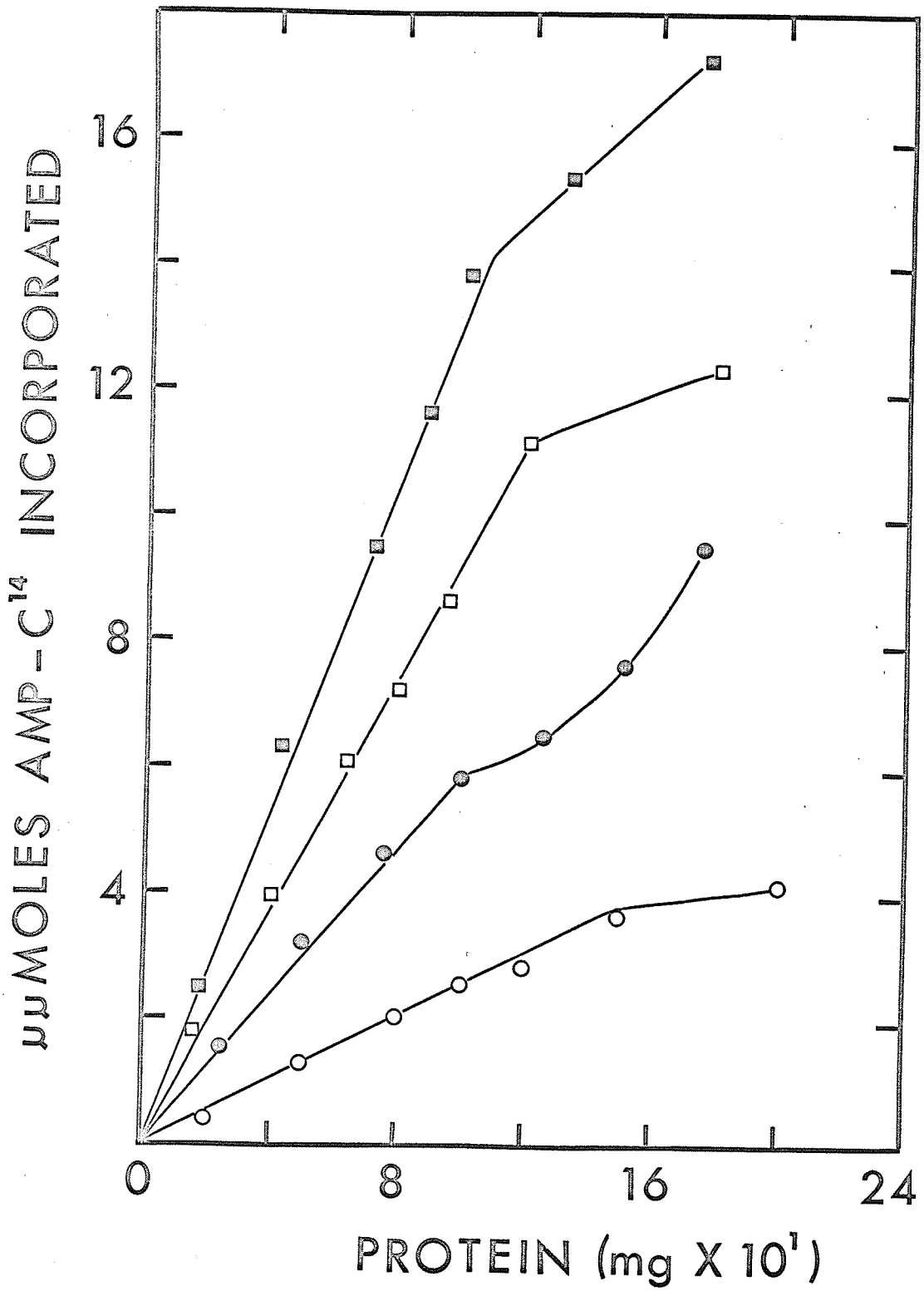

μμMOLES AMP-C14 INCORPORATED
16
12
8
4
0
8
16
24
PROTEIN (mg X 10¹)

Figure 2: The relationship between protein concentration and enzyme activity of " regen " nuclei. Activity was measured under the standard conditions of assay. Incubations were carried out for 5 min in the absence of AmS (open circles), 5 min in the presence of 0.35 M AmS (closed circles), 15 min in the absence of AmS (open squares), and 15 min in the presence of 0.35 M AmS.

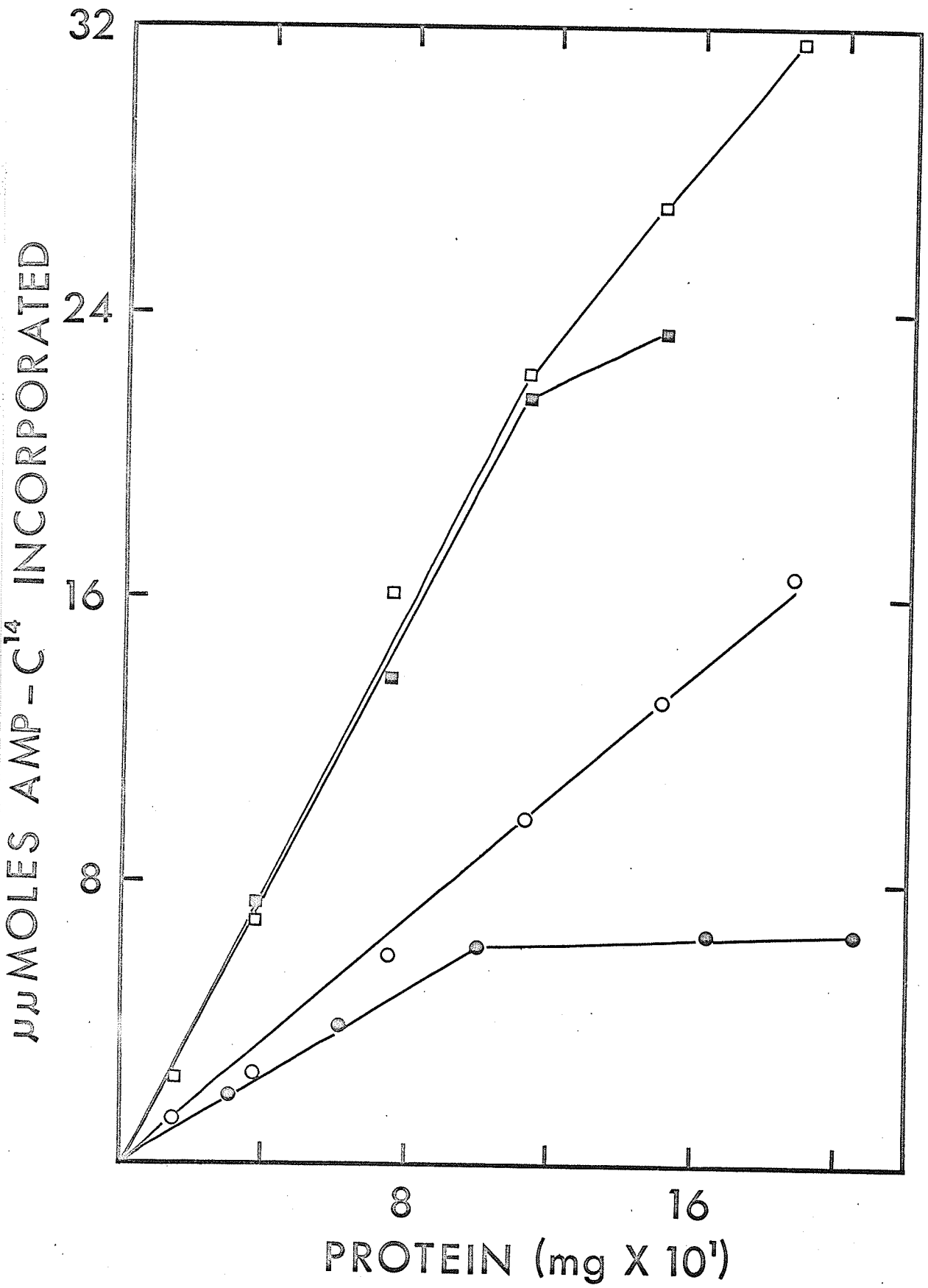

32
24
16
8
μμ MOLES AMP - C14 INCORPORATED
8
16
PROTEIN (mg X 10¹)

Figure 3: The relationship between pH and enzyme activity of " viral " nuclei. Activity was measured under the standard conditions of assay but at differing pH's. Incubations were carried out for 5 min in the absence of AmS (open circles) and 15 min in the presence of 0.35 M AmS (closed circles)

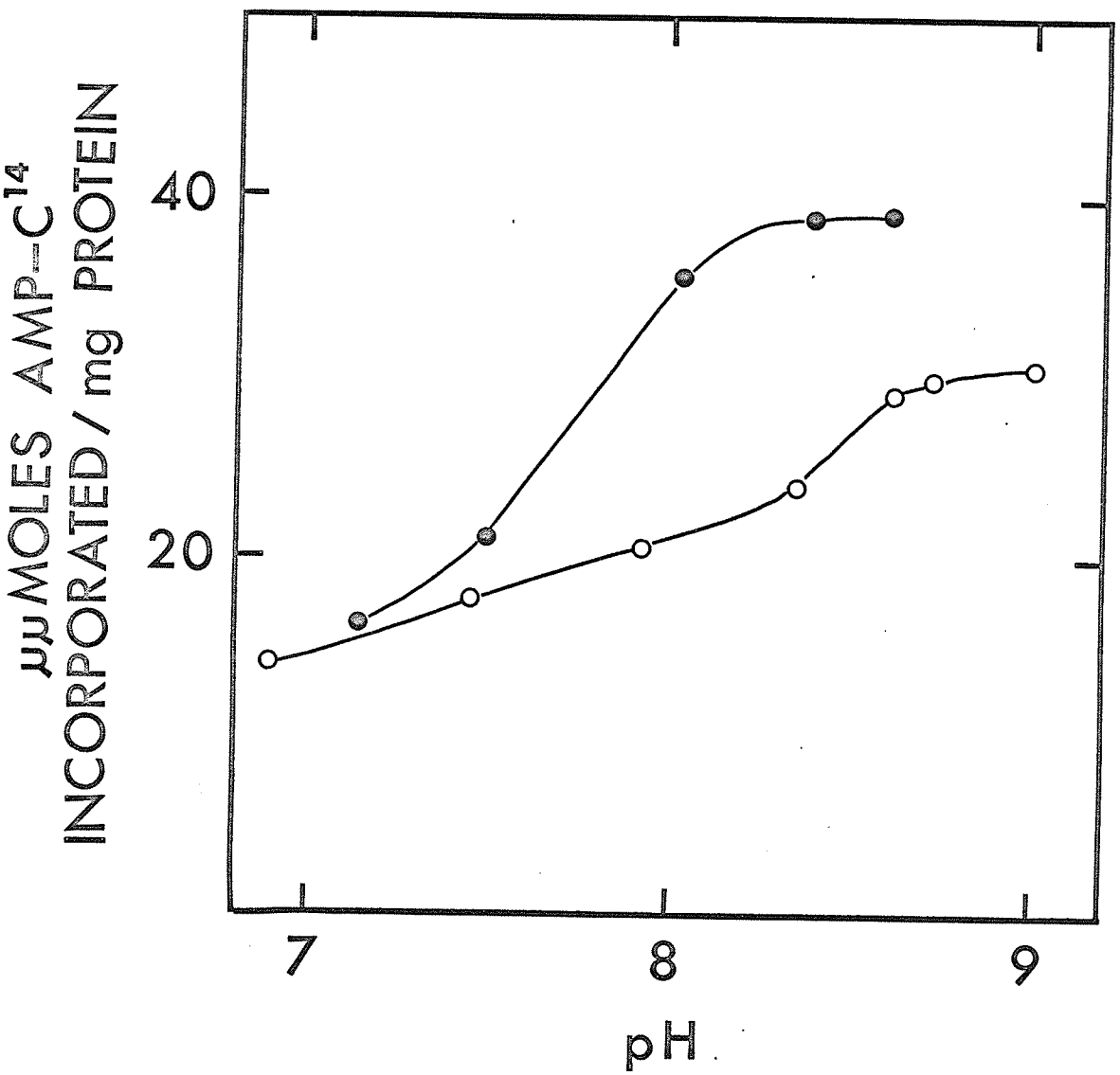

μμMOLES AMP—C14
INCORPORATED / mg PROTEIN
40
20
7
8
9
pH

Figure 4: The relationship between pH and enzyme activity of " regen " nuclei. Activity was measured under the standard conditions of assay but at differing pH's. Incubations were carried out for 5 min in the absence of AmS (open circles) and 15 min in the presence of 0.35 M AmS (closed circles).

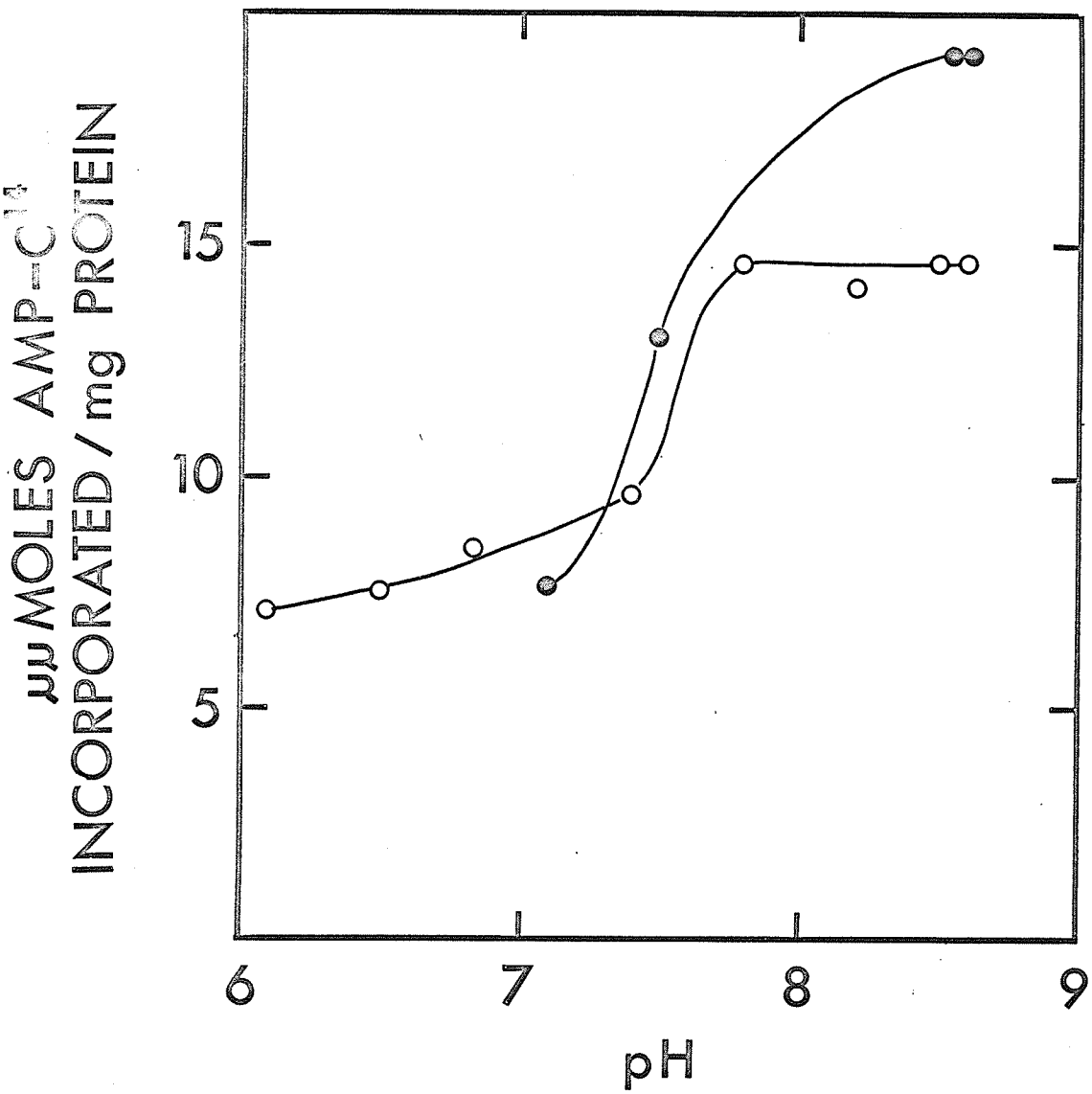

μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
15
10
5
6
7
8
9
pH

Figure 5: Effect of DNA on RNA polymerase activity. Activity was measured under the standard conditions of assay but the amount of added DNA was varied. The effect of DNA on the 5-min (open circles) and 15-min (closed circles) activity of " regen " nuclei, and the 5-min (open squares) activity of " viral " nuclei is shown.

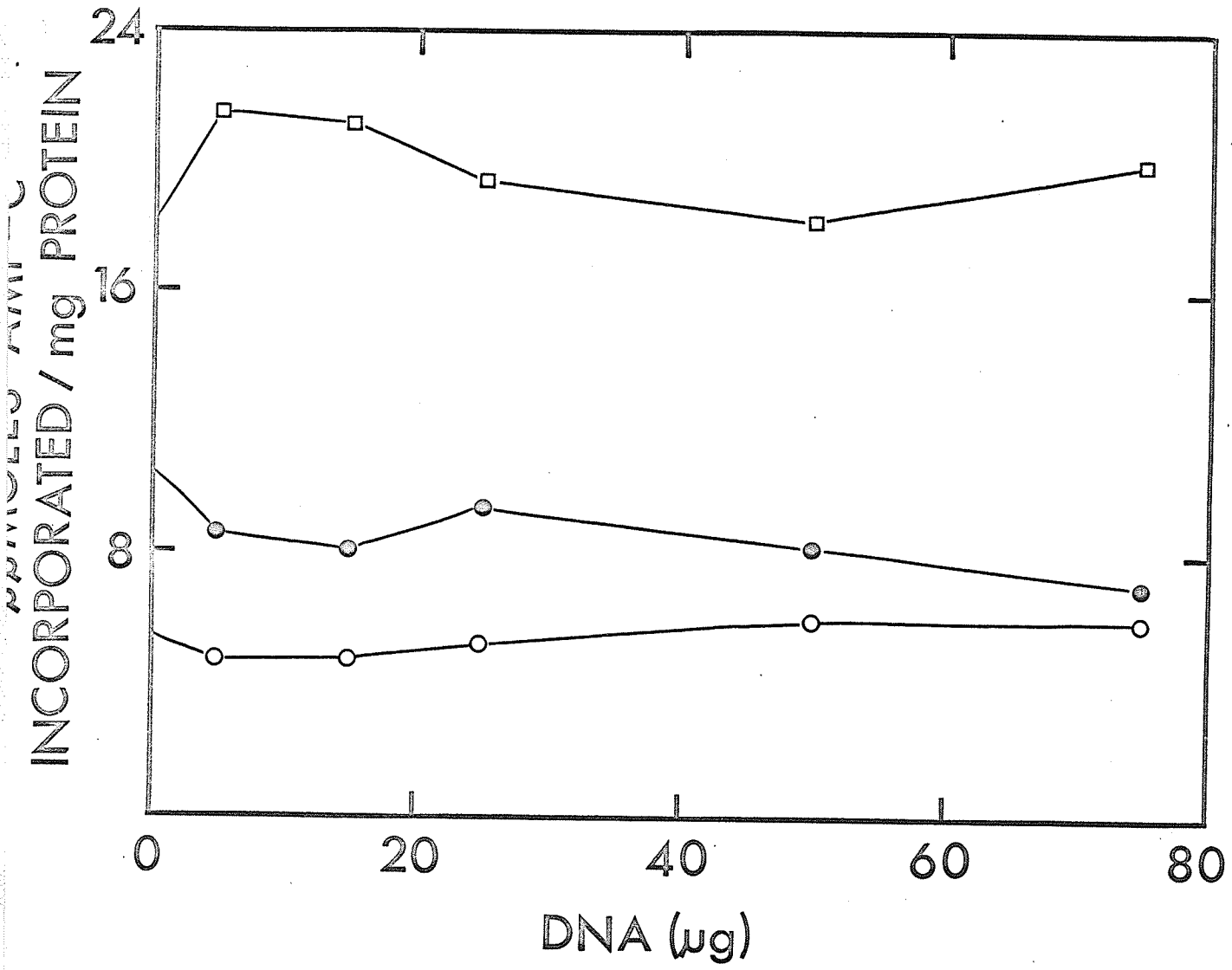

INCORPORATED / mg PROTEIN
24
16
8
0
20
40
60
80
DNA (ug)

Figure 6: Effect of nucleoside triphosphates on RNA polymerase activity. Activity was measured under the standard conditions of assay but the amount of added nucleoside triphosphates was varied. The effect of nucleoside triphosphates on the 5-min (open circles) and 15-min (closed circles) activity of " regen " nuclei and 5-min (open squares) activity of " viral " nuclei is shown.

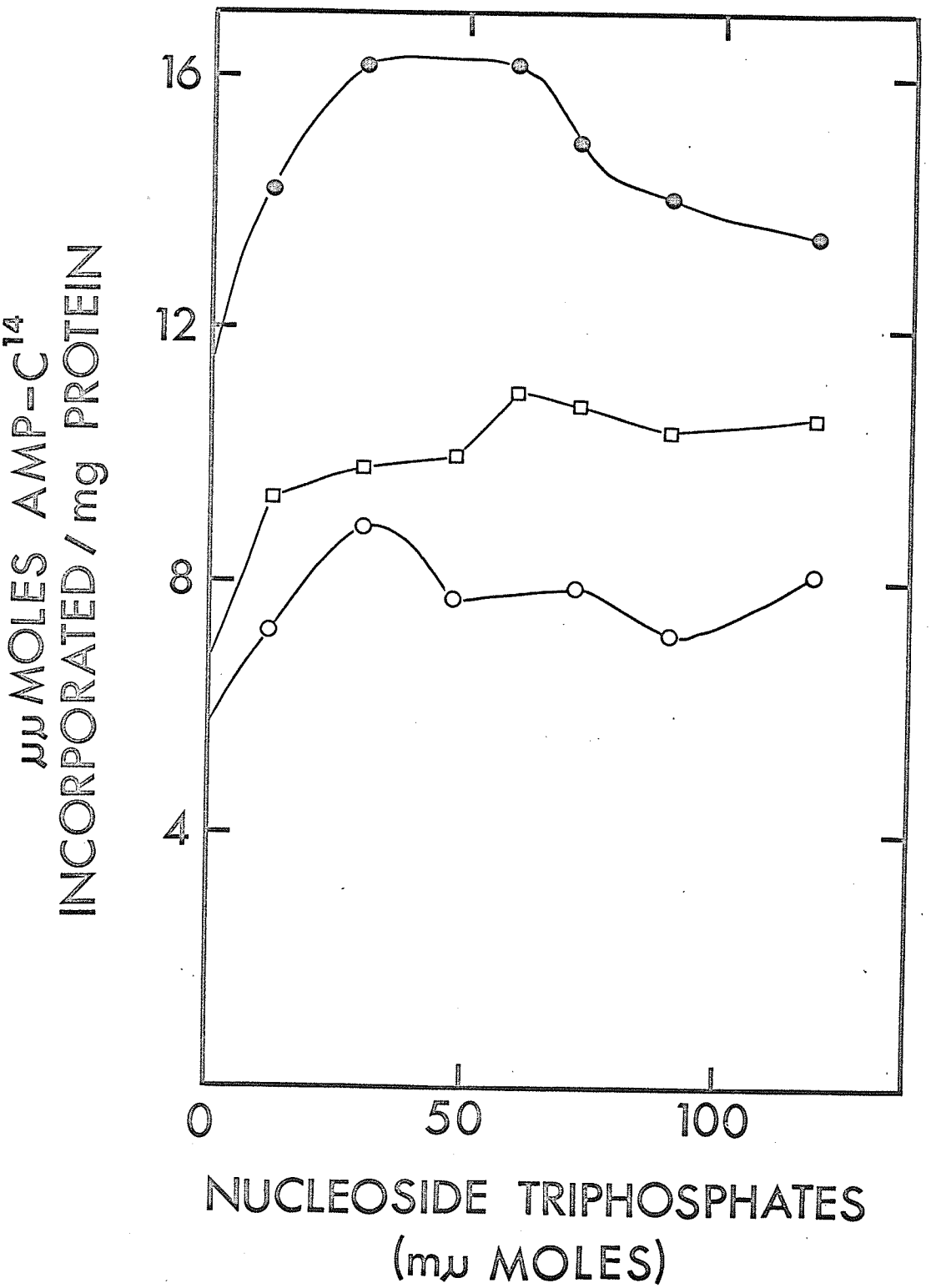

16
12
8
4
0
50
100
μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
NUCLEOSIDE TRIPHOSPHATES (mμ MOLES)

Figure 7: Effect of divalent cations on the 5-min enzyme activity of " vird " nuclei. Activity was measured under the standard conditions of assay but with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

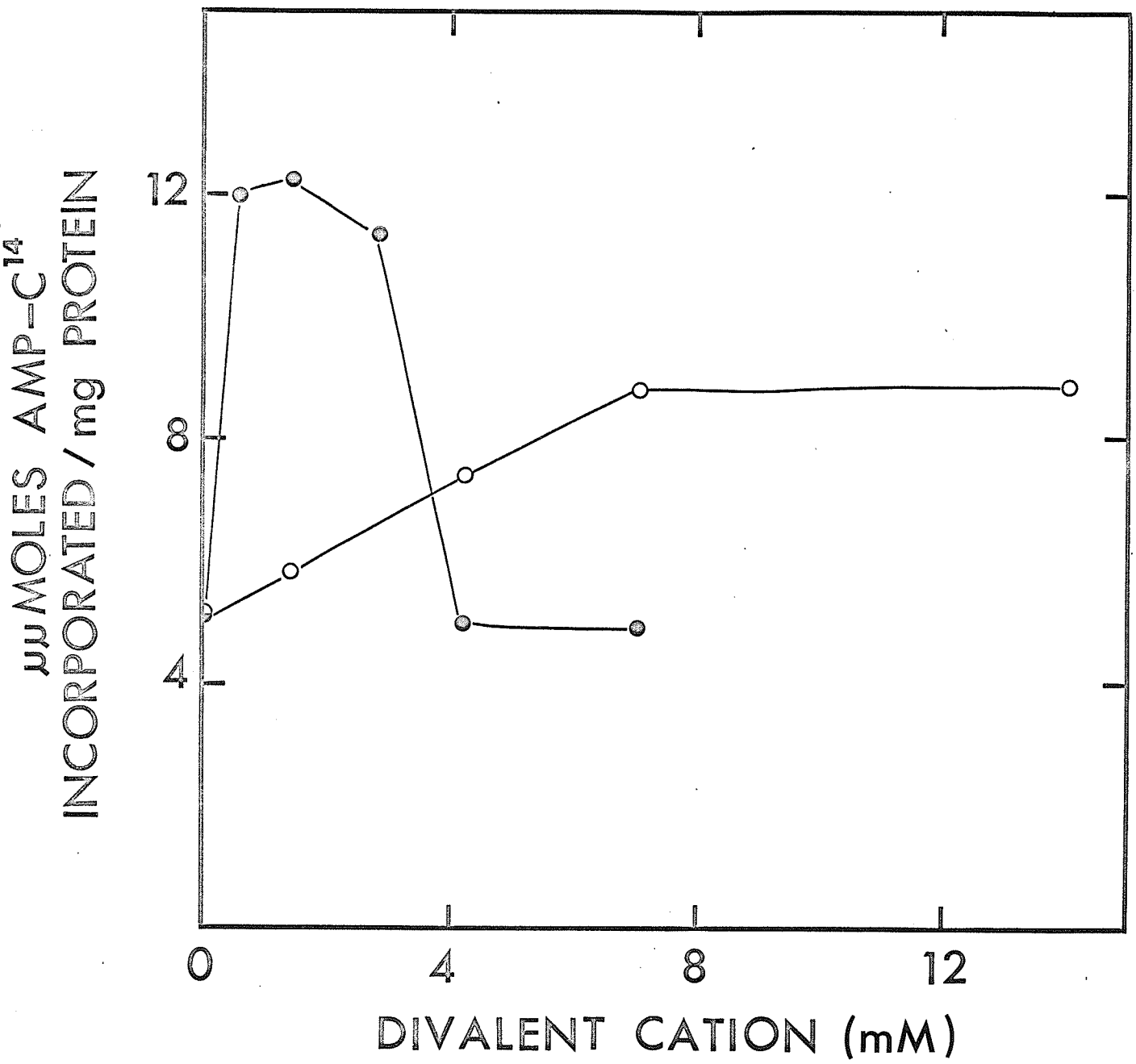

μμ MOLES AMP–C14 INCORPORATED / mg PROTEIN
12
8
4
0
4
8
12
DIVALENT CATION (mM)

Figure 8: Effect of divalent cations on the 15-min enzyme activity of " viral " nuclei. Activity was measured under the standard conditions of assay but with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

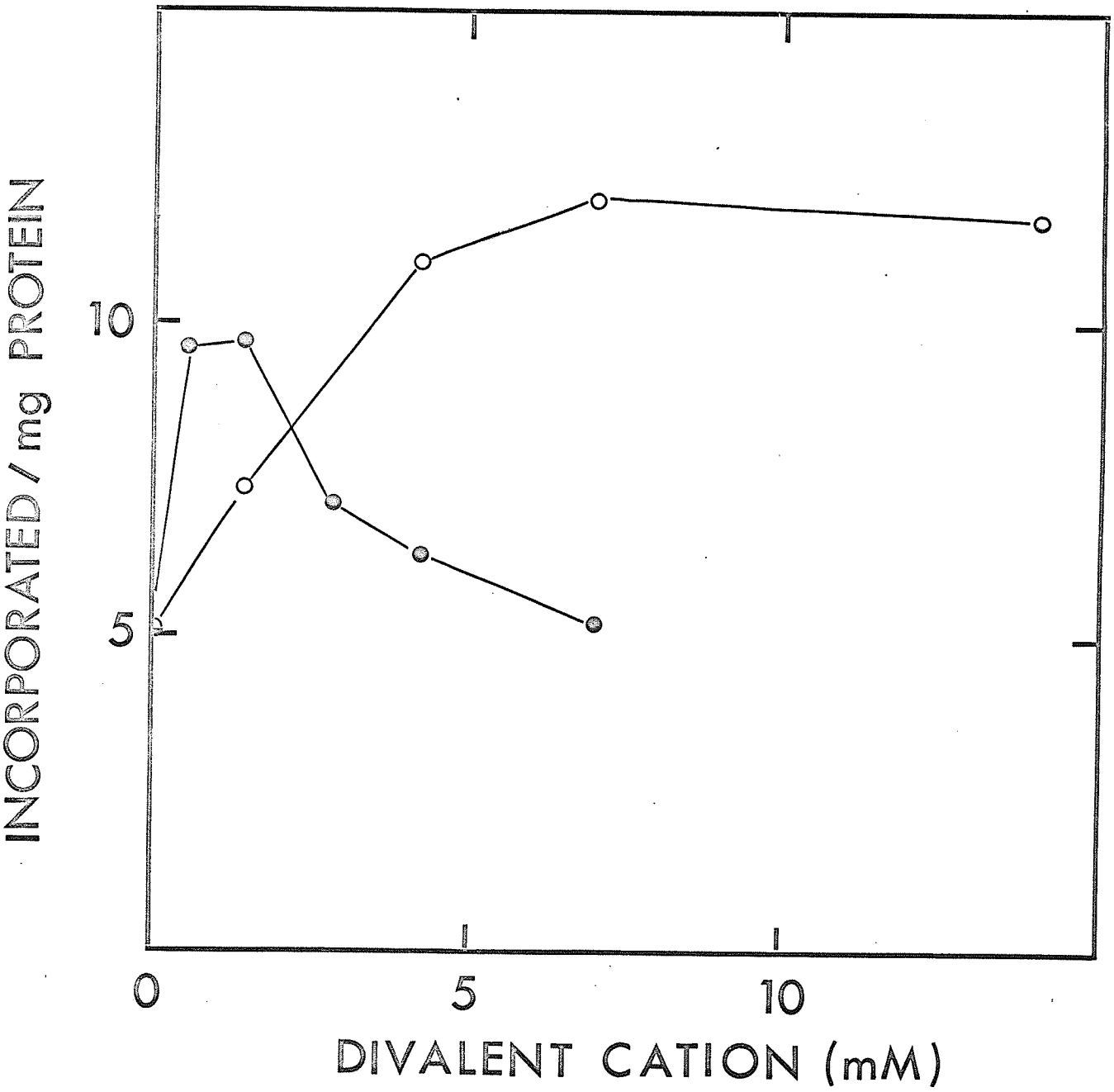

μμMOLES AMP-C14
INCORPORATED / mg PROTEIN
10
5
0
5
10
DIVALENT CATION (mM)

Figure 9: Effect of divalent cations on the 5-min enzyme activity of " viral " nuclei in the presence of 0.35 M AmS. Activity was measured under the standard conditions of assay but with AmS at a final concentration of 0.35 M and with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

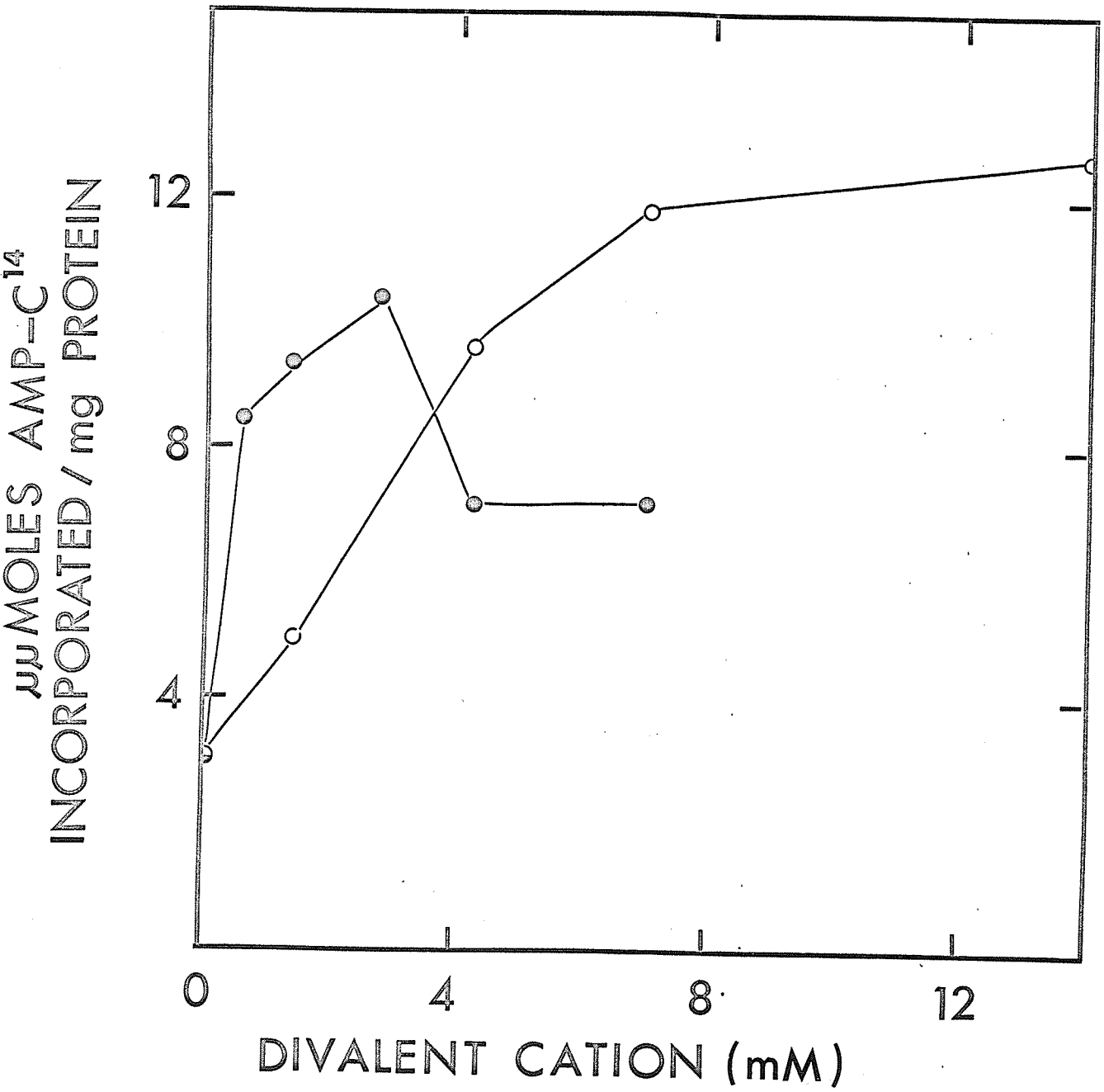

μμ MOLES AMP–C14 INCORPORATED / mg PROTEIN
12
8
4
0
4
8
12
DIVALENT CATION (mM)

Figure 10: Effect of divalent cations on the 15-min enzyme activity of " viral " nuclei in the presence of 0.35 M AmS. Activity was measured under the standard conditions of assay but with AmS at a final concentration of 0.35 M and with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

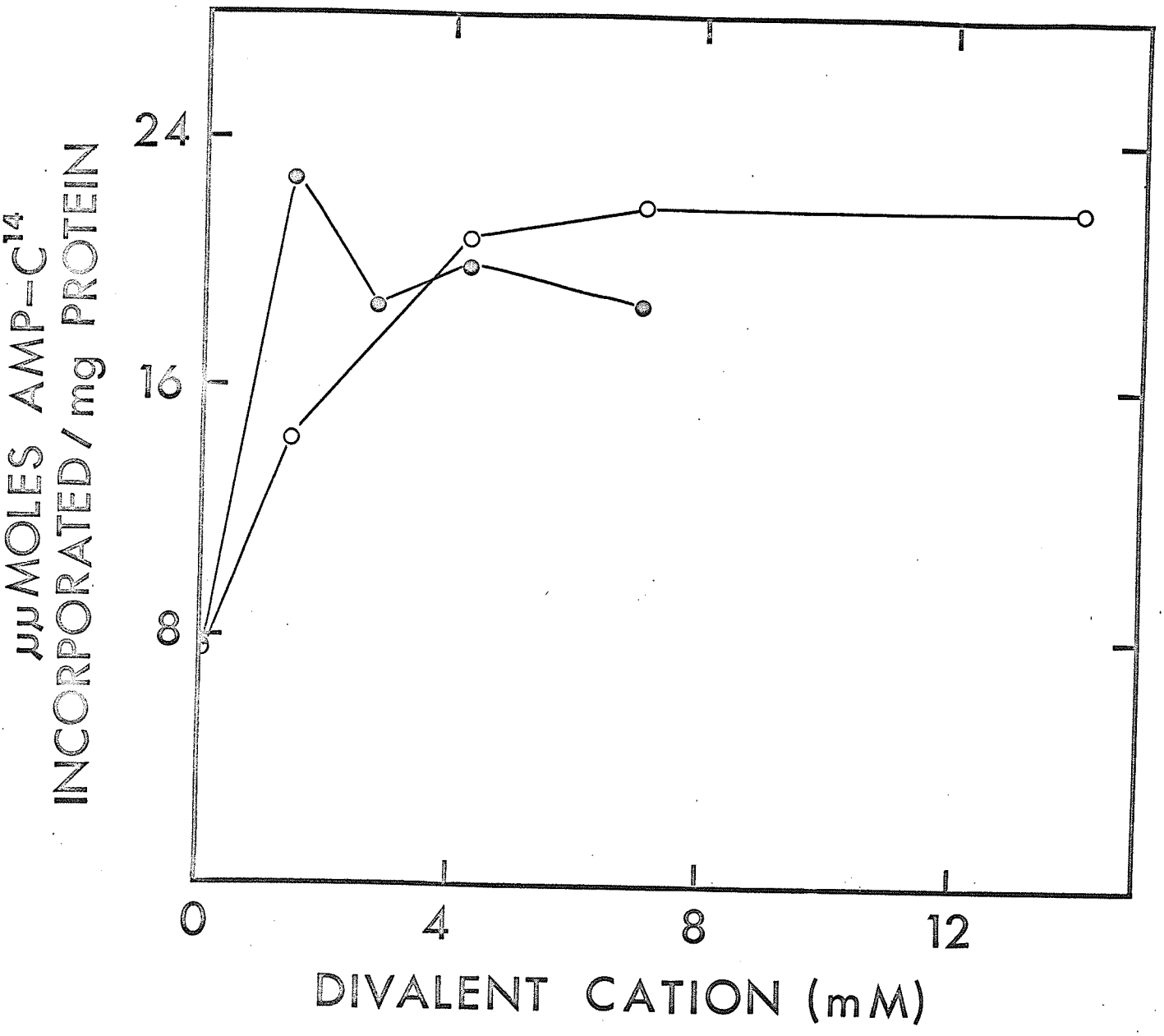

μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
24
16
8
0
4
8
12
DIVALENT CATION (mM)

Figure 11: Effect of divalent cations on the 5-min enzyme activity of " regen " nuclei. Activity was measured under the standard conditions of assay but with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

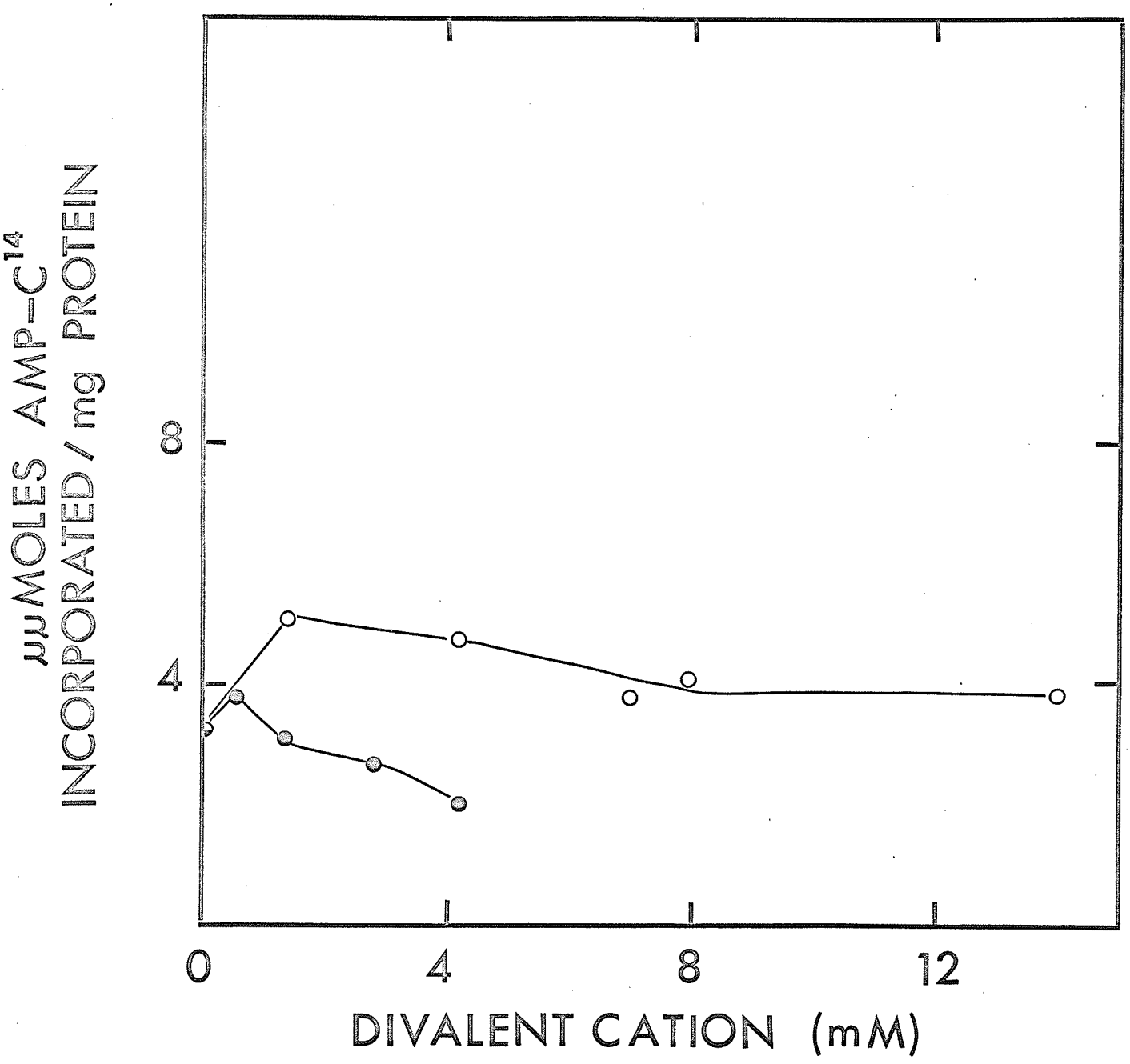

μμMOLES AMP-C14
INCORPORATED / mg PROTEIN
8
4
0
4
8
12
DIVALENT CATION (mM)

Figure 12: Effect of divalent cations on the 15-min enzyme activity of " regen " nuclei. Activity was measured under the standard conditions of assay but with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

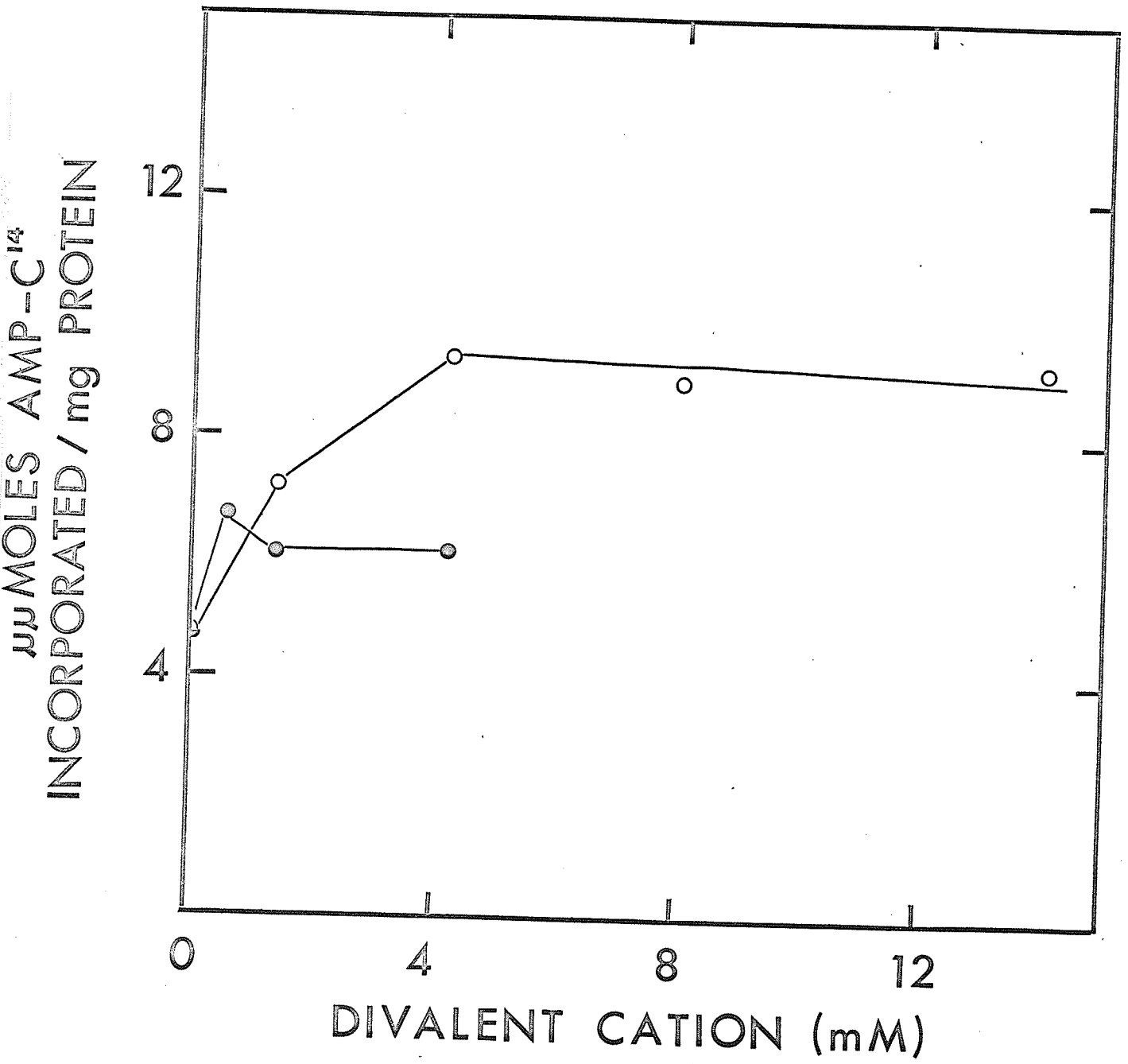

μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
12
8
4
0
4
8
12
DIVALENT CATION (mM)

Figure 13: Effect of divalent cations on the 5-min enzyme activity of " regen " nuclei in the presence of 0.35 M AmS. Activity was measured under the standard conditions of assay but with AmS at a final concentration of 0.35 M and with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

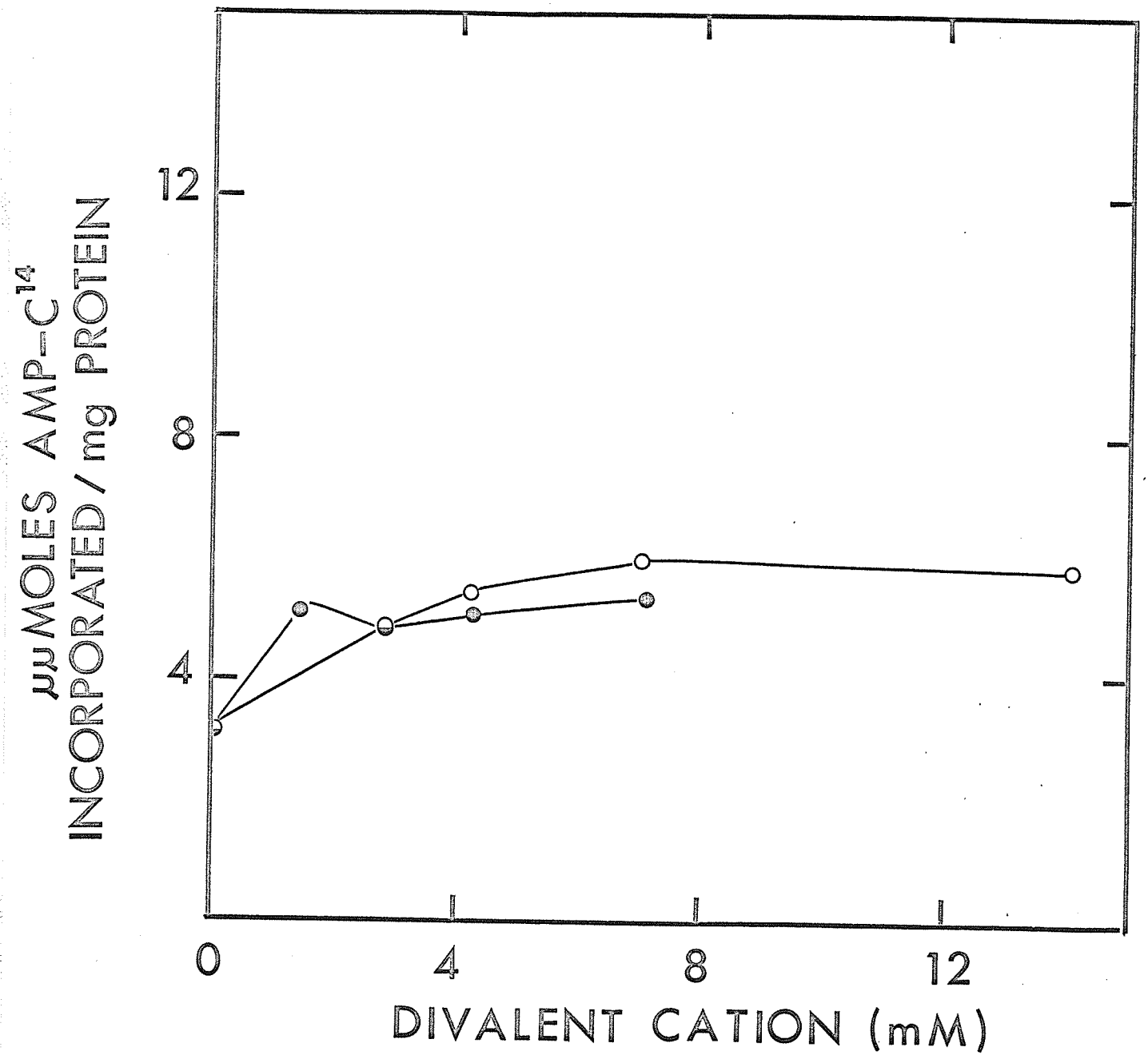

μμMOLES AMP–C14
INCORPORATED / mg PROTEIN
12
8
4
0
4
8
12
DIVALENT CATION (mM)

Figure 14: Effect of divalent cations on the 15-min enzyme activity of " regen " nuclei in the presence of 0.35 M AmS. Activity was measured under the standard conditions of assay but with AmS at a final concentration of 0.35 M and with varying concentrations of Mg^{++} (open circles) or Mn^{++} (closed circles).

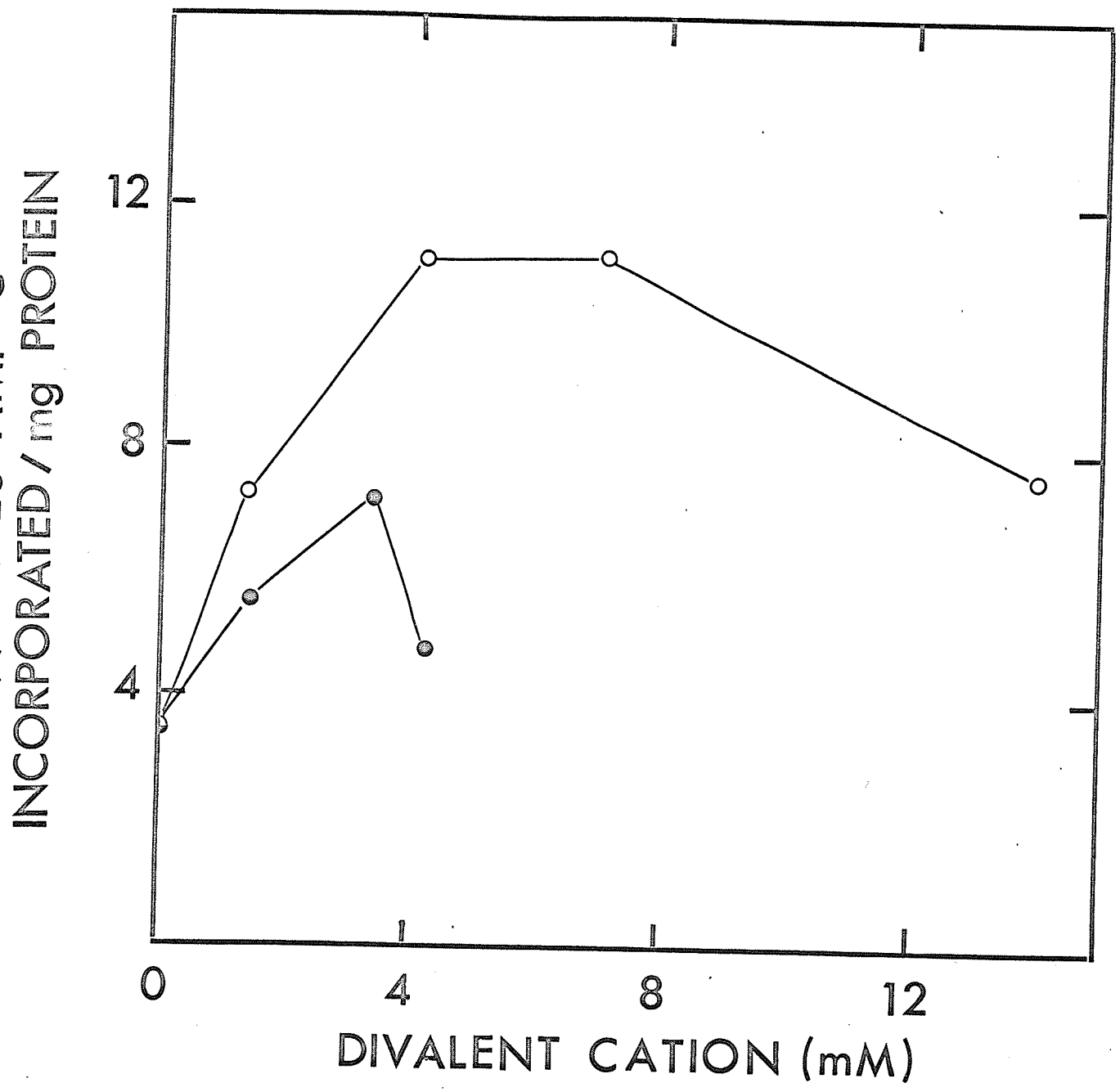

μμ MOLES AMP–C14 INCORPORATED / mg PROTEIN
12
8
4
0
4
8
12
DIVALENT CATION (mM)

Figure 15: Effect of AmS on RNA polymerase activity of " viral " nuclei. Activity was measured under the standard conditions of assay but with varying concentrations of AmS for both the 5-min (open circles) and 15-min (closed circles) activity.

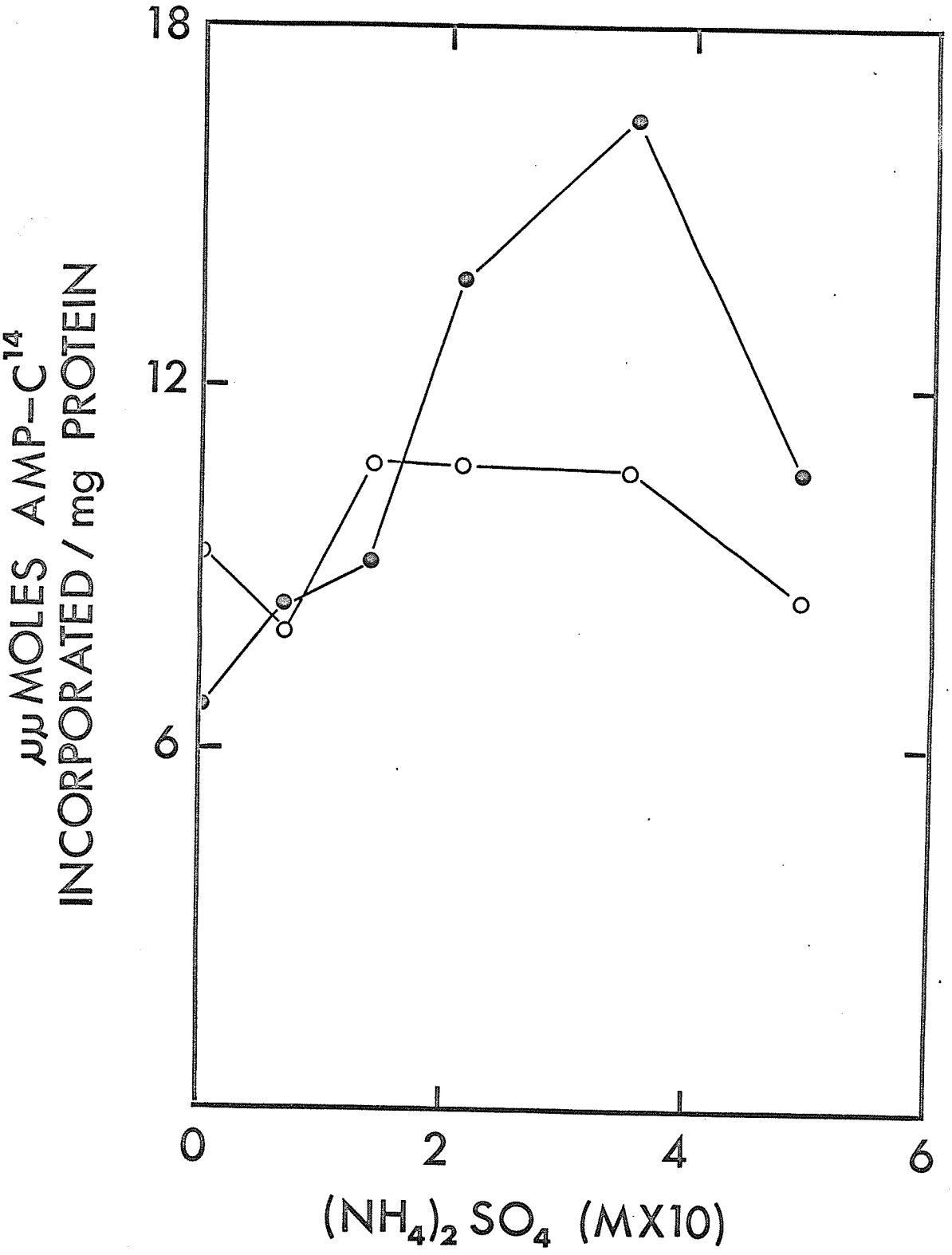

18
12
6
0
2
4
6
μμ MOLES AMP–C14 INCORPORATED / mg PROTEIN
(NH4)2 SO4 (MX10)

Figure 16: Effect of AmS on RNA polymerase activity of " regen " nuclei. Activity was measured under the standard conditions of assay but with varying concentrations of AmS for both the 5-min (open circles) and 15-min (closed circles) activity.

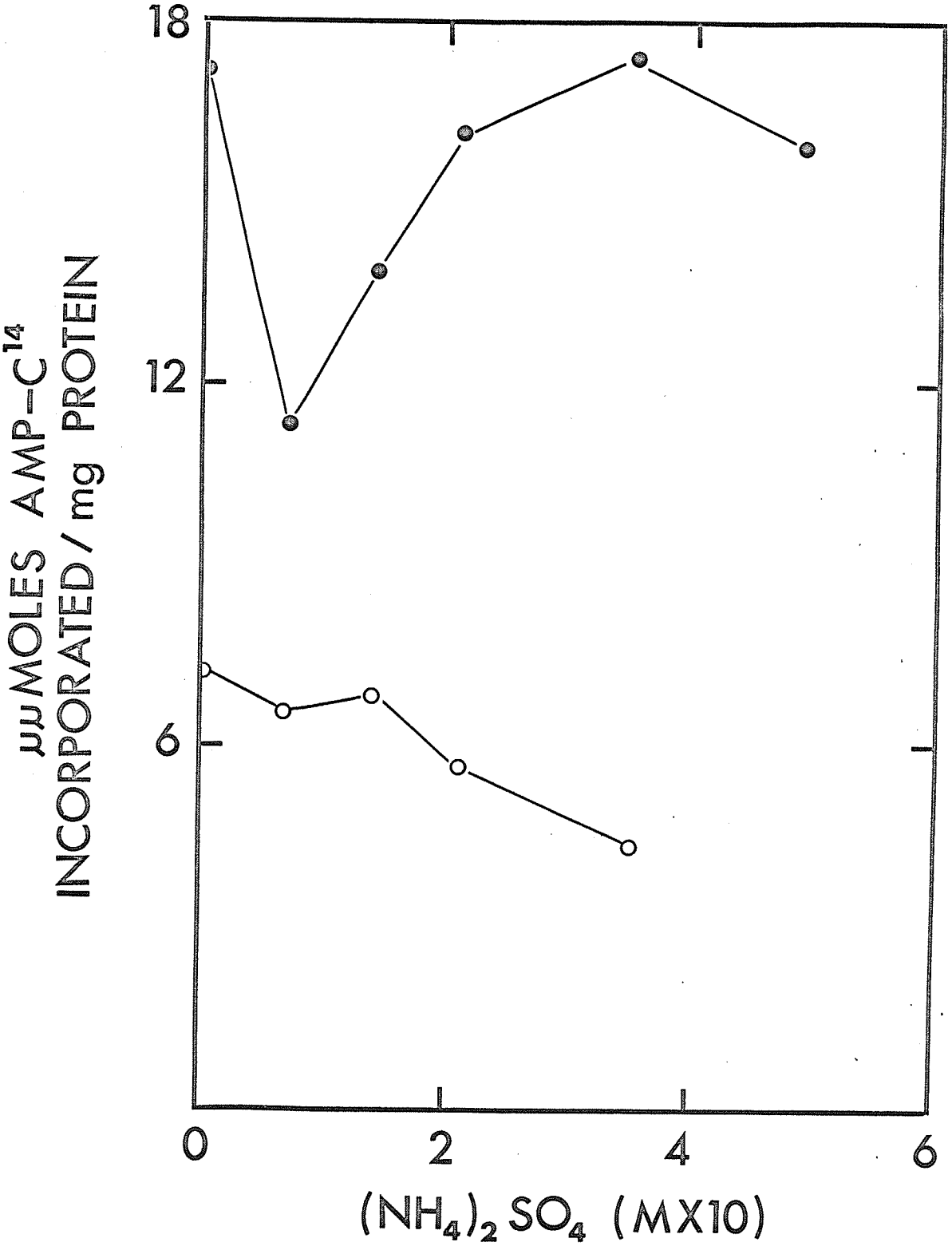

18
12
6
0
2
4
6
μμMOLES AMP–C14 INCORPORATED / mg PROTEIN
(NH4)2 SO4 (MX10)

Figure 17: Effect of KCl on RNA polymerase activity. Activity was measured under the standard conditions of assay but with varying concentrations of KCl for both the 5-min (open circles) and 15-min (closed circles) activity of " viral " nuclei, and the 5-min (open squares) and 15-min (closed squares) activity of " regen " nuclei. (The activity of " viral " nuclei was measured using ATP-H^3 rather than ATP-C^{14}).

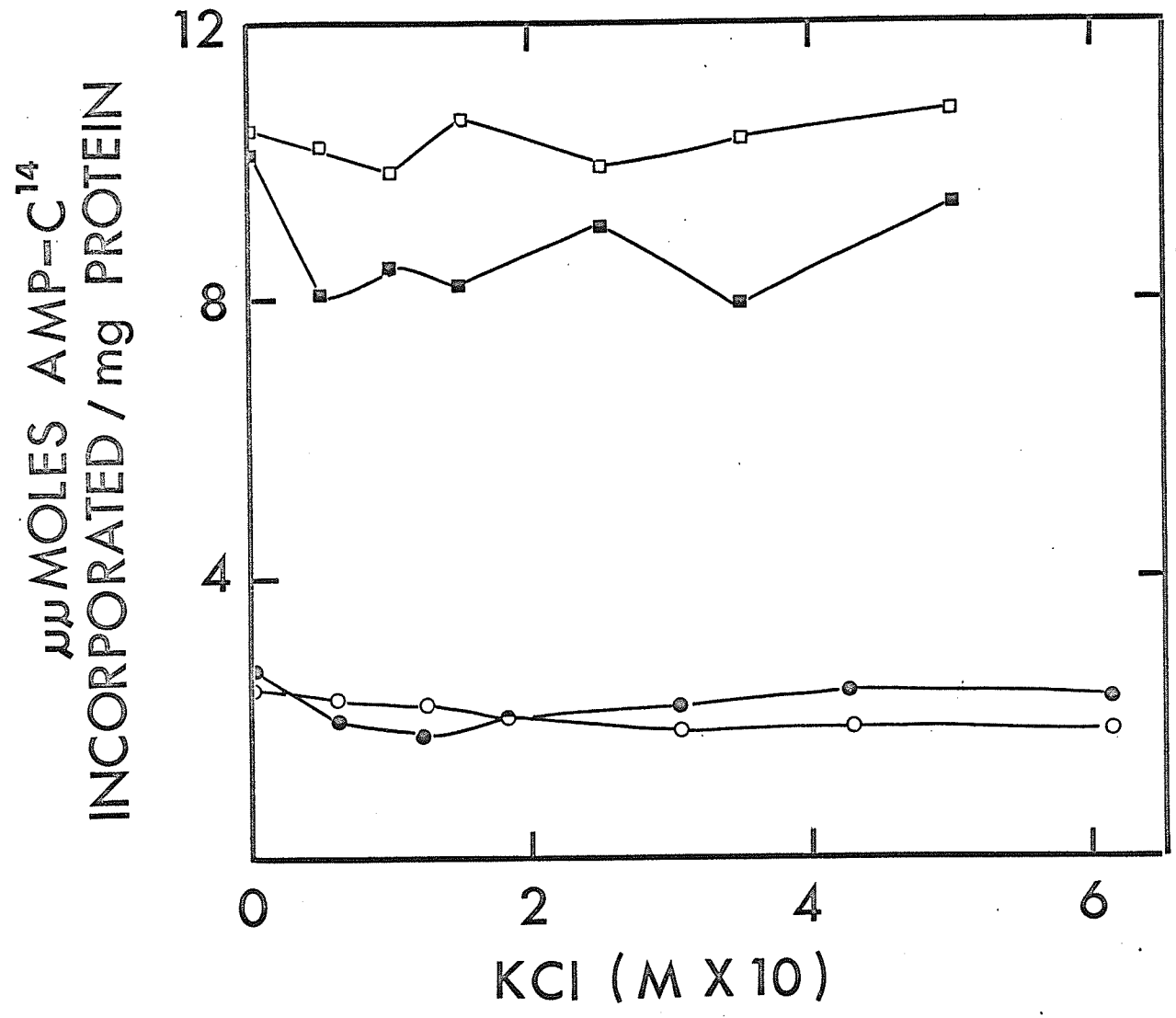

12
8
4
0
2
4
6
μμMOLES AMP-C14 INCORPORATED / mg PROTEIN
KCl (M X 10)

Figure 18: Effect of KCl on the 15-min enzyme activity of " viral " nuclei in the presence of 0.35 M AmS. Activity was measured under the standard conditions of assay but with AmS at a final concentration of 0.35 M and with varying concentrations of KCl.

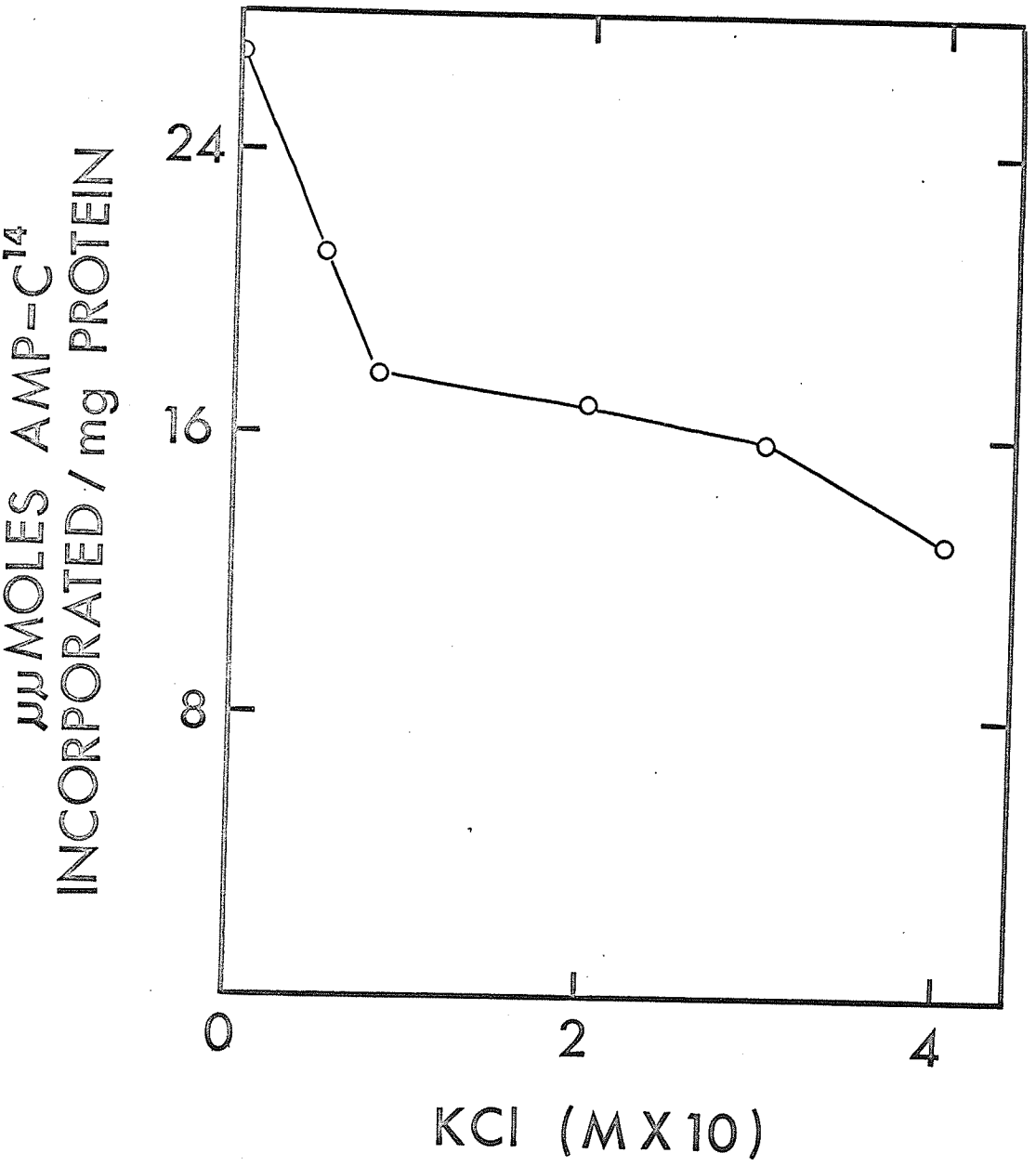

μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
24
16
8
0
2
4
KCl (M X 10)

Figure 19: Effect of spermine on RNA polymerase activity. Activity was measured under the standard conditions but with varying concentrations of spermine. The effect of spermine on the 5-min (open circles) and 15-min (closed circles) activity of " viral " nuclei and on the 5-min (open squares) and 15-min (closed squares) activity of " regen " nuclei is shown.

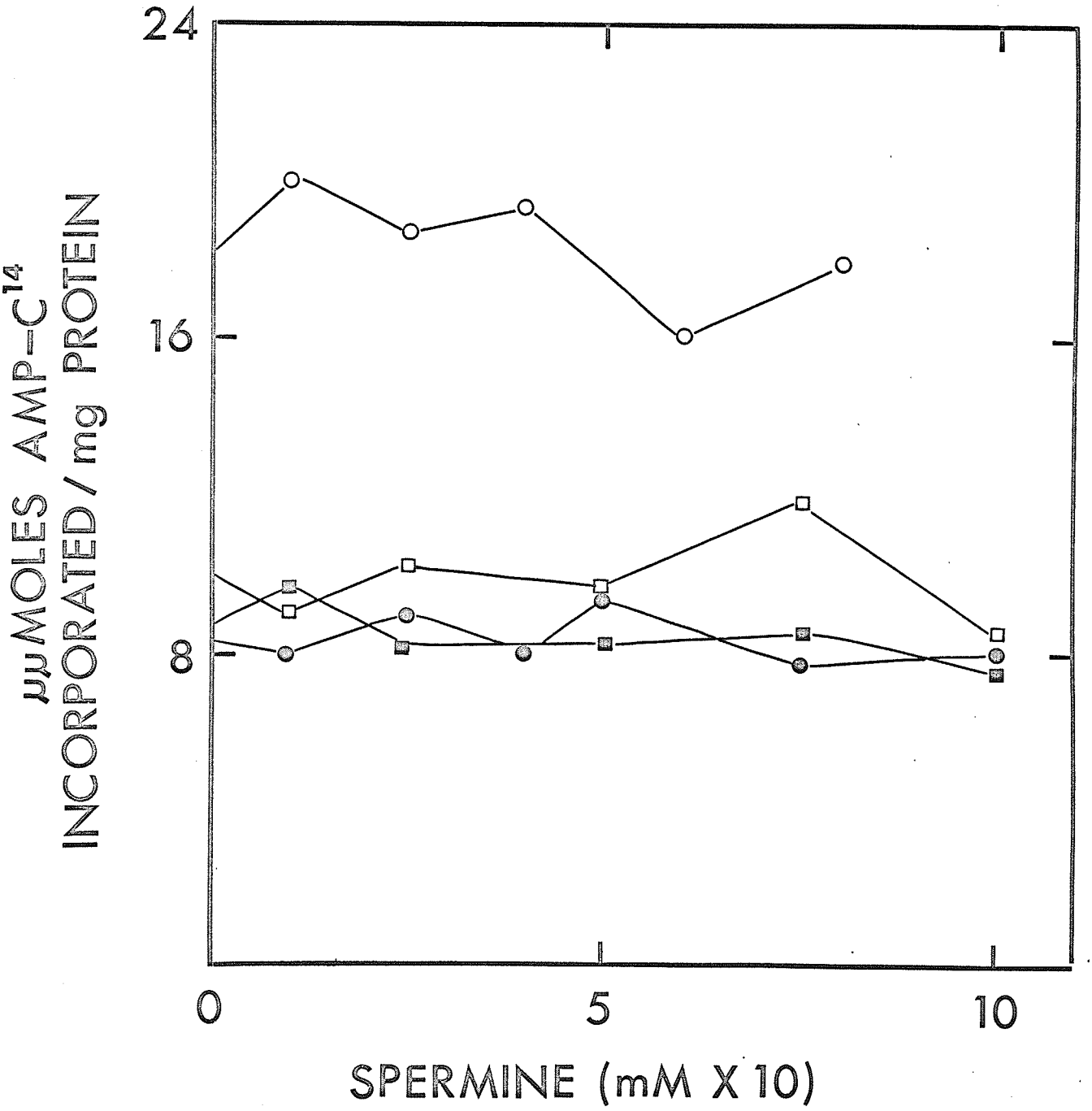

24
16
8
0
5
10
μμMOLES AMP-C14 INCORPORATED / mg PROTEIN
SPERMINE (mM X 10)

Figure 20: Effect of spermidine on RNA polymerase activity. Activity was measured under the standard conditions but with varying concentrations of spermidine. The effect of spermidine on the 15-min (open circles) and 5-min (closed circles) activity of " viral " nuclei and on 15-min (open squares) activity of " regen " nuclei is shown.

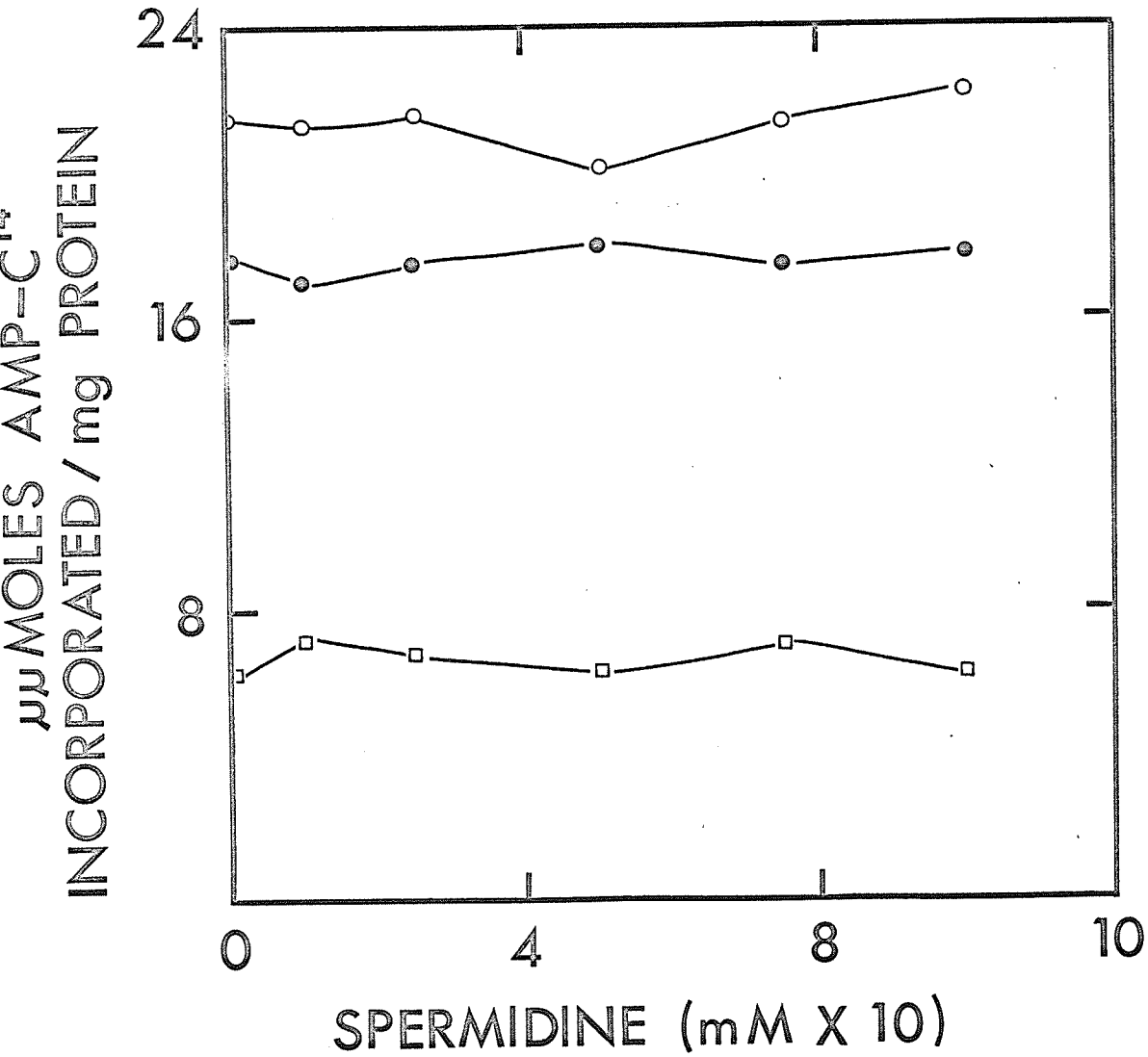

24
16
8
0
4
8
10
μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
SPERMIDINE (mM X 10)

Figure 21: Effect of spermine on the 15-min enzyme activity of " viral " nuclei in the presence of 0.35 M AmS. Activity was measured under the standard conditions of assay but with AmS at a final concentration of 0.35 M and with varying concentrations of spermine.

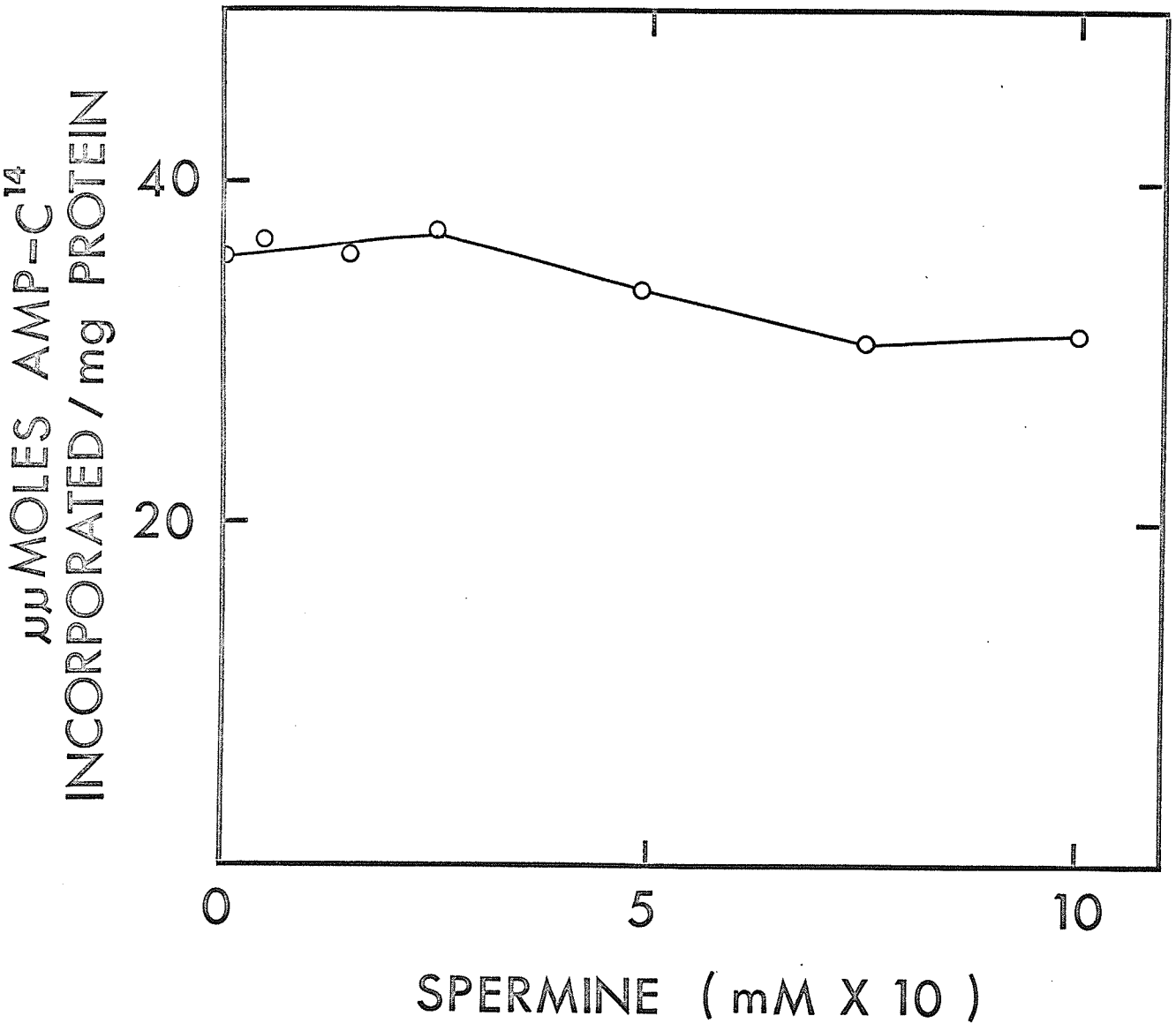

μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
40
20
0
5
10
SPERMINE (mM X 10)

Figure 22: The relationship between time and enzyme activity of " viral " nuclei. Activity was measured under the standard conditions of assay in the absence of AmS (open circles) and presence of 0.35 M AmS (closed circles).

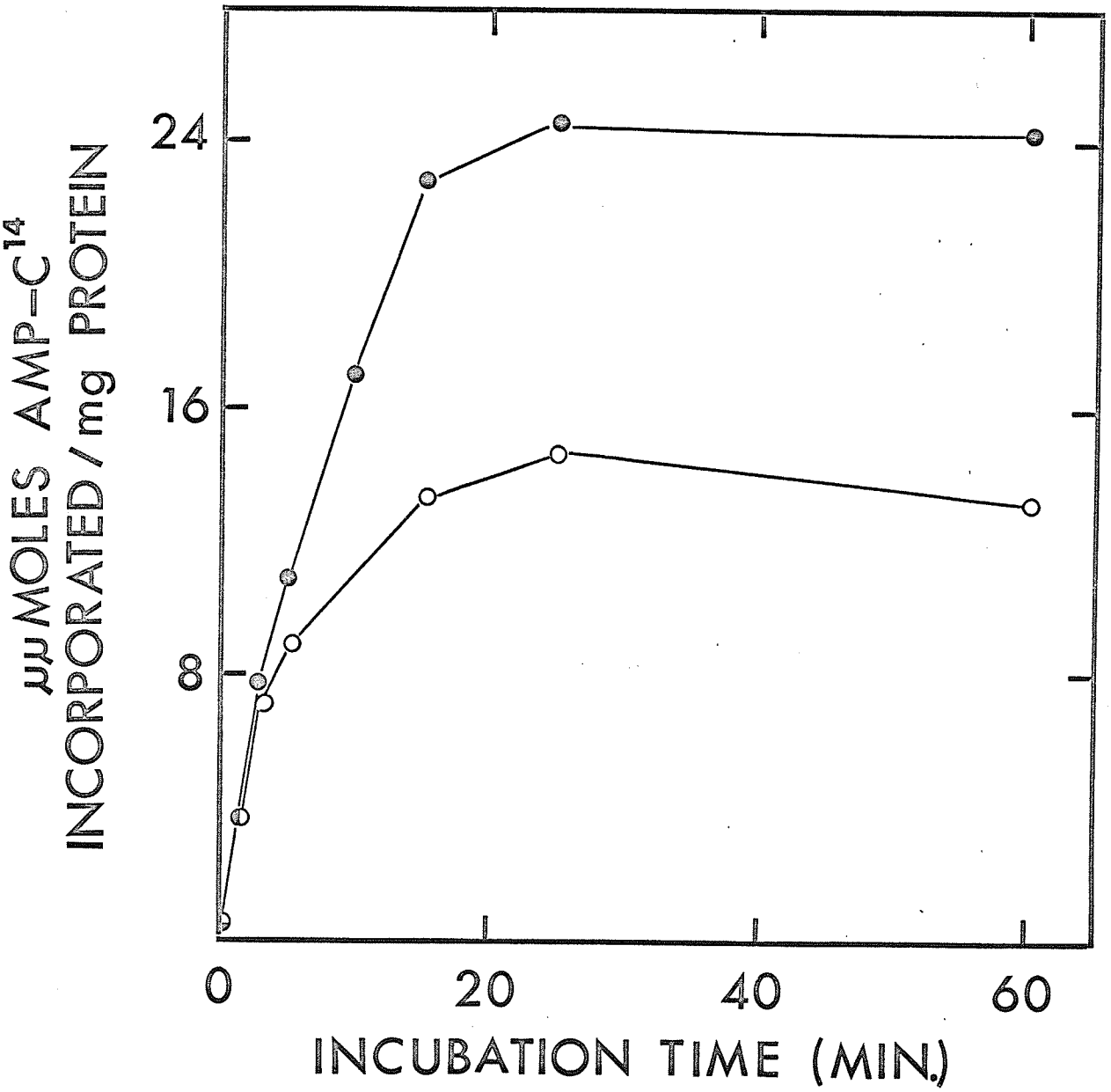

μμ MOLES AMP–C14 INCORPORATED / mg PROTEIN
24
16
8
0
20
40
60
INCUBATION TIME (MIN.)

Figure 23: The relationship between time and enzyme activity of " regen " nuclei. Activity was measured under the standard conditions of assay in the absence of AmS (open circles) and presence of 0.35 M AmS (closed circles).

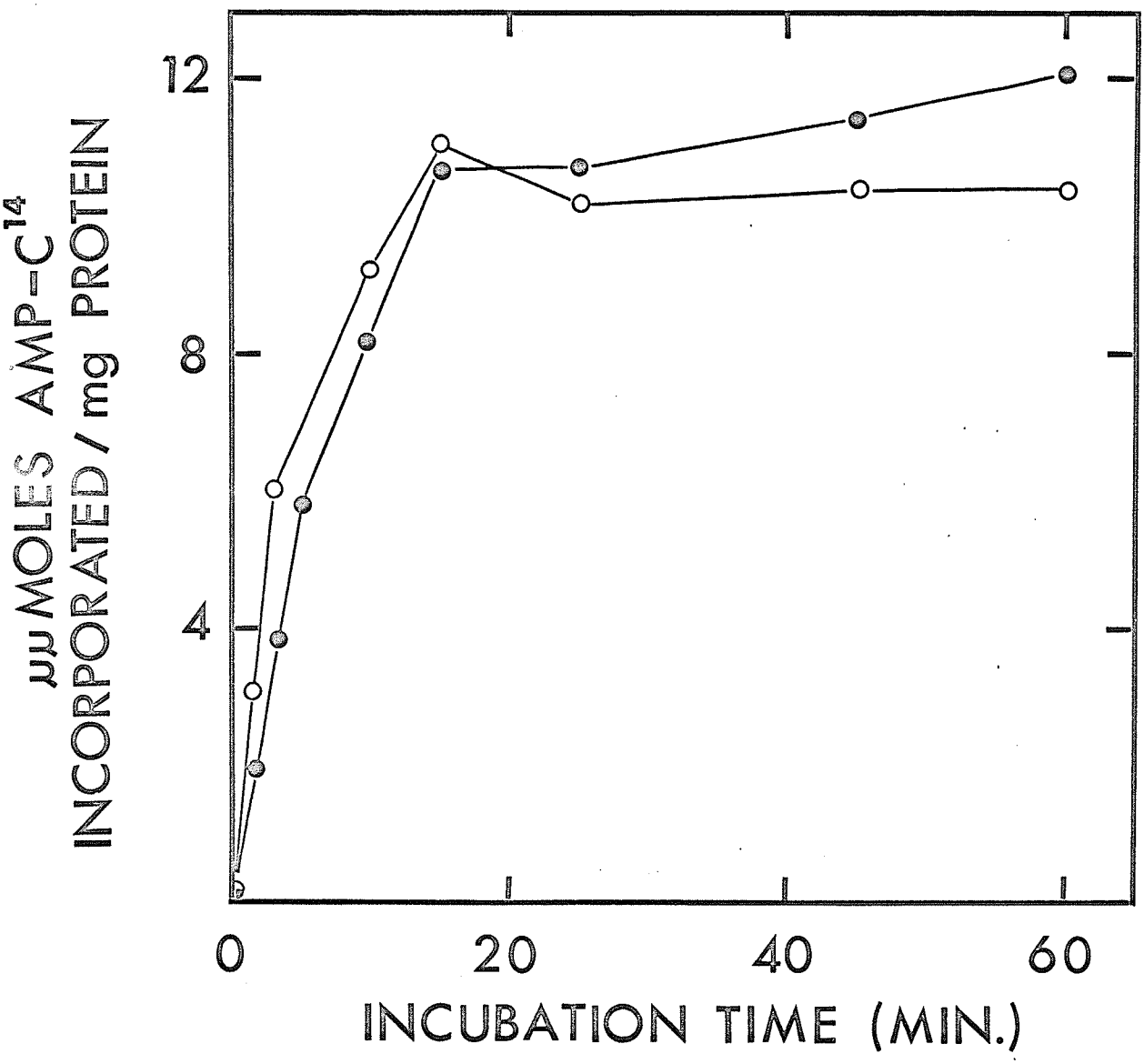

μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
12
8
4
0
20
40
60
INCUBATION TIME (MIN.)

Figure 24: The initial relationship between time and enzyme activity. Activity was measured under the standard conditions of assay for both " viral " nuclei in the absence (open circles) and presence (closed circles) of 0.35 M AmS and " regen " nuclei in the absence (open squares) and presence (closed squares) of 0.35 M AmS.

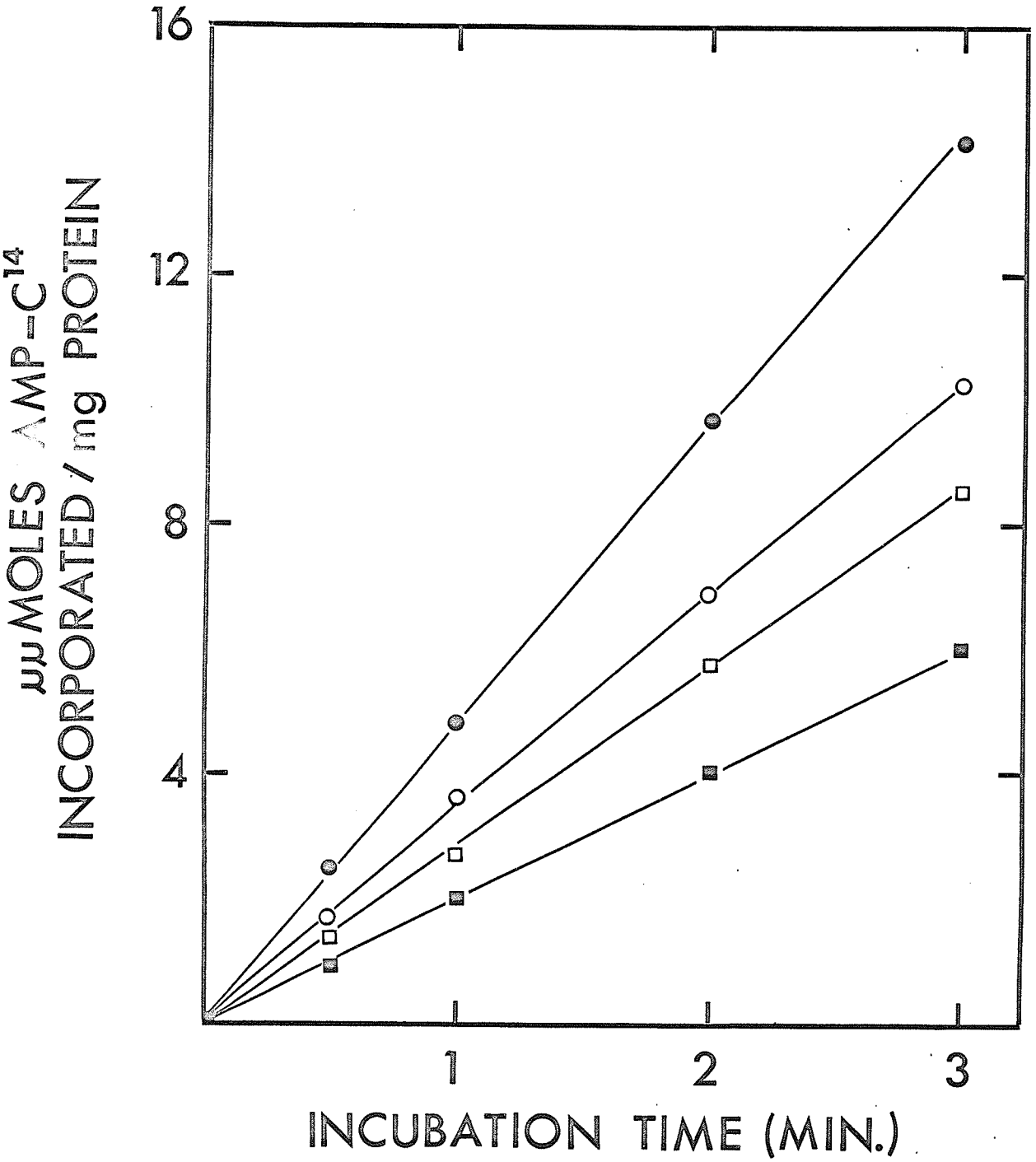

16
12
8
4
1
2
3
μμ MOLES AMP-C14 INCORPORATED / mg PROTEIN
INCUBATION TIME (MIN.)

Figure 25: Correlation between the initial rate of RNA polymerase activity and percent erythroblasts. Activity was measured under the standard conditions of assay for various incubation periods up to 3 min both for " viral " nuclei in the absence (open circles) and presence (closed circles) of 0.35 M AmS and for " regen " nuclei in the absence (open squares) and presence (closed squares) of 0.35 M AmS.

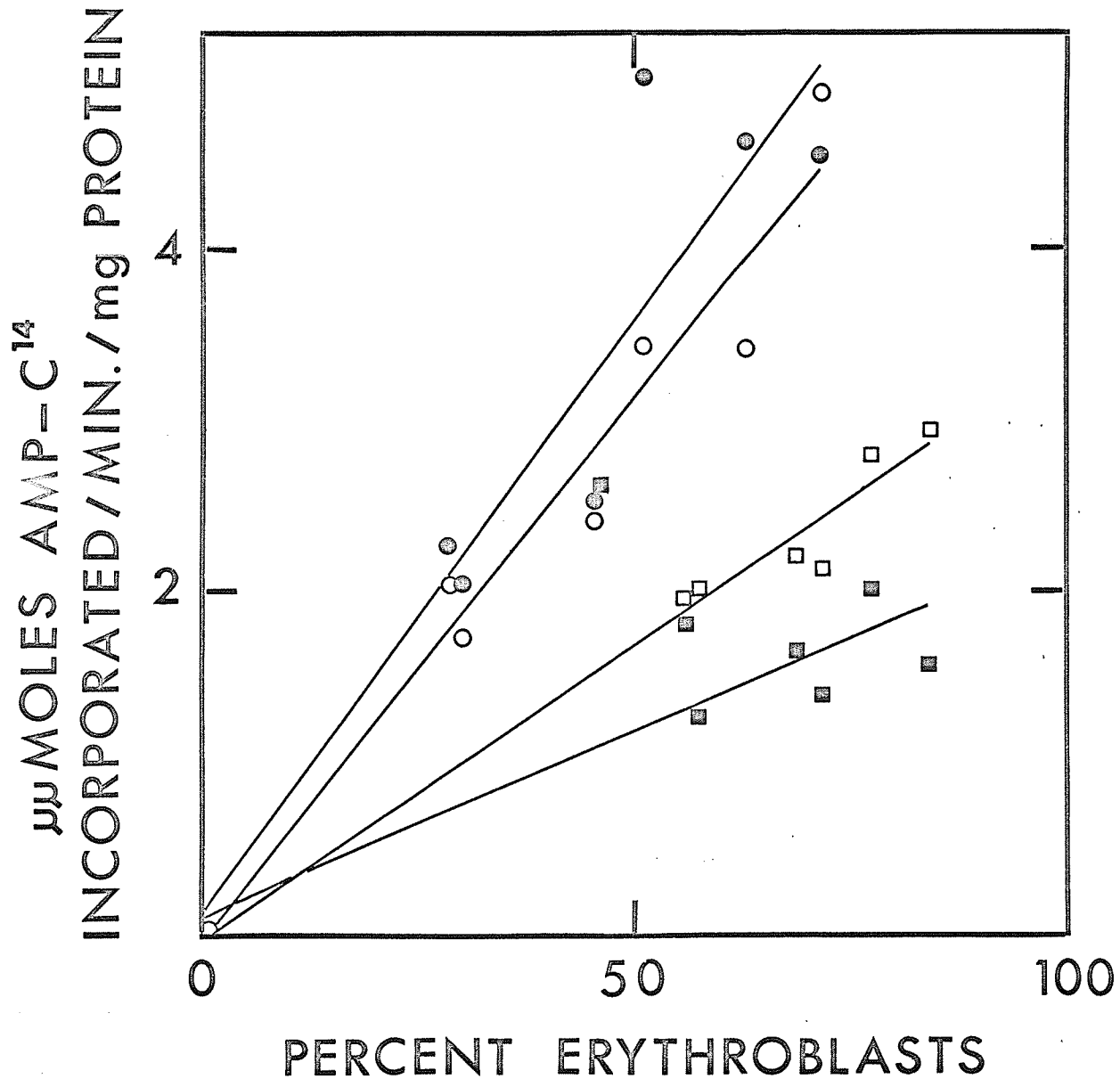
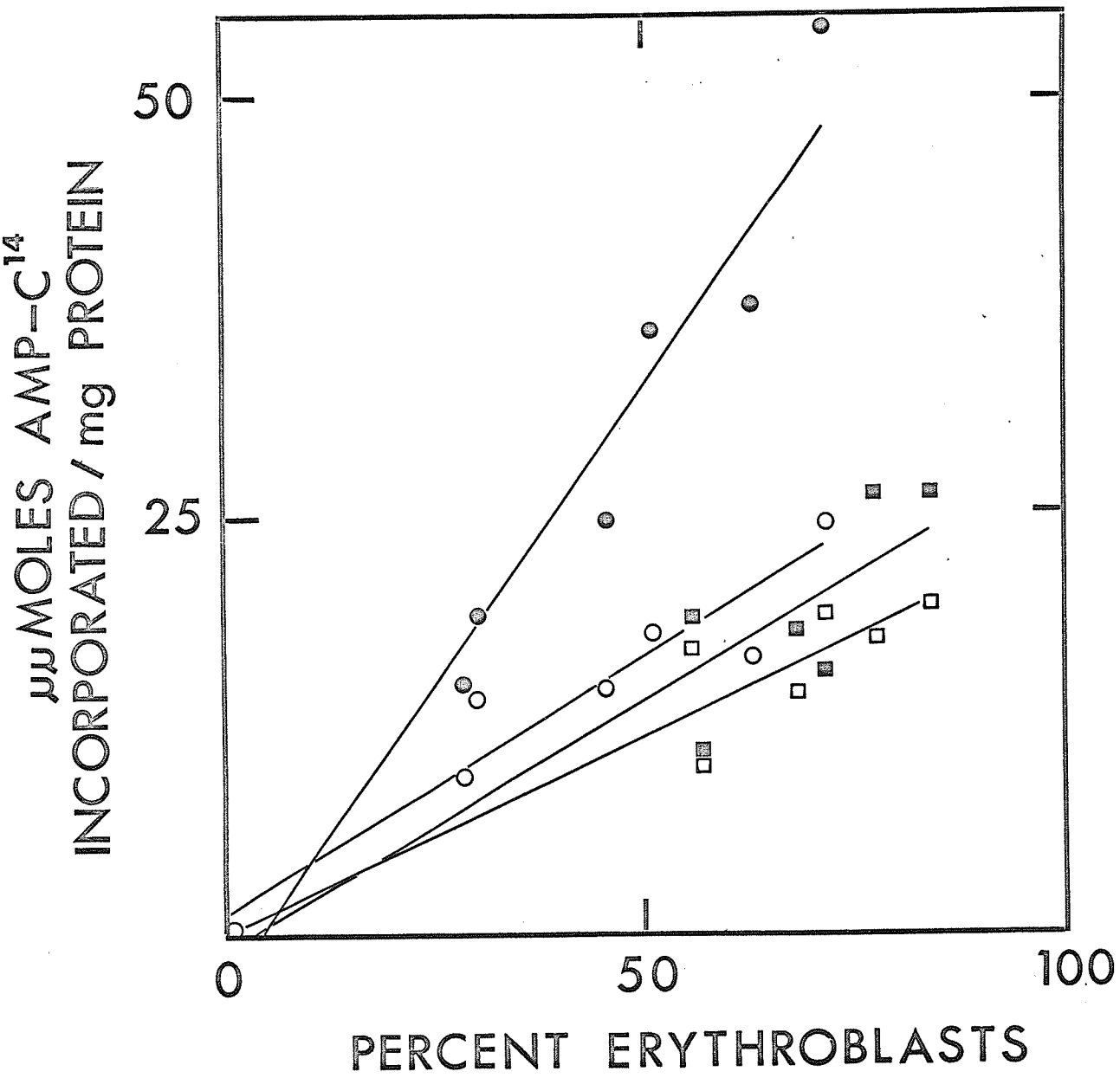

μμ MOLES AMP–C14 INCORPORATED / MIN. / mg PROTEIN
4
2
0
50
100
PERCENT ERYTHROBLASTS

Figure 26: Correlation between the total RNA polymerase activity and percent erythroblasts. Activity was measured under the standard conditions of assay for incubation periods up to 60 min for both " viral " nuclei in the absence (open circles) and presence (closed circles) of 0.35 M AmS and for " regen " nuclei in the absence (open squares) and presence (closed squares) of 0.35 M AmS. The total activity was maximum in all cases at 25 min and this value was plotted.


50
25
0
50
100
μμ MOLES AMP–C14 INCORPORATED / mg PROTEIN
PERCENT ERYTHROBLASTS

TABLES

Table I: Effect of inhibitors on RNA polymerase activity. Activity was measured under the standard conditions of assay with or without 10 μg of added inhibitor.

Table I

Effect of Inhibitors on RNA Polymerase Activity

Inhibitor			Activity ($\mu\mu$moles AMP-C^{14} incorporated/mg protein)			
			"Viral"		"Regen"	
			0 AmS	+ AmS	0 AmS	+ AmS
Act. D (10 ug)	5-min activity	0 Act. D	18.05	39.05	6.45	11.70
		+ Act. D	2.29	5.03	0.93	1.55
		% Inhibition	87.8	87.1	85.6	86.8
	15-min activity	0 Act. D	11.60	14.56	11.70	17.40
		+ Act. D	1.37	1.52	1.55	2.05
		% Inhibition	91.5	89.6	86.8	88.2
DNase (10 ug)	5-min activity	0 DNase	-	-	6.45	-
		+ DNase	-	-	1.93	-
		% Inhibition	-	-	70.1	-
	15-min activity	0 DNase	11.60	14.56	11.70	-
		+ DNase	3.64	4.13	3.36	-
		% Inhibition	68.1	71.6	71.3	-
RNase (10 ug)	5-min activity	0 RNase	9.04	-	5.75	-
		+ RNase	1.54	-	1.12	-
		% Inhibition	83.0	-	80.6	-
	15-min activity	0 RNase	11.60	14.56	-	17.40
		+ RNase	1.31	1.47	-	2.16
		% Inhibition	91.1	89.9	-	87.6

Table II: Cell composition of enzyme preparations.

Table II

Cell Composition of Enzyme Preparations

(Differential Cell Counts %)

Sample		Erythroblasts & Immature Red Blood Cells	Erythrocytes	Thromboblasts	Immature Thromboblasts	Thrombocytes	Metagranulo-blasts	Heterophils	Basophils	Lymphocytes	Nuclei from Broken Cells
"Regen"	(1)	77.5	18.2	0.4	0.0	0.8	0.0	1.5	0.6	0.6	0.4
	(2)	68.6	26.5	0.0	0.3	1.2	0.0	0.6	0.3	1.9	0.6
	(3)	84.1	11.0	0.9	0.0	0.9	0.0	0.9	0.0	1.8	0.4
	(4)	55.9	39.3	0.0	0.0	1.5	0.0	2.1	0.4	0.8	0.0
	(5)	57.1	39.3	0.6	0.0	0.0	0.0	2.4	0.0	0.6	0.0
	(6)	71.7	24.9	0.0	0.0	0.4	0.0	1.3	1.7	0.0	0.0
"Viral"	(1)	62.9	31.2	0.8	0.0	1.7	0.0	2.0	0.0	1.4	0.0
	(2)	28.5	66.1	1.1	0.8	0.8	0.0	1.1	0.8	0.8	0.0
	(3)	51.0	45.2	0.5	0.4	1.3	0.1	0.9	0.3	1.3	0.0
	(4)	30.0	65.5	0.3	0.3	0.9	0.0	0.9	0.6	0.9	0.6
	(5)	45.2	49.8	0.0	0.4	2.4	0.4	0.8	0.0	0.4	0.6
	(6)	71.5	23.2	1.0	0.7	0.7	0.0	1.1	0.7	0.8	0.3

Table III: Correlation between RNA polymerase activity and percent erythroblasts in " viral " preparations. Activity was measured under the standard conditions of assay in the presence or absence of 0.35 M AmS. Both the initial rates (obtained during the first 3 min of incubation) and total activity (at 25 min) were determined.

Table III

Correlation between RNA Polymerase Activity and Percent Erythroblasts in ''Viral'' Preparations

Erythroblasts (%)	Activity ($\mu\mu$moles AMP-C^{14} incorporated/mg protein)			
	0 AmS activity/ min	+ AmS activity/ min	0 AmS activity/ 25 min	+ AmS activity/ 25 min
0.0	0.00	0.00	0.00	0.00
28.5	2.05	2.28	9.49	14.94
30.0	1.73	2.04	14.02	19.03
45.2	2.41	2.52	14.67	24.57
51.0	3.43	5.00	17.95	36.00
62.9	3.41	4.64	16.50	37.62
71.5	4.91	4.53	24.49	54.21
y=bx + a				
a	-0.0302	0.1203	1.4619	-2.8652
b	0.0628	0.0698	0.3005	0.7140
Standard error of b	±0.0067	±0.0125	±0.0440	±0.0758
Standard error of estimate (S.E.E.)	±0.0067	±0.0626	±2.1957	±3.7832
Correlation coefficient (r)	0.9725	0.9278	0.9503	0.9729
P	0.001	0.001	0.01	0.001

Table IV: Correlation between RNA polymerase activity and percent erythroblasts in " regen " preparations. Activity was measured under the standard conditions of assay in the presence or absence of 0.35 M AmS. Both the initial rates (obtained during the first 3 min of incubation) and total activity (at 25 min) were determined.

Table IV

Correlation between RNA Polymerase Activity and Percent Erythroblasts in ''Regen'' Preparations

Erythroblasts (%)	Activity ($\mu\mu$moles AMP-C^{14} incorporated/mg protein)			
	0 AmS activity/ min	+ AmS activity/ min	0 AmS activity/ 25 min	+ AmS activity/ 25 min
0.0	0.00	0.00	0.00	0.00
55.9	1.97	1.83	17.13	18.82
57.1	2.01	1.29	10.20	10.65
68.6	2.21	1.65	14.33	18.23
71.7	2.14	1.40	19.18	15.82
77.5	2.81	2.01	17.82	26.10
84.1	2.94	1.58	19.55	26.36
$y=bx + a$				
a	-0.0034	0.1423	0.1850	-1.1441
b	0.0340	0.0211	0.2336	0.2988
Standard error of b	±0.0025	±0.0047	±0.0358	±0.0594
Standard error of estimate (S.E.E.)	±0.1465	±0.2720	±2.2381	±3.4473
Correlation coefficient (r)	0.9865	0.8959	-0.9381	0.9139
P	0.001	0.01	0.01	0.01