Relation of Ornithine Decarboxylase and Tyrosine Kinase Activity in the Jejunal Mucosa *In Vivo* (43978)

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Abstract. **Our aim was to study the relationship between jejunal mucosal activity of ornithine decarboxylase and tyrosine kinase during proliferation in adolescent rats *in vivo*. Their relationship in the proliferating intestinal mucosa under *in vivo* conditions has not been reported before. From the results of *in vitro* studies, it was speculated that tyrosine kinase activity modulated ornithine decarboxylase activity during colonic mucosal proliferation (Majumdar AP. Am J Physiol 259:G626–G630, 1990). Jejunal mucosal hyperplasia was induced by Type I diabetes and suppressed in both control and diabetic rats by administration of difluoromethylornithine. Jejunal mucosal weight and enzyme activity were determined after 3, 6, and 10 days, and tyrosine-specific phosphorylated proteins after 10 days of induction of diabetes. Difluoromethylornithine suppressed jejunal mucosal proliferation and tyrosine kinase activity after the 6- and 10-day study periods. After the 3-day study period although jejunal mucosal growth was suppressed, tyrosine kinase activity was not. Activity of tyrosine kinase and ornithine decarboxylase were highly significantly correlated at all time periods in both control and diabetic rats. Tyrosine-specific phosphorylated proteins of 34, 54, 80, and 200 kDa proteins were observed in jejunal mucosa of both control and diabetic rats. In the difluoromethylornithine-treated rats, phosphorylation of the above proteins was negligible while the phosphorylation of a 14-kDa protein was prominent. We speculate that *in vivo* ornithine decarboxylase activity may be modulating tyrosine kinase activity and that phosphorylation of a 14-kDa protein was associated with suppressed mucosal growth in difluoromethylornithine-treated rats.**
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Activity of the enzymes ornithine decarboxylase and tyrosine kinase increase during the process of cell division. However, the relationship between these enzymes and how they influence cell multiplication is not well understood. In studies *in vitro*, exposure of rat colonic mucosal explants to gastrin greatly enhanced the activity of both ornithine decarboxylase and tyrosine kinase in the explants (1). While the maximal stimulation of tyrosine kinase occurred within 10–15 min of exposure to gastrin, ornithine decarboxylase activity was significantly stimulated 2 hr after exposure to this hormone. Administration of difluoromethylornithine (an irreversible inhibitor of ornithine decarboxylase) completely abolished the gastrin-mediated stimulation of ornithine decarboxylase activity, but not of tyrosine kinase activity. On the other hand, administration of genistein (a specific irreversible inhibitor of tyrosine kinase) caused a total suppression of the gastrin-induced stimulation of both tyrosine kinase and ornithine decarboxylase activity. Because genistein abolished these responses in the mucosal explants, it was thought that tyrosine kinase modulated the response of ornithine decarboxylase to the mitogens in these *in vitro* studies (1).

In studies *in vivo*, administration of bombesin for 2 weeks to rats resulted in significant increases in mu-

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cosal DNA synthesis and activity of ornithine decarboxylase and of tyrosine kinase (2). Activity of ornithine decarboxylase was also reported to be elevated during intestinal mucosal hyperplasia noted after induction of streptozotocin diabetes in rats (3, 4). Administration of difluoromethylornithine abolished the mucosal hyperplasia in the diabetic rats and markedly reduced mucosal ornithine decarboxylase activity. The above observations suggest that, similar to the situation *in vitro,* activity of ornithine decarboxylase and of tyrosine kinase are important for intestinal mucosal growth *in vivo*. However, the relationship between the activity of ornithine decarboxylase and of tyrosine kinase during intestinal mucosal hyperplasia has not been established *in vivo*. Tyrosine-specific phosphorylated proteins of 55, 80, and 100 kDa were found to be associated with intestinal mucosal proliferation in studies *in vitro* (5). The present studies were undertaken to investigate the relationship between intestinal mucosal growth and its ornithine decarboxylase and tyrosine kinase activities *in vivo* in control and streptozotocin diabetic rats.

Materials and Methods

Rats. Sprague-Dawley adolescent male albino rats (150–160 g; Harlem, Indianapolis, IN) were divided into weight-matched control and diabetic groups. One half of each group was given difluoromethylornithine in drinking water. Housing conditions, feeding method, induction and documentation of diabetes, administration of DFMO, and collection and processing of scrapped mucosa were similar to those described in previous publications (3, 4). Rats were studied on the 3rd, 6th, and 10th day after induction of diabetes.

Determination of Ornithine Decarboxylase Enzyme Activity. Ornithine decarboxylase–specific activity was determined by the amount of $^{14}CO_2$ released from L-(1-^{14}C)-ornithine by the mucosal homogenates. This method has been described previously (3, 4).

Determination of Tyrosine Kinase Activity. Tyrosine kinase activity was determined by a modification method described by Majumder *et al.* (6). Six hundred (600) mg of mucosal scrapings were homogenized in 1 ml of homogenizing buffer (10 m*M* HEPES, pH 7.4, 150 m*M* NaCl, 1 m*M* $MgCl_2$, 1 m*M* Na_3VO_4, 20 μg/ml leupeptin, 20 mg/ml pepstatin, 20 mg/ml aprotonin, 2 m*M* phenylmethylsulfonyl fluoride and 0.05% Triton X-100) and immediately centrifuged at 30000*g* for 30 min. The supernatant was suspended in 350 μl of ice-cold homogenizing buffer. An aliquot of the supernatant containing 40 μg protein was added to a reaction mixture in a final volume of 100 μl, containing 3 μmol of Tris HCl (pH 7.4), 3 μmol ATP, 0.6 μCi [γ-^{32}P] ATP (New England Nuclear, Boston, MA) and 150 μg of polymeric L-Glu-L-Tyr (4:1) (Sigma Chemical Co., St. Louis, MO) as substrate. The reaction was run at room temperature and stopped after 10 min by spotting 40 μl of the reaction mixture onto 3 cm^2 Whatman No. 3 MM filter paper. The filters were washed with 20 ml of washing solution containing 12% TCA and 10 m*M* sodium pyrophosphate and rinsed with 95% ethanol. The filters were air dried and counted in 10 ml of scintillation cocktail (Ultima Gold-Packard Instruments, Chicago, IL). The tyrosine kinase–specific activity was expressed as picomoles of ^{32}P incorporated per milligram of protein.

Determination of Tyrosine-Specific Phosphorylated Proteins. Tyrosine-specific protein phosphorylation was determined in the jejunal mucosal homogenates of the rats at the 10th day study period by modifying the method described by Dangott *et al.* (7). An aliquot containing 100 μg protein from the homogenate of the mucosal scrapings (used for the determination of tyrosine kinase specific activity) was incubated at 4°C for 1, 3, 5, 8, 12, and 15 min with a reaction mixture containing 3 μmol HEPES (pH 7.4), 3 μmol $MgCl_2$, 1 nmol of Na_3VO_4, 0.6 μCi [γ-^{32}P] ATP and 0.01% Triton X-100. The reaction was terminated by adding 40 μl of buffer containing 62.5 m*M* Tris HCl, pH 6.8, 10% sodium dodecyl sulfate, 10% glycerol, 10% mercaptoethanol, and 0.05% bromophenol blue, and incubating at 95°C for 4–5 min. The reaction mixture was briefly vortexed and then centrifuged for 10 sec. The supernatant was electrophoresed for about 3 hr on a 12% polyacrylamide gel (1.5 mm) containing 0.1% sodium dodecyl sulfate. After electrophoresis the gels were fixed for 10 hr at room temperature, with mild agitation in fixing buffer (methanol, acetic acid, and water 5:1:4) and treated with 1 *M* KOH at 57°C for 2 hr in a shaker water bath. Following this treatment, gels were washed twice with distilled H_2O with mild agitation for 20 min, fixed overnight in 10% acetic acid and 10% methanol in 1:1 proportion, and dried under vacuum using a Bio-Rad gel dryer at 80°C. The gels were then exposed to autoradiography film (New England Nuclear) for 3 days at −70°C. Prestained molecular weights (Amersham Radiochemicals, Chicago, IL) standards were electrophoresed concurrently. Radioactivity of the tyrosine specific phosphorylated proteins were quantitated using a phosphoimager (Packard Instruments, Chicago, IL).

Statistical Analysis. Mean and SEM were calculated for all parameters determined. Data from 7–13 separate rats were used to calculate mean values for a given parameter. Significance was determined using analysis of variance (ANOVA). The unpaired student *t* test was used to determine statistical significance between corresponding mean values. Values of $P < 0.05$

were considered to indicate statistically significant difference between the corresponding mean values.

Results

Data pertaining to the rats studied are shown in Table I.

Body Weight. Throughout the study period, control rats gained weight, while diabetic rats lost weight. Body weight change in the difluoromethylornithine-treated rats was not significantly different from that in the corresponding nontreated control and diabetic rats.

Blood Glucose. Mean ± SEM blood glucose (mg/dl) in diabetic rats (400 ± 20) was over 2-fold greater ($P < 0.01$) than in control rats (168 ± 15) at all three time periods studied. In the difluoromethylornithine-treated rats, blood glucose levels were similar to those in the corresponding untreated control and diabetic rats.

Water and Food Intake. Diabetic rats were polydipsic and polyphagic compared with control rats. In the difluoromethylornithine-treated rats, water and food intake was similar to the corresponding untreated control and diabetic rats.

Data for the jejunal mucosal parameters studied are shown in Table II.

Jejunal Mucosal Weight. Weight of the scraped jejunal mucosa expressed as weight per centimeter became significantly higher in the nontreated diabetic than in the nontreated control rats after the 6th day of induction of diabetes ($P < 0.05$). In the difluoromethylornithine-treated control and diabetic rats, jejunal mucosal weight was significantly lower than in the corresponding control and diabetic rats at all three time periods studied ($P < 0.01$). Weight of the jejunal mucosa increased significantly over the 10-day study period in the control, diabetic, and difluoromethylornithine-treated control and diabetic rats ($P < 0.05$).

Jejunal Mucosal DNA Content. Mean ± SEM DNA content of the jejunal mucosal (not shown on Table II) was determined on the 10th day after induction of diabetes and was significantly greater ($P < 0.05$) in the diabetic (25 ± 2) than in the control (19 ± 2) rats. Administration of difluoromethylornithine had significantly ($P < 0.05$) reduced mean jejunal mucosal DNA content in both the control (8 ± 3) and the diabetic (10 ± 3) rats.

Jejunal Mucosal–Specific Activity of Ornithine Decarboxylase. As depicted in Table II jejunal mucosal activity of ornithine decarboxylase in the nontreated rats was significantly lower in the control than in the diabetic rats at all three study periods ($P < 0.05$ to $P < 0.001$). In both control and diabetic nontreated rats jejunal mucosal ornithine decarboxylase activity increased by 50% between the 3rd and 6th day, and by 20% between the 6th and 10th day study periods. On the 10th day study period, mean jejunal mucosal ornithine decarboxylase activity was over 2-fold and significantly greater than the corresponding values at the 3rd day study period ($P < 0.01$). In the difluoromethylornithine-treated rats, activity of ornithine decarboxylase in the jejunal mucosal was significantly lower than in the corresponding nontreated control and diabetic rats ($P < 0.01$). On the 3rd and 6th day study periods, difluoromethylornithine reduced ornithine decarboxylase activity by around 50%, and on the 10th day study period by over 80%.

Jejunal Mucosal–Specific Activity of Tyrosine Kinase. In both control and diabetic nontreated rats jejunal mucosal activity of tyrosine kinase increased by 50% between the 3rd and 6th day, and by around 100% between the 6th and 10th day study periods. Mucosal activity of tyrosine kinase was significantly lower in control than in diabetic rats at 3, 6, and 10 days after induction of diabetes ($P < 0.01$). Administration of difluoromethylornithine in control rats sup-

Table I. Data Pertaining to the Rats Studied

	Control rats			Diabetic rats[a]		
	3rd day	6th day	10th day	3rd day	6th day	10th day
Change in body wt (g/day)						
−DFMO[b]	2.5 ± 0.3	3.8 ± 0.8	7.1 ± 0.5[c]	−1.0 ± 0.6	−1.5 ± 0.5	−3.1 ± 0.5
+DFMO	2.4 ± 0.8	3.9 ± 0.9	7.4 ± 0.5[c]	−1.2 ± 0.5	−1.5 ± 0.6	−2.8 ± 0.4
Water intake (ml/day)						
−DFMO	22 ± 3	30 ± 3	40 ± 2[c]	40 ± 3	80 ± 5	110 ± 2.0[c]
+DFMO	20 ± 3	33 ± 3	41 ± 2[c]	39 ± 3	82 ± 4	112 ± 3.0[c]
Food intake (g/day)						
−DFMO	10 ± 2	15 ± 2	24 ± 2	15 ± 1	23 ± 2	39 ± 3[c]
+DFMO	11 ± 3	14 ± 2	26 ± 2	17 ± 2	23 ± 2	35 ± 3[c]

Note. Values are means ± SEM.

[a] All mean values for diabetic rats are significantly different than corresponding mean values in control rats ($P < 0.01$–0.001).

[b] DFMO, difluoromethylornithine; −DFMO, untreated rats; +DFMO, rats administered DFMO in drinking water.

[c] Mean value at the 10th day study period significantly different than the corresponding mean value at the 3rd day study period ($P < 0.05$–0.001).

Table II. Jejunal Mucosal (J muc) Parameters Studied

	Control rats			Diabetic rats		
	3rd day	6th day	10th day	3rd day	6th day	10th day
J muc wt (mg/cm)						
−DFMO[a]	39 ± 2	46 ± 4	59 ± 4[b]	46 ± 3	59 ± 4	76 ± 3[b,c]
+DFMO	25 ± 3	32 ± 3	45 ± 3	34 ± 2	43 ± 2	49 ± 2
ODC activity[d]						
−DFMO	6.3 ± 0.7	9.9 ± 0.8	12.1 ± 1.2[b]	10.7 ± 1.0	14.8 ± 1.2	34.8 ± 2.8[b,c]
+DFMO	3.1 ± 0.6	4.6 ± 0.6	2.0 ± 0.1	6.8 ± 0.4	7.2 ± 0.3	6.0 ± 0.5
Tyr K activity[e]						
−DFMO	1.0 ± 0.1	1.5 ± 0.2	3.0 ± 0.2[b]	1.8 ± 0.1	3.1 ± 0.2	7.2 ± 0.4[b,c]
+DFMO	0.8 ± 0.1[f]	0.3 ± 0.1	0.5 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1

Note. Values are means ± SEM.
[a] DFMO, difluoromethylornithine; −DFMO, untreated rats; +DFMO, rats administered DFMO in drinking water.
[b] Mean value in rats studied on the 10th day significantly different than corresponding mean value at 3 days.
[c] Mean value in diabetic rats significantly different than corresponding mean value in control rats $P < 0.05$.
[d] Picomoles of $^{14}CO_2$ released/per milligram protein.
[e] Picomoles of ^{32}P incorporated per milligram protein in 10 min.
[f] Mean value in control + DFMO rats not significantly different than corresponding mean value in control −DFMO rats.

pressed tyrosine kinase activity at the 6th and 10th but not at the 3rd day study period, while in diabetic rats administration of difluoromethylornithine suppressed tyrosine kinase activity at all three study periods ($P < 0.01$).

Correlations Between DNA Content and Specific Activity of Ornithine Decarboxylase and of Tyrosine Kinase in the Jejunal Mucosa. The correlation between jejunal mucosal DNA content and specific activity of ornithine decarboxylase and tyrosine kinase for rats on the 10th day study period is shown Figure 1. Significant ($P < 0.05$) linear positive correlations were observed between jejunal mucosal DNA content and its ornithine decarboxylase and between DNA and tyrosine kinase activity.

Correlation Between Jejunal Mucosal Ornithine Decarboxylase and Tyrosine Kinase Activity. Figure 2 depicts the highly significant ($P < 0.001$) positive linear correlation between jejunal mucosal ornithine decarboxylase and its tyrosine kinase activity in the control (C), diabetic (D), and difluoromethylornithine (DFMO)–treated control (C + DFMO) and diabetic (D + DFMO) rats studied on the 10th day after induction of diabetes. Similar highly significant positive correlations between jejunal mucosal ornithine decarboxylase and tyrosine kinase activity were observed in the rats studied on the 3rd day ($r = 0.96$; $P < 0.001$) and the 6th day ($r = 0.86$; $P < 0.001$), and in the combined data of all the rats studied at the three periods ($r = 0.98$; $P < 0.001$).

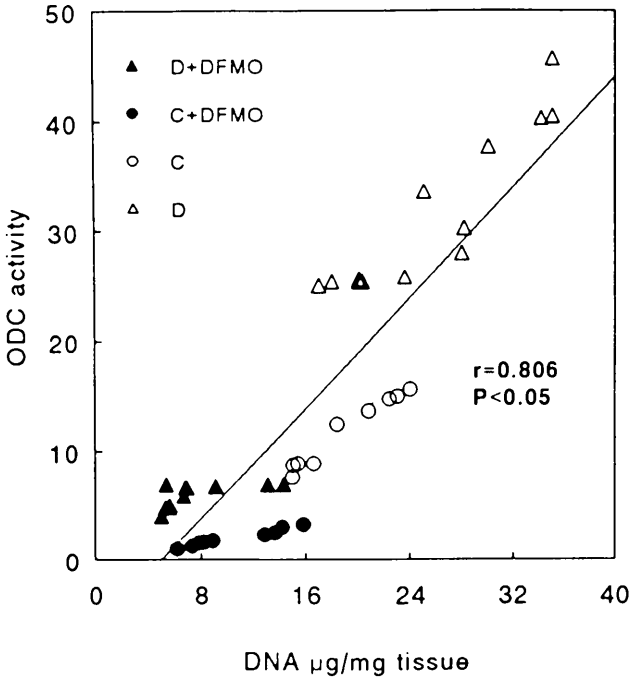

Figure 1. Correlation between DNA content and specific activity of ornithine decarboxylase (ODC) in the jejunal mucosa of control (C), diabetic (D), and difluoromethylornithine (DFMO)–treated control (C + DFMO) and diabetic (D + DFMO) rats studied on the 10th day after induction of diabetes. The symbols represent mean of duplicate determination in individual rats. ODC activity was determined as picomoles of $^{14}CO_2$ released by mucosal homogenates from ^{14}C-L-ornithine per milligram of protein per hour. In the combined data of the four groups of rats studied, a significant positive correlation was observed between DNA content and ODC activity in the jejunal mucosa.

Jejunal Mucosal Tyrosine-Specific Phosphorylated Proteins. Tyrosine-specific phosphorylated proteins in the jejunal mucosa are shown in Figure 3, a and b. The proteins showing tyrosine specific phosphorylation in the jejunal mucosa of the rats studied on the 10th day after induction of diabetes showed molecular weight of 14, 34, 54, 80, and 200 kDa in both the control and diabetic rats. In the nontreated rats tyrosine-specific phosphorylation of all 5 proteins increased significantly ($P < 0.05$) over the first 3–8 min in control rats and over the first 3–10 min in the diabetic rats. In the difluoromethylornithine control and diabetic treated rats, tyrosine-specific phosphoryla-

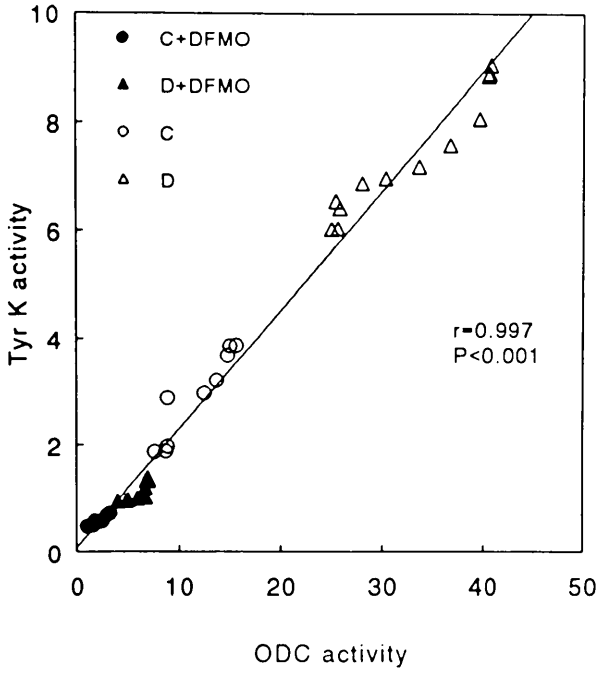

Figure 2. Correlation between specific activity of ornithine decarboxylase (ODC) and specific activity of tyrosine kinase (TyrK) in jejunal mucosa of control (C), diabetic (D), and difluoromethylornithine (DFMO)–treated control (C + DFMO) and diabetic (D + DFMO) rats studied on the 10th day after induction of diabetes. The symbols represent mean of duplicate determinations in individual rats. In the combined data of the four groups of rats studied, a highly significant direct correlation was observed between ODC activity and TyrK activity in the jejunal mucosa.

tion of the 34-, 54-, 80-, and 200-kDa proteins was markedly suppressed. However, tyrosine-specific phosphorylation of the 14-kDa protein became prominent especially in the diabetic rats. Densitometric analysis revealed statistically significant higher ($P < 0.01$) rates of tyrosine-specific phosphorylation in diabetic than in control rats after the 5-min incubation period. In the difluoromethylornithine-treated control and diabetic rats, except for tryrosine-specific phosphorylation of the 14-kDa protein, tyrosine phosphorylation of the 34-, 54-, 80-, and 200-kDa proteins was minimal.

Discussion

The findings of jejunal mucosal hyperplasia and enhanced mucosal ornithine decarboxylase activity in the streptozotocin diabetic rats in the present study confirm our previous observations (3, 4). Similar to the results of our earlier study, the administration of difluoromethylornithine suppressed both mucosal proliferation and mucosal ornithine decarboxylase activity in the control and diabetic rats (4). In a previous study, oral administration of difluoromethylornithine to control rats reduced jejunal mucosal ornithine decarboxylase activity at 2 days, and jejunal mucosal DNA and protein contents after 4 days of treatment (8). Administration of polyamines with difluoromethylornithine completely prevented the inhibition of mucosal growth caused by difluoromethylornithine (9). The association of intestinal mucosal hyperplasia with enhanced mucosal tyrosine kinase activity (in the diabetic rats) is consistent with reports of other investigators in which increased mucosal DNA synthesis was associated with enhanced ornithine decarboxylase and tyrosine kinase activity after administration of bombesin (2).

In studies *in vitro*, exposure of rat colonic mucosal explants to gastrin (1) and exposure of gastric mucosal explants to bombesin (2), respectively, resulted in a profound stimulation of both tyrosine kinase and ornithine decarboxylase activities compared with the corresponding basal levels. In the gastric explants, while increase in tyrosine kinase activity occurred within 10–15 min of exposure to gastrin, ornithine decarboxylase activity was significantly stimulated 2 hr after exposure to the hormone. Administration of difluoromethylornithine completely abolished the gastrin-mediated stimulation of ornithine decarboxylase, but not of tyrosine kinase activity, whereas genistein (a specific inhibitor of tyrosine kinase) caused a total suppression of the gastrin-induced stimulation of both tyrosine kinase and ornithine decarboxylase activity (1). These observations suggest that tyrosine kinase activity may be modulating ornithine decarboxylase activity. The findings in the control rats at the 3-day study period (Table II), where administration of difluoromethylornithine significantly suppressed activity of ornithine decarboxylase but not that of tyrosine kinase, are similar to the above *in vitro* observation.

The relationship between tyrosine kinase and ornithine decarboxylase activity during hyperplasia of the jejunal mucosa is obvious from the highly significant positive correlation between activity of these two enzymes (Fig. 2). It is not possible to determine definitely which enzyme modulates the activity of the other. The finding that at the 3-day study period mucosal proliferation in the control rats was significantly suppressed by administration of difluoromethylornithine, but activity of tyrosine kinase was not, suggested that the intestinal mucosal hyperplasia and its tyrosine kinase activity may have been modulated by the mucosal ornithine decarboxylase activity, most likely *via* the effect of ornithine decarboxylase on levels of one or more of the polyamines (putrescine, spermidine, and spermine) in the intestinal mucosa. Because polyamines are needed in DNA replication and RNA and protein synthesis (10, 11), it seems possible that the polyamines could regulate tyrosine kinase activity.

The relationship between tyrosine kinase activity and the polyamines, and their role in the control of the intestinal epithelial cell proliferation remains to be further investigated. In the porcine spleen, the cytosolic protein tyrosine kinase activity was reported to be

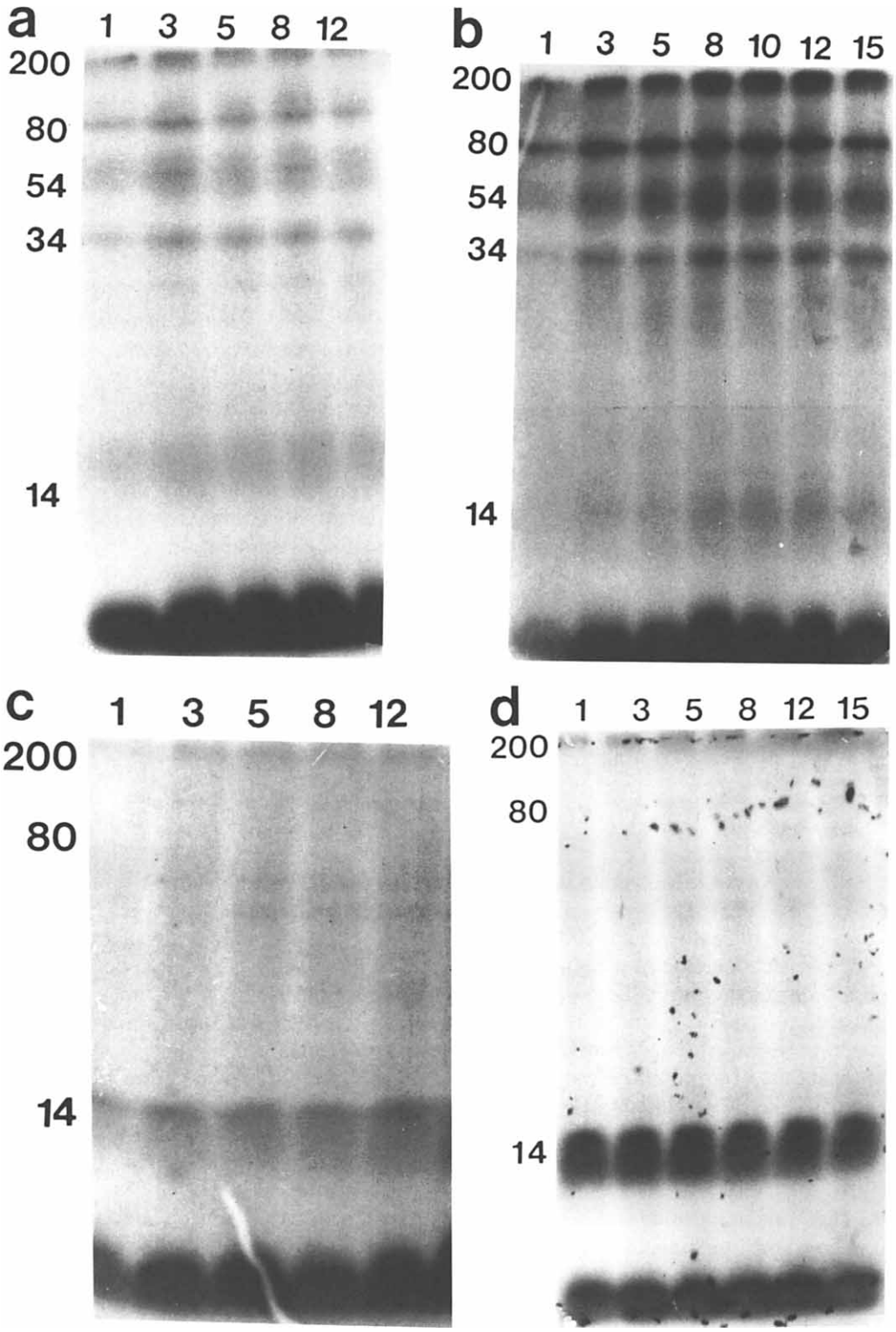

Figure 3. Tyrosine-specific phosphorylated proteins in the jejunal mucosal homogenates of representative nontreated and difluoromethylornithine-treated control and diabetic rats. Tyrosine-specific phosphorylation of the proteins was determined at 1, 3, 5, 8, 10, 12, and 15 min, after the addition of [^{32}P] ATP to the jejunal mucosal homogenates. In the nontreated control (a) and diabetic (b) rats, the tyrosine-specific phosphorylated proteins had molecular weights of 34, 54, 80, and 200 kDa. Tyrosine-specific phosphorylation of all these proteins was greater ($P < 0.05$) in the diabetic than control rats. In the difluoromethylornithine control (c) and diabetic (d) treated rats, tyrosine-specific phosphorylation of the 34-, 50-, 80-, and 200-kDa proteins was minimal but the tyrosine specific phosphorylation of a 14-kDa protein became prominent.

stimulated by about 2-fold by spermine and spermidine using (Val 5) angiotensin II as substrate (12). Similarly, the cytosolic protein tyrosine kinase from human erythrocytes appeared to be stimulated by the three polyamines in a dose-dependent manner with spermine having the highest stimulatory effect (13). The higher content of the polyamines and activity of tyrosine kinase in diabetic than in control rats (3) *in vivo* suggested that the increased polyamines content could have enhanced tyrosine-specific phosphorylation of some mucosal proteins important in mucosal proliferation.

In studies *in vitro* of the intestinal crypt cell line (IEC-6), exposure to gastrin resulted in cell proliferation which was associated with phosphorylation on tyrosine of a similar group of proteins with molecular weight of 42, 60, 78, and 120 kDa (14). This phosphorylation occurred as early as 5 min and lasted for only 15 min. The authors suggested that the gastrin-induced proliferation of the IEC-6 cell line was due to stimulation of tyrosine kinase and/or inhibition of tyrosine phosphatase in the cells (14). In isolated gastric mucosal cells obtained from young rats, exposure to gastrin was associated with a 2- to 3-fold rise in tyrosine-specific phosphorylation of 55-, 80-, and 100-kDa membrane proteins (5). In the present *in vivo* study, the mucosal membrane proteins that were tyrosine-specifically phosphorylated showed molecular weights of 34, 54, 80, and 200 kDa. The identity of these proteins is unknown at this time. The observation that the tyrosine-specific phosphorylation of the 34-, 54-, 80-, and 200-kDa protein was suppressed and that of a 14-kDa protein became prominent by administration of difluoromethylornithine suggested that tyrosine-specific phosphorylation of these protein was important in jejunal mucosal proliferation in both control and diabetic rats. It seems probable that the polyamines could influence the tyrosine-specific phosphorylation of these proteins.

In studies *in vitro* of IEC-6 cells lines derived from intestinal crypts, depletion of polyamines by administration of difluoromethylornithine resulted in decreased cell proliferation and in the expression of c-*fos*, c-*myc*, and c-*jun* proto-oncogenes. Exogenous spermidine reversed these inhibitory effects of difluoromethylornithine (15). A cascade of kinase and phosphatase reactions follow growth factor/receptor interactions and result in the expression of some of the oncogenes, such as c-*fos*, c-*myc*, and c-*ras* (16). Expression of products of these gene products has been demonstrated to be critical for the progression of cells through G and into S phase (17). Thus, the polyamines seem to be regulating the cell cycle in the intestinal epithelium *via* their ability to regulate proto-oncogene expression.

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