Structural Proteins of Oncogenic Ribonucleic Acid Viruses

**INTERSPEC II, A NEW INTERSPECIES ANTIGEN***

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**SUMMARY**

Two Rauscher murine leukemia virus polypeptides with apparent molecular weights of 69,000 and 71,000 have been purified and characterized. These polypeptides contained a previously undescribed interspecies antigenic determinant of mammalian oncogenic C type RNA viruses (interspec II), as was demonstrated by the reaction of the murine antigen with anti-feline leukemia virus serum. Radioimmunoassay analysis showed that both polypeptides were precipitated by the anti-feline virus serum, indicating that they each contained the interspecies determinant or that they were closely associated and coprecipitated. The interspec II antigen was distinguished from the known gs-3 interspecies antigen (interspec I) and the virus RNA-dependent DNA polymerase by protein purification, physical properties, and immunological analysis. The new antigen was not detected by competition radioimmunoassay in uninfected mouse cells or mouse cells productively infected with vesicular stomatitis virus.

The structural proteins of oncogenic C type RNA viruses have proven to be valuable reagents for analysis of virus gene expression and for characterization of viral particles. Recently, these proteins have become increasingly important in the search for possible oncogenic viruses in human neoplasia. Some of the most useful of these proteins are those containing antigenic determinants found in other viruses as well (group-specific (gs) antigens). Two classes of such antigens are known: (a) those that are found only in viruses of the same animal species (species-specific antigens), and (b) those that are present in viruses from animals of different species (interspecies antigens).

The presence of an interspecies antigen in C type RNA tumor viruses was first described by Geering et al. (1, 2). The protein containing this antigenic determinant has been purified in several laboratories and shown to be a major internal component of C type viruses with a molecular weight of about 30,000 (3-12). The antigen has been termed gs-3 (1, 2) or gs-interspec (5), but in this paper it will be referred to as interspec I so as to distinguish it from other interspecies antigens. Recently it was found that the RNA-dependent DNA polymerases of mammalian C type viruses also contain interspecies antigenic determinants. Antiserum against the polymerase of either murine virus or feline virus inhibited the DNA polymerase of mouse, rat, cat, or hamster viruses (13, 14).

We now report the isolation and characterization of other major Rauscher murine leukemia virus polypeptides containing an interspecies antigen. Until a standard nomenclature for the proteins of C type viruses is developed, we shall refer to this antigen as interspec II.

**METHODS**

**Purification of Virus Proteins**—Frozen Rauscher MuLV,1 92 mg of total protein in 50 ml of 50 mM sodium citrate, was thawed and dialyzed against 2 liters of 5 mM Tris-HCl, pH 7.6, and 1 mM EDTA for 4 hours with one change of dialysate. The virus was then centrifuged at 108,000 X g for 2 hours, and the pellet, 60 mg of protein, suspended in 20 ml of 5 mM Tris-HCl, pH 9.2, 1 mM EDTA, and 2 mM KCl. This virus suspension was sonicated for a total of 90 s in a Branson Sonifier, and then centrifuged at 108,000 X g for 2 hours. The supernatant, 18 mg of protein, was dialyzed for 15 hours against 2 liters of 10 mM N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid pH 6.5, and 1 mM EDTA, with one change of dialysate. The virus was then centrifuged and applied to a column (1.5 x 10 cm) of phosphocellulose (Whatman P-11) previously equilibrated with the same buffer. The column was washed with the starting buffer and then eluted at a rate of 10 ml per hour, with a total of 200 ml of buffer solution containing 10 mM BES, pH 6.5, 1 mM EDTA and a linear gradient of 0 to 1.0 mM KCl. Fractions of 2 ml were collected. Following the flow through of unabsorbed proteins, two major protein fractions were eluted. The first (phosphocellulose Fraction I) contained 6.2 mg of protein, and eluted at 2.5 to 15 mM KCl. The second phosphocellulose fraction contained 3.9 mg of protein and eluted at 20 to 27 mM KCl. Both of these fractions contained protein which was precipitated by anti-feline leukemia virus serum as well as with anti-MuLV serum. Phosphocellulose Fraction I

1 The abbreviations used are: MuLV, murine leukemia virus; BES, N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; FeLV, feline leukemia virus; RD, rhabdomyosarcoma; PBS, phosphate-buffered saline.
was found to contain two polypeptides (see "Results"), which were not further purified. The protein of the second phosphocellulose fraction was precipitated by the addition of (NH₄)₂SO₄ to 75% saturation and collected by centrifugation. The precipitate was suspended in 10 mM BES, pH 6.5, and 1.0 M NaCl, dialyzed, and applied to a column of Sephadex G-200 (1.5 × 50 cm) equilibrated in the same buffer. Fractions of 2.0 ml were collected at a flow rate of 10 ml per hour. The major peak (Sephadex fraction), 2 mg of total protein, contained an apparently homogeneous protein of 30,000 daltons (see "Results").

Purification of the Rickard FeLV 27,000-dalton structural protein containing the feline interspec I determinant was carried out in the same manner by phosphocellulose and Sephadex column chromatography.

**Antiserum**—Anti-Rauscher MuLV serum and anti-Rickard FeLV serum were obtained from rabbits injected with purified virus particles that had been degraded by incubation at 45°C for 1 min with 0.2% sodium dodecyl sulfate or 0.4% Triton X-100 and mixed with an equal volume of Freund's complete adjuvant. In some experiments, as indicated, the immunoglobulin was partially purified by Na₂SO₄ precipitation as described by Kekwick (15). Rabbit and goat antisera against the purified interspec I and interspec II antigens were prepared by multiple injections of purified proteins in Freund's complete adjuvant followed by booster injections in Freund's incomplete adjuvant. Horse anti-rabbit IgG was generously provided by Dr. R. Porter, Oxford University. Rabbit IgG containing antibodies against anti-rabbit IgG was kindly provided by Dr. E. Scolnick, and interspec II antigens were prepared by multiple injections of purified proteins in Freund's complete adjuvant followed by booster injections in Freund's incomplete adjuvant. Horse anti-rabbit IgG was generously provided by Dr. R. Porter, Oxford University. Rabbit IgG containing antibodies against anti-rabbit IgG was kindly provided by Dr. E. Scolnick, Meloy Labs, Springfield, Va.

**Radioimmunounassay**—Quantitative analysis of viral antigens was carried out by radioimmunounassay as described by Hunter (16). A similar assay has recently been reported by Scolnick et al. (17), Parks and Scolnick (18), and Oroszlan et al. (19). The reaction mixture contained the following: 0.03 ml of normal rabbit serum diluted 6-fold in a buffer solution containing 20 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM EDTA, and 2 mg per ml of Fraction V bovine serum albumin, 0.01 ml of [³²I]-labeled virus antibody (2 to 5 ng of protein containing 10⁶ to 10⁷ cpm per ng, as indicated), and 0.01 ml of diluted antiserum, as indicated. All antigen and antibody proteins were diluted in a buffer solution containing 20 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM EDTA, and 20 mg per ml of crystalline bovine serum albumin. The reaction mixture was incubated at 37°C for 3 hours, after which 0.03 ml of horse anti-rabbit IgG was added to precipitate the antigen-antibody complex. The mixture was incubated for 12 hours at 2-4°C. Cold buffer solution (0.5 ml) containing 20 mM Tris-HCl, pH 7.6, 100 mM NaCl, and 1 mM EDTA was added, and the precipitate was collected by centrifugation at 4°C. The pellet was washed twice with the same buffer solution, and the [³²I]-labeled antigen present in the precipitate was measured in a gamma counter.

The same reaction mixture was used for the competition radioimmunounassay. Competing proteins were diluted in a buffer solution containing 20 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM EDTA, and 90 mg per ml of crystalline bovine serum albumin, and were added in 0.01 ml immediately before the antiserum. When virus particles or cells were tested, Triton X-100, 0.4% final concentration, was added to the virus or cell preparation, and the suspension was incubated at 37°C for 10 min. In this case, the buffer solution used for serial dilution of the fractions also contained 0.4% Triton X-100.

Purified viral proteins, 2 to 5 μg, were labeled with [³²I] (10⁴ to 10⁶ cpm per ng of protein) by substitution of iodine into tyrosine by the chloramine-T procedure as described by Hunter (16). The protein of the second phosphocellulose fraction was precipitated by the addition of (NH₄)₂SO₄ to 75% saturation and collected by centrifugation. The precipitate was suspended in 10 mM BES, pH 6.5, and 1.0 M NaCl, dialyzed, and applied to a column of Sephadex G-200 (1.5 × 50 cm) equilibrated in the same buffer. Fractions of 2.0 ml were collected at a flow rate of 10 ml per hour. The major peak (Sephadex fraction), 2 mg of total protein, contained an apparently homogeneous protein of 30,000 daltons (see "Results").

**RNA-dependent DNA Polymerase**—Enzyme activity was measured in reaction mixtures of 0.1-ml total volume, containing 5 μmoles of Tris-HCl, pH 8.0, 1 μmole of dithiothreitol, 2 μmoles of KCl, 1 μmole of MgCl₂, 2.5 μmoles of [³H]TTP (140 cpm per pmole), 0.03% Nonidet P-40, 5 μmoles of poly(rA), 0.17 μmoles of oligo(dt₁₂-₁₈), and 1.0 to 10 μg total protein of purified Rauscher MuLV or virus subfractions.

After incubation at 37°C for 30 min the reactions were terminated by the addition of 3.0 ml of cold 5% trichloroacetic acid containing 20 mM NAPPP₁. The acid-insoluble radioactivity was collected on Whatman GF/C glass fiber discs, washed with the trichloroacetic acid-PP₁ solution, and measured by liquid scintillation spectrometry.

In experiments testing the effect of rabbit antipolymerase antibodies, the purified antisera immunoglobulin and equiva- lent control immunoglobulin were added to complete reaction mixtures prior to incubation. The polymerase activity present with 3 to 4 μg of purified virus was 50% inhibited by 15 to 20 μg of immunoglobulin protein. Proteins competing with the polymerase for antibody binding were tested in the same manner and were added immediately prior to the antiserum.

**Viruses**—Rauscher murine leukemia virus was kindly provided by Drs. S. Mayyas and D. Larson of the John L. Smith Memorial for Cancer Research. The virus was propagated in a BALB/c mouse bone marrow culture (JLS-V9) continuously infected with Rauscher virus as described by Wright et al. (20), harvested, and purified as described previously (21), and suspended in 50 mM sodium citrate.

A rat-tropic Kirsten strain of murine sarcoma virus, propagated in a clone of productively infected, transformed normal rat kidney cells (29), was a generous gift of Drs. M. Nicolson and R. M. McAllister, Children's Hospital of Los Angeles.

The Rickard strain of feline leukemia virus propagated in the F-422 suspended cell culture derived from the thymus gland of a leukemic cat (23) was received as a gift from Dr. J. Hoekstra, Rush-Presbyterian-St. Luke's Medical Center, Chicago. The virus was also obtained from Electro-Nucleonic Laboratories, Inc.

Gardner feline sarcoma virus, kindly provided by Dr. E. Scolnick, was propagated by Electro-Nucleonic Laboratories, Inc., in a cat embryo cell line (24).

The Gardner-Arnstein strain of feline leukemia virus propagated in a human rhabdomyosarcoma cell line (25) and RD-114, a C virus strain derived from RD cells that had been transplanted in a kitten (RD-114 cells) (26), were kindly provided by Drs. R. M. McAllister and M. Nicolson.

The Mason Piifer monkey virus propagated in a co-cultivated monkey mammary tumor cell line (27), was kindly provided by Dr. M. Ahmed of the John L. Smith Memorial for Cancer Research.

Woolly monkey virus (simian sarcoma virus, type 1), propagated in a productively infected and transformed fibroblastic cell line (HF) derived from a skin biopsy of a marmoset (28), was generously provided by Drs. F. Deinhardt and J. Hoekstra. Woolly monkey leukemia virus propagated in a clone of normal rat kidney cells nonproductively transformed by Kirsten murine sarcoma virus (29) was a gift of Dr. E. Scolnick.

Gibbon ape lymphosarcoma virus propagated in a virus-producing line of gibbon tumor cells (30) was a gift from Dr. J. Hoekstra. Gibbon ape leukemia virus propagated in the A204...
Fig. 1. Polyacrylamide gel electrophoresis of Rauscher MuLV proteins. Electrophoresis in a high resolution 5 to 20% gradient polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate was carried out as described by Baum et al. (34). A, standard proteins: 4 μg of cytochrome c (mol wt 11,700), 4 μg of chymotrypsinogen (mol wt 25,500), 5 μg of ovalbumin (mol wt 43,000), 3 μg of bovine serum albumin (mol wt 68,000), 8 μg of phosphorylase A (mol wt 94,000). B, Rauscher MuLV, 60 μg of protein. C, phosphocellulose Fraction I (interspec II), 7.5 μg of protein. D, Sephadex fraction (interspec I), 3.5 μg of protein.

RESULTS

Protein Purity and Molecular Weight—The homogeneity and molecular weight of the purified murine virus proteins were analyzed by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate. The phosphocellulose Fraction I contained two polypeptides with apparent molecular weights of 69,000 and 71,000 (Fig. 1C). These polypeptides correspond to major components of the crude virus and, as shown below, were found to comprise 5 to 10% of the protein of undegraded virus and to contain the interspec II antigen. In recent experiments it was found that this purified fraction also contains small amounts of sialic acid, glucosamine, galactose, and possibly fucose (data not shown), suggesting that these polypeptides comprise glycoprotein components of the virus. In this case the actual molecular weight of the molecules may be lower as the migration of glycoproteins in sodium dodecyl sulfate polyacrylamide gel electrophoresis is not an accurate measurement of size.

The Sephadex fraction contained an apparently homogeneous component of molecular weight 30,000 (Fig. 1D). This protein is the major structural component of the virus, and is known to contain interspec I, the previously reported interspecies antigenic determinant (gs-3 or gs-interspec) (1-10).

Gel Diffusion Evidence of Two Interspecies Antigens—Each of the fractions purified from Rauscher MuLV contained protein which reacted with anti-Felv serum as well as with anti-MuLV serum, indicating that both murine virus proteins contained antigens common to feline virus (Fig. 2). Moreover, the precipitin patterns of nonidentity indicated that the antigenic determinants were different. This was true for all of the determinants bound by the antimurine antibodies as well as the interspecies determinants bound by the antifeline antibodies. The
diffuseness of the precipitin line of interspec II was commonly observed and was often seen to resolve into two distinct bands; this is possibly related to the separation of the two polypeptides of this protein fraction. There appeared to be partial merging between the precipitin of interspec I and interspec II with antifeline serum but we believe this is an artifact due to nonspecific absorption of antiserum by the proteins or to adherence of the proteins to one another. As shown below, antiserum against each of the purified interspec antigens was monospecific even in the highly sensitive radioimmunoassay.

Radioimmunoassay of Interspec II.—In order to determine whether one or both of the polypeptides contained the interspec II antigen, binding of the proteins by anti-MuLV serum and anti-FeLV serum was quantitatively measured by the radioimmunoassay. All of the acid-precipitable radioactivity of the 

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Cell Fractionation and Separation of Interspec II from DNA Polymerase.—Another virus protein reactive with antisera directed against viruses of different species is the RNA-dependent DNA polymerase (13, 14). For this reason, several experiments were carried out to distinguish interspec II polypeptides from the virus polymerase, especially as the mass of the enzyme has been reported as 70,000 daltons (37). Studies of the fractionation of interspec II polypeptides and the polymerase protein showed that they could be completely separated from one another (Table I). All of the antigenic activity of the interspec II but none of the polymerase was present in a supernatant fraction after freezing and thawing the virus, whereas almost all of the polymerase and none of the antigen remained in the particulate fraction after sonication. In other experiments (data not shown) it was found that interspec II and the polymerase were antigenically distinct as well. Anti-interspec II serum with a titer of 1:12,000 against 5 ng of interspec II antigen by radioimmunoassay did not inhibit the polymerase activity of 3.4 μg of Rauscher virus even when added in vast excess (a final dilution of 1:40 in the enzyme reaction mixture). Moreover interspec II failed to absorb antipolymerase IgG. As much as 0.8 μg of interspec II added with 3.4 μg total of virus protein did not show competition for antipolymerase antibody under conditions of limiting antibody in the reaction mixture. We have

Because the molecular weights of the interspec II polypeptides were similar to that of bovine serum albumin, the possible presence of the interspec II antigens in fetal calf serum was tested by the competition radioimmunoassay. There was no competition of fetal calf serum with interspec II for antibody binding, tested to a 3000-fold greater concentration of serum proteins. We conclude that the interspec II polypeptides are not constituents of fetal calf serum.

Comparison of Interspec I and II by Monospecific Antisera—Antisera prepared against the two purified proteins reacted only with their specific interspecies type, when analyzed either by gel diffusion (Fig. 5) or radioimmunoassay (Fig. 6), thus providing further evidence that these antigens represent two distinct classes of interspecies determinants.

Comparison of Interspec I and II by Competition Radioimmunoassay—Additional evidence that the interspecies antigenic determinants of interspec II differed from those of interspec I was obtained by use of the competition radioimmunoassay. In these experiments competition between the interspecies determinants of the two murine proteins for binding by antiFeLV serum was tested with both 125I-interspec I and 125I-interspec II antigens (Fig. 4). Binding of MuLV 125I-interspec I antigen by anti-FeLV serum was not blocked by addition of interspec II; nor was binding of the MuLV 125I-interspec II antigen by anti-FeLV serum blocked by FeLV interspec I. In control experiments binding of the labeled proteins was blocked by unlabeled counterparts, as expected. Thus, no antigenic cross-reactivity could be demonstrated between the interspecies determinants of the two proteins by this highly sensitive assay.

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2 The extent of release of the interspec II polypeptides by freezing and thawing has been variable with different batches of virus, the ionic environment, and virus concentration. In every case, the proteins have been completely solubilized by combined freezing and thawing and sonication.
FIG. 5. Gel diffusion analysis of goat anti-interspec I and rabbit anti-interspec II sera. The procedure was as described in Fig. 2. The antigens were 2 μg of interspec I and 10 μg of interspec II, and 25 μl of undiluted antiserum was added as indicated.

FIG. 6. Radioimmunoassay analysis of anti-interspec I and anti-interspec II sera. The assay was performed as described under “Methods” with A, 5 ng of 125I-MuLV interspec II antigen (10,000 cpm per ng), or B, 2.5 ng of 125I-MuLV interspec I antigen (18,600 cpm per ng). Rabbit anti-interspec I or rabbit anti-interspec II serum was added as indicated.

concluded that interspec II is not an antigenic determinant of the virus DNA polymerase.

Species Distribution of Interspec II—The distribution of interspec II in different animal cell viruses was tested by use of the competition radioimmunoassay with 125I-interspec II and anti-FeLV antiserum (Table II). Murine and feline viruses contained equivalent amounts of the competing protein. In contrast, none of the primate viruses or RD-114, at protein concentrations up to 100-fold greater than that of the murine and feline viruses, showed any competition. Nor was the protein found in AMV, herpes virus, nononcogenic viruses, or mycoplasma.

Analysis of Uninfected Cells—In studies of the genetic origin of the interspec II antigen, analysis was made of uninfected cells to investigate the possibility that this protein is constitutively expressed in the host. Because of the possibility that interspec II is a membrane component, this experiment was also carried out with host cells infected with vesicular stomatitis virus, another membrane maturing virus. Under assay conditions which could detect interspec II at a concentration 10^-4 of the total cell protein, none was found in the uninfected cell or cells productively infected with vesicular stomatitis virus (Fig. 7). In control experiments, a competing protein was readily detected in cells productively infected with Rauscher MuLV.

DISCUSSION

It may be hypothesized that the interspecies antigens of the mammalian animal cell RNA tumor viruses are components of proteins that are involved in specific functions common to these viruses and that have been conserved during evolution of different species. These structures could be expected to include

| Fraction | Total protein | Interspec II total protein | Polymerase activity |
|----------|---------------|---------------------------|---------------------|
| 1. Virus | 30 | 3 | 4.4 |
| 2. Frozen-thawed | Supernatant: 7, Pellet: 21 | 3, 0.1 | 7.2 |
| 3. Sonicated | Supernatant: 9, Pellet: 12 | 0.1, <0.001 | 0.9, 11.0 |

* Rauscher MuLV, propagated and purified as previously described (16), was concentrated by high speed centrifugation, suspended in 20 mM Tris, pH 7.6, 100 mM NaCl, and 1 mM EDTA; 30 mg of total protein was dialyzed against 2 liters of 5 mM Tris, pH 7.6, and 1 mM EDTA for 2 hours (Fraction 1). The virus was then frozen in Dry Ice and methanol and thawed at 25°C in a water bath, and the suspension centrifuged at 108,000 × g for 2 hours. The supernatant was retained (Fraction 2 supernatant), and the pellet (Fraction 2 pellet) was suspended in 5 mM Tris, pH 9.2, 1 mM EDTA, 1 mM dithiothreitol, 1% Triton X-100, and 20% glycerol, sonicated, and centrifuged at 108,000 × g for 2 hours. The soluble fraction was retained (Fraction 3 supernatant). The pellet (Fraction 3 pellet) was solubilized in 20 mM Tris, pH 9.0, 1 mM dithiothreitol, 1% Triton X-100, and 20% glycerol.

† Interspec II was measured by competition radioimmunoassay as described under “Methods” with 5 ng of 125I-interspec II antigen (16,000 cpm per ng) as antigen and a 1:450 final dilution of rabbit anti-FeLV serum. Before assay, Triton X-100, 0.4% final concentration, was added to each fraction, except the sonicated pellet which already contained the detergent, and the fractions were incubated for 10 min at 37°C. The buffer solution used for the serial dilution of the antigen also contained 0.4% Triton X-100. The concentration of interspec II in the virus fractions was calculated by comparing the competition of the fraction to that of pure interspec II antigen.

‡ Virus RNA-dependent DNA polymerase was measured as described under “Methods.”
active sites of the RNA-dependent DNA polymerase, the RNA packaging protein or proteins, and the structural proteins that determine the configuration of the particles. Only three such proteins with interspecies homology are now known, interspec II (1, 2), the RNA-dependent DNA polymerase (13, 14), and interspec II, but there could well be others present as minor components of the virus or not expressed as strong antigens. It appears reasonable to expect that the maximum number of such proteins is governed by the size of the virus genome. Although genetic evidence of proteins coded for by the virus is available only for the DNA polymerase (38), the selective nature of the interspec antigenic determinants and the absence of any evidence for these proteins in uninfected cells strongly suggests that they are virus coded. At this time the apparent mass of probable virus-specific proteins is about 400,000 daltons, comprising the DNA polymerase and the proteins containing the type-specific, species-specific, and interspec II determinants (1–10, 37). As the total mass of the viral RNA is about 10^6 daltons, many more virus proteins could be accommodated if the genome is non-repetitive. However, if the genome is polyploid and contains 3 to 4 repeating 35 S subunits of 3.0 to 3.5 × 10^6 daltons, as is suggested by genetic studies, then these proteins already appear to comprise the apparent limit of what might be coded for by such a subunit.

At this time the interspec II polypeptides and monospecific antisera are useful reagents for virus characterization and for analysis of viral gene expression. The species distribution of interspec II shows a strong homology between the murine and feline sarcoma or leukemia viruses, as is also demonstrated by interspec II and the polymerase protein. It can be expected that rat and hamster viruses also contain the interspec II determinant because of the known similarity of these viruses (10, 12, 32). No evidence of antigenic homology between interspec II and proteins of RD-114 and primate viruses was obtained from competition radioimmunoassay analysis. We believe, however, that this lack of competition is due to a low affinity of the antisera because of the known similarity of these viruses (10, 12, 32).

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Structural Proteins of Oncogenic Ribonucleic Acid Viruses: INTERSPEC II, A NEW INTERSPECIES ANTIGEN
Mette Strand and J. Thomas August

J. Biol. Chem. 1973, 248:5627-5633.

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Additions and Corrections

Vol. 248 (1973) 4956-4964

In Buchanan, Bob B., and Peter Schurmann. Regulation of Ribulose 1,5-Diphosphate Carboxylase in the Photosynthetic Assimilation of Carbon Dioxide.

Page 4957, in the section on "Purification of Ribulose-1,5-P Carboxylase" Paragraph 5, Line 4; Paragraph 6, Line 8; Paragraph 7, Line 3; Paragraph 8, Line 3; Paragraph 9, Line 3, all concentrations of Tris·HCl and N-tris(hydroxymethyl)methylglycine buffer should read:

M instead of mM

Vol. 248 (1973) 5627-5633

In Strand, Mette, and J. Thomas August. Structural Proteins of Oncogenic Ribonucleic Acid Viruses. Interspecific Assimilation of Carbon Dioxide. II, a New Interspecies Antigen.

Page 5627, right-hand column, Lines 3 to 5 from the bottom should read:

mg of protein, and eluted at 25 to 150 mM KCl. The second phosphocellulose fraction contained 3.9 mg of protein and eluted at 200 to 270 mM KCl.

Vol. 248 (1973) 4756-4762

In Winters, Mary Ann, and Mary Edmonds. A Poly(A) Polymerase from Calf Thymus. Purification and Properties of the Enzyme.

Page 4758, left-hand column, Line 4 should read:

... which has been equilibrated with 0.02 M potassium phosphate, pH 6.0 instead of 0.20 M potassium phosphate.

Vol. 248 (1973) 6471-6478

In So1off, Melvyn S., and Theodore L. Swartz. Characterization of a Proposed Oxytocin Receptor in Rat Mammary Gland.

Page 6471, "Summary," Lines 27 and 28; Page 6475, right-hand column, Line 5; Page 6477, left-hand column, Lines 15 and 16:

iodoacetate should read: iodoacetamide.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.