THE INFLUENCE OF THE SOW'S ADRENAL ACTIVITY ON THE ABILITY OF THE PIGLET TO ABSORB IgG FROM COLOSTRUM

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To study modifications in adrenal activity, 18 pregnant Yorkshire sows were infused intravenously with either ACTH, metyrapone or saline between days 104 and 114 postbreeding. During this time the sows were bled twice daily. At birth the piglets were force fed pooled bovine colostrum until 6 h of life and bled at birth, 6h, 1, 2, 4, 8, 12, 16 and 21 days of age. Cortisol, corticosterone, and 11-deoxycortisol were measured in sow serum and the concentrations of bovine and porcine immunoglobulin G (IgG) were measured in piglet serum. ACTH significantly increased the levels of cortisol, corticosterone and 11-deoxycortisol in sows. Metyrapone did not produce a significant reduction in the levels of cortisol or corticosterone. but the concentration of 11-deoxycortisol was significantly elevated in comparison to the controls. Piglets from sows treated with ACTH (A pigs) had significantly more bovine IgG than controls (C pigs) at 6 h of life, and maintained higher levels at the other sampling times. Piglets from sows treated with metyrapone (M pigs) had significant increased serum concentrations of bovine IgG from 6 h to 21 days. The concentration of bovine IgG at 6 h was about 71% higher in M pigs than in the C pigs. At birth, M pigs were heavier than A pigs, and C pigs were the lightest of the three groups.

Key words: Piglets corticosteroids, IgG, absorption, colostrum

[Effets de l'activité des surrénales des truies sur l'aptitude des porcelets à absorber l'IgG du colostrum.]

Titre abrégé: Activité des surrénales et absorption de l'IgG chez les porcelets.

Pour étudier les effets de changements dans l'activité endocrine des glandes surrénales, nous avons pratiqué une injection intraveineuse lente d'ACTH, de métyrapone ou d'une solution saline sur 18 truies Yorkshire gravides entre le 104e et le 114e jour suivant l'accouplement. Pendant cette péride, les truies ont été saignées deux fois par jour. Les porcelets ont été gavés de colostrum bovin pendant les six premières heures de leur vie et saignés dès la naissance, six heures après, puis à l'âge de 1, 2, 4, 8, 12, 16 et 21 jours. Nous avons mesuré la teneur en cortisol, en corticostérone et en 11-désoxycortisol du sérum des truies et la teneur en immunoglobuline G (IgG) bovine et porcine chez les porcelets. L'ACTH a provoqué une hausse significative des teneurs en cortisol, en corticostérone et en 11-désoxycortisol du sérum des truies. Le métyrapone n'a pas donné de baisse significative des teneurs en cortisol ni des teneurs en corticostérone, mais il a entraîné une hausse significative de la teneur en 11-désoxycortisol par rapport aux témoins. Les porcelets des truies traitées à l'ACTH (porcelets A) présentaient une teneur en IgG bovine significativement plus élevée que les témoins (porcelets C) à 6 h et ils ont maintenu ces teneurs plus élevées pour toutes les autres périodes d'échantillonnage. Les porcelets des truies traitées au métyrapone (porcelets M) présentaient des teneurs en IgG bovine significativement plus élevées de 6 h à 21 jours. La teneur en

IgG bovine à 6 h était environ 71% plus élevée chez les porcelets M que chez les porcelets C. À la naissance, les porcelets M étaient plus lourds que les porcelets A et les porcelets C étaient les plus légers des trois groupes.

Mots clés: Corticostéroïdes chez les porcelets, IgG, absorption, colostrum

Piglet mortality is still one of the main factors contributing to the potential loss of pork farmers' income. One mechanism, to increase the chances of survival of the newborn piglet, it to ensure that the piglet acquires adequate immunological protection as early as possible. Therefore, the rate at which the piglet can absorb immunoglobulins may be a determining factor for improving the chances of survival. It is known that, once a piglet ingests colostrum, the period during which a significant quantity of macromolecules can be absorbed is limited to about 12–24 h (Payne and Marsh 1962; Speer et al. 1959).

Glucocorticoids produce a variety of effects throughout the body (Wilcke and Davis 1982), their specificity depending on their actions on the target cell. Glucocorticoids can increase gluconeogenesis, protein and fat mobilization and cellular turnover rate (Nelson 1980). They can also produce an early cessation in immunoglobulin absorption in rats (Clark 1971). This last effect is a consequence of an increased rate of replacement of the absorptive epithelial cells in the intestine by mature cells which are incapable of absorbing macromolecules (Patt and Eberhart 1974). The role of glucocorticoids in those cells of the pig's small intestine which are capable of immunoglobulin absorption has not been clearly estabished. With injections of ACTH, Patt and Eberhart (1976) increased serum corticosteroid levels in ceasarean-derived piglets, without affecting IgG absorption. Metyrapone injections to these piglets reduced circulating corticosteroid concentrations and resulted in subnormal concentrations of immunoglobulin G (IgG) in 14-h-old piglets.

Depression of calf serum corticosteroid with metyrapone produced a trend toward reduced serum immunoglobulins (Johnston and Oxender 1979). When parturition was rapidly induced with a synthetic glucocorticoid (dexamethasone) in cows, the capability of the calves to absorb IgG was not affected (Muller et al. 1975). However, sustained exposure of the pregnant cow to a slow releasing synthetic corticosteroid (dexamethasone TMA) shortened gestation length and reduced by half the postnatal absorption of IgG (Bailey et al. 1973; Husband et al. 1973). It has been suggested that this effect could be a consequence of the length of time the fetus was exposed to the synthetic corticosteroid (Muller et al. 1975; Stott 1980).

The present study was designed to evaluate the response of the adrenal gland in pregnant sows to ACTH and metyrapone and to investigate the relationship between maternal adrenal function in late gestation and the ability of the newborn piglet to absorb IgG from colostrum.

MATERIALS AND METHODS

Eighteen pregnant Yorkshire sows from the Arkell Swine Research Station herd of the Ontario Ministry of Agriculture and Food were used. The sows were randomly allocated to either a metyrapone, ACTH or control group. All sows were fitted with an indwelling cannula (Bate and Hacker 1985) in the ear vein on day 102 postbreeding and allowed to rest for 2 days. During this period the cannulae were flushed twice daily with 10 mL of heparinized saline (40 IU heparin/mL). On day 104 postbreeding, the treatments were infused intravenously, using 500 mL/day of 0.9% sodium chloride solution (Travenol Lab. Malton, Ont.) as the medium. Seven sows (A sows) received 1 IU of porcine ACTH·kg^{-1}·day^{-1} (Sigma Chemical Co. St. Louis, Mo.) until day 114 postbreeding. Six sows (M sows) were given 5 mg of metyrapone·kg^{-1}·day^{-1} (Sigma Chemical Co. St. Louis Mo.) on day 104. This dosage was reduced by

20% of the initial amount each consecutive day and from day 109 these sows received only saline solution. Five sows of the control group (C sows) received saline solution throughout the infusion period.

From day 104 postbreeding the sows were bled through the cannulae twice daily between 0730 to 0830 h and 1930 to 2030 h. At birth the piglets were weighed, identified, bled and separated from the sow and kept in a box at 35°C. Piglets were force fed 25 mL/kg of a pooled bovine colostrum which was maintained frozen (Foley and Otterby 1978) until parturition and then warmed to 37°C before feeding. Feeding was done at 30 min, 2, 4 and 6 h of life. Six hours after birth of the first piglet, all piglets were returned to the sow simultaneously and allowed to suckle freely. However, those piglets younger than 6 h received their respective dosages of bovine colostrum while having access to sow colostrum. The piglets were bled from the suborbital sinus with a 2.5 cm × 20-gauge disposable needle at birth, 6 h, 1, 2, 4, 8, 12, 16, and 21 days of age. Blood collected from sows and piglets was cooled to 4°C within 1 h of collection, allowed to clot for 24 h and centrifuged for 10 min. The serum was transferred to plastic vials and stored at −17°C until assayed.

The experimental design was a complete randomized design and comparisons between treatments were done by contrasting treatments and control. Analysis of covariance and regression was required to determine correlations between hormonal parameters in the sow and IgG in the litters. The statistical analysis was done using General Linear Models from a SAS package (Goodnight 1979).

Hormone Assays

Serum corticosterone was measured by a modification of the assay described by Etches (1976). Fifty microlitres of sow serum was extracted with 2×3 mL iso-octaine (Fisher Spectranalyzed). Extractions were carried out in racks containing 40 tubes, which were shaken for 2 × 2 min in a wrist action shaker after addition of solvents. The tubes were frozen and the progesterone containing organic phase was discarded. Subsequently, 5 mL of methylene chloride (Fisher Spectranalyzed) were added to the samples and extracted as before. The aqueous phase was frozen to the walls of the tubes and the organic phase transferred to 16×100 mm glass disposable tubes. After evaporation under N_2, 500 μL of corticosterone antiserum (Miles Lab. Inc., Elkhart, Indiana) were added to the tubes, vortexed for 15 sec and incubated overnight in a room at 4°C.

The next day, 200 μL of dextran coated charcoal (3.75 g Norit A and 0.375 g dextran T70/L of tris buffer pH 8) were added to each tube and allowed to equilibrate for 10 min from the point of middle addition. All charcoal additions were carried out at 4°C. The tubes were then centrifuged at 1720 × *g* and 4°C for 10 min and 500 μL of supernatant removed for scintillation counting.

Serum cortisol was measured using the procedure and reagents of Autopak Test Delivery System Ind. (Horsham, Pa.). The manual assay procedure was followed but the assays were counted and calculated using a Concept 4 automatic radioimmunoassay counter attached to a Hewlett Packard 9815A microcomputer system.

Serum 11-deoxycortisol was measured using the reagents and the procedure recommended by Miles Scientific Company, without modification.

The procedures published by Abraham (1975) were used to validate the corticosterone assay. For cortisol and 11-deoxycortisol, the methods as validated by Micromedic and Miles laboratories were used. The double iso-octaine extraction removed over 99% of the progesterone in serum and a maximum of 4% of the corticosterone when no progesterone was available. Methylene chloride extracted an average of 88% of the corticosterone in the serum.

The slope of the inhibition curves for corticosterone standards and various dilutions of known porcine serum were not different ($P>0.05$) when compared using Student *t* test (Steel and Torrie 1980). The cross-reactivities of the corticosterone antiserum have been published (Miles Lab. Inc. Elkhart, Ind.).

The sensitivity of the corticosterone assay was 0.28 ng of corticosterone. The recoveries for 0.25, 0.75, 1.0 and 2.0 ng of corticosterone added to porcine serum were 0.29, 0.89, 1.03 and 2.35 ng, respectively.

The intra- and inter-assay coefficients of variation were 8.38 and 10.98%, 5.03 and 5.96%, 6.53 and 17.98% for corticosterone, cortisol and 11-deoxycortisol, respectively.

Immunoglobulin assay

The radial immunodiffusion procedure used was a modification of the method described by Man-

cini et al. (1965). To prepare the 1-mm diffusion plates, 26 mL of a 3% agarose stock solution (Bio Rad Laboratories, Richmond, Calif.) in phosphate buffer saline (PBS), pH 7.3 were diluted to 1.5°C by adding an equal volume of PBS containing anti-IgG protein (Cappel Laboratories, Inc. Cochranville, Pa.). The solution, maintained at 56%, was poured on a sheet of gel bond NF film of 203 × 254 mm (FMC corporation, Bio products, Rockland, Maine). The sheet was maintained horizontally on a warm leveling table and the gel was allowed to cool and solidify for 1 h. Each plate was perforated with 300 wells made with a 2-mm-diameter well cutter.

The standards were prepared by dissolving purified IgG (Cappel Laboratories, Inc. Cochranville, Pa.) with PBS to provide concentrations of 0, 1, 2.5, 5, 10, 15, 30 and 50 mg/mL. Each plate included two sets of standards and at least four duplicates of a control serum. Two microlitres of piglet serum, control, or standard were placed in each well, all in duplicate, on the same plate. After the addition of the samples, the plates were allowed to diffuse on a moist environment at 4°C for 22 h.

The ring diameter of the precipitate was measured with a stereo microscope with a scale incorporated into the lens. The concentration of IgG was calculated by interpolation between the standards. The value of each standard and sample was determined as the area of the ring minus the area of the well.

There was no cross-reaction between porcine and bovine IgG and bovine and porcine anti-IgG, respectively. Each plate had one type of anti-IgG depending on the measurement required. The intra-and inter-plate coefficients of variation were determined as 9.0 and 12.6% for bovine IgG and 2.9 and 12.3% for porcine IgG, respectively.

RESULTS

All A sows responded with an increase ($P<0.001$) in serum cortisol concentration following the treatment (Fig. 1A). These elevated levels were maintained until ACTH was withdrawn on day 114. M sows did not show a difference ($P>0.05$) in the concentration of serum cortisol when compared to the control group. M and C sows maintained a stable concentration of cortisol at about 25 mg/mL throughout the infusion period. The patterns of corticosterone in the three groups of sows were very similar to those of cortisol (Fig. 1B). The levels of corticosterone were higher ($P<0.01$) in A sows when compared to the C sows but M sows maintained concentrations similar ($P>0.05$) to the C sows. The net values for corticosterone were lower ($P<0.01$) than those for cortisol.

The concentration of 11-deoxycortisol in C sows remained at a basal level but increased ($P<0.01$) in a similar pattern to cortisol in A sows (Fig. 1C). M sows also had an increase in serum 11-deoxycortisol

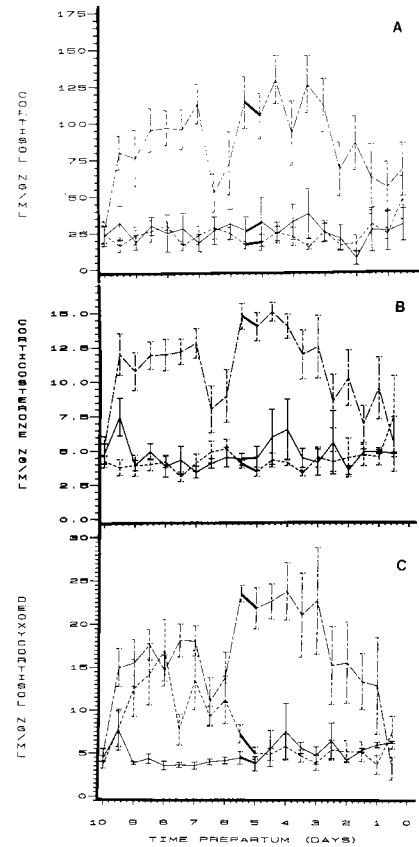

Fig. 1. Concentration of serum cortisol (A), corticosterone (B) and 11-deoxycortisol (C) in sows treated with ACTH (— - — -), metyrapone (- - - -) or saline (——). The thick line joins the 10th sample after beginning of infusion and the 10th sample prior to parturition.

($P<0.01$) during the period of infusion. When metyrapone was replaced by saline, the concentration of 11-deoxycortisol dropped almost immediately to the basal levels.

The concentrations of bovine IgG in piglet serum reached maximal levels between 6 h and 1 day of life. Piglets from the control group (C pigs) had the lowest levels of bovine IgG (Fig. 2A) reaching a maximum concentration of 14.5 mg/mL at 1 day. The piglets from A sows (A pigs) had higher concentrations of bovine IgG than the controls during the experimental period, but this difference was only significant at 6 h ($P<0.05$) when levels reached 19 mg/mL. The concentration of bovine IgG in pigs from M sows (M pigs) showed a substantial increase by 6 h reaching concentrations 71% higher ($P<0.01$) than those of the C pigs. The concentration of bovine IgG decreased thereafter; nevertheless, differences in bovine IgG between C pigs and M pigs were maintained until day 21 ($P<0.05$).

The levels of porcine IgG followed similar patterns to those of bovine IgG (Fig. 2B) although the large variability made it impossible to statistically differentiate the groups.

The lowest birth weight was recorded for C pigs, followed by those of A pigs while M pigs were the heaviest. These differences ($P<0.05$) lasted at least 4 days (Table 1). Weights taken after this time do not reflect experimental effects because several litters in the experiment became affected with exudative epidermitis. This condition primar-

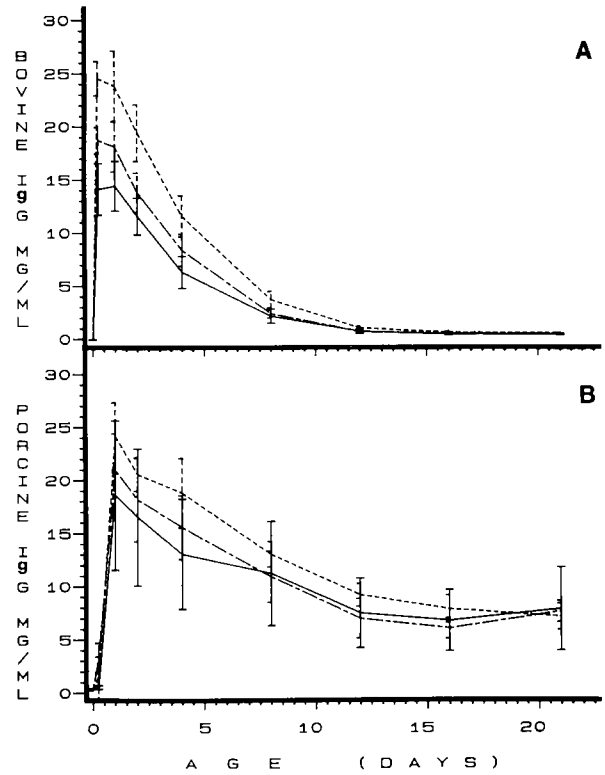

Fig. 2. Concentrations of bovine IgG (A) and porcine IgG (B) in serum of piglets from sows treated with ACTH (— – — –), metyrapone (- - - - -) or saline (———).

Table 1. Effect of treatment on piglet body weight (kg) during the first 4 days of life

Age (days)	Treatment										
	ACTH				Metyrapone				Control		
	Mean	SE	N†	P‡	Mean	SE	N†	P‡	Mean	SE	N†
0	1.24	0.03	59	0.024	1.32	0.03	54	0.003	1.13	0.03	50
0.25	1.28	0.03	59	0.012	1.35	0.03	52	0.001	1.15	0.03	47
1	1.38	0.04	59	0.022	1.51	0.03	52	0.001	1.24	0.04	46
2	1.50	0.04	58	0.004	1.61	0.04	52	0.001	1.32	0.04	45
4	1.81	0.06	58	0.048	1.95	0.05	52	0.003	1.65	0.05	43

†Number of piglets.
‡*P* value when compared to controls.

ily affects pigs of 5–35 days of age (Underdahl and Twiehaus 1978). No correlation was found between birth weight and bovine or porcine IgG absorption or litter size.

Sows from the ACTH, metyrapone and control groups farrowed a total of 62, 59 and 55 piglets, respectively. From these, 3, 5 and 5 piglets or 4.83, 8.47 and 9.09% of the piglets were stillborn. By 21 days the mortality in the three respective groups reached 22, 20 and 32% of the piglets born alive. Most of the mortality occurred between days 5 and 16 reflecting the impact of the exudative epidermitis in the litters (Fig. 3). Treatment did not influence gestation length. Within any treatment, there was no difference in IgG absorption when males were compared to females, neither was there a treatment × sex interaction. Absorption of porcine IgG was not influenced by the amount of bovine IgG absorbed. Birth order did not affect bovine IgG absorption. As previously described (Parker et al. 1980), those piglets which failed to survive the experimental period were lighter than the surviving littermates at birth ($P<0.05$).

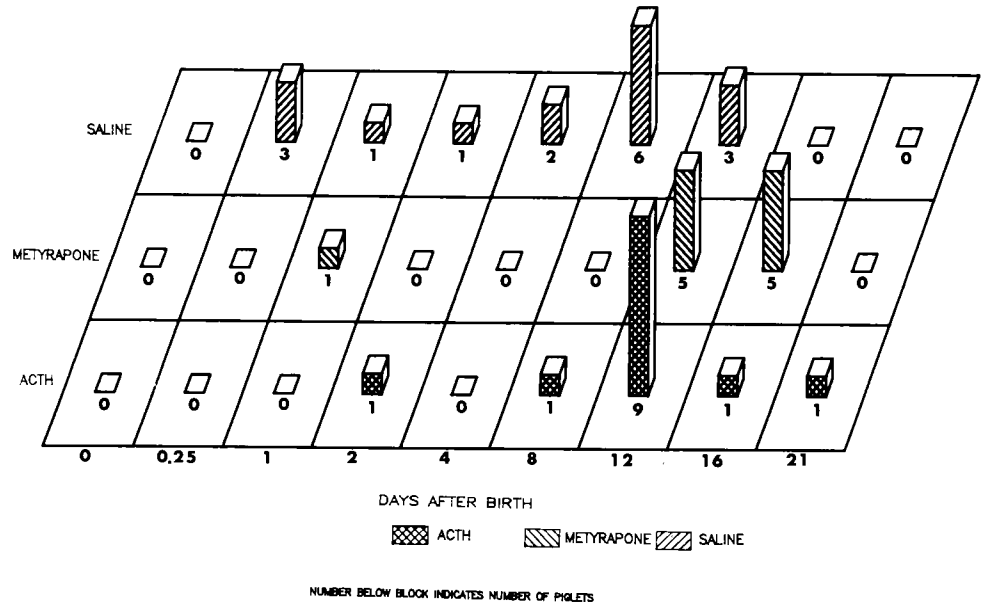

Fig. 3. Mortality of piglets up to 3 wk of age from sows treated with ACTH, metyrapone or saline.

DISCUSSION

The increase in cortisol, corticosterone and 11-deoxycortisol in A sows clearly reflected the response of the adrenal cortex to the treatment. The lack of change in the cortisol and corticosterone concentrations of M sows initially suggested a lack of response of the sows' adrenals to the treatment. However, the profile of 11-deoxycortisol during the metyrapone infusion period indicated that the blockage of the biosynthetic step, 11-deoxycortisol or cortisol, had occurred as expected (Liddle et al. 1958). This suggests that the dosage of metyrapone may have been large enough to initially produce adrenal inhibition. Therefore, the maintenance of basal levels of maternal glucocorticoids could have resulted from a decrease in glucocorticoids, which may have stimulated pituitary ACTH release and the adrenal may have begun its characteristic compensatory growth (Chart et al. 1958). The consequence of this compensatory growth may have been an increase in the amount of 11β-hydroxylase (Wilson et al. 1968) to a level with which the metyrapone could not compete. Chart et al. (1958) demonstrated that within 4 h of initiating metyrapone treatment the adrenal could increase its weight by 50%. If that was the case, the sampling frequency used in this experiment could not detect this type of response.

The substantial increase in IgG absorption by M piglets is evident but difficult to interpret. It is doubtful that an accumulation of 11-deoxycortisol in response to metyrapone could be responsible for the increased absorption, as A piglets did not increase IgG absorption dramatically despite the large increase in 11-deoxycortisol found in A sows. However, if an increase in fetal glucocorticoid production in M pigs resulted from a faster drainage of the hormones from the fetal to the maternal pool, it is plausible that the mechanism which increased IgG absorption in the ACTH and metyrapone treatment could be similar. That is, an increase in the concentration of glucocorticoids in the fetal compartment. Increased birth weight, which certainly is a beneficial effect by itself, can not be directly involved because of the lack of a correlation found between body weight and serum IgG concentration. The possibility remains that metyrapone crossed the placental barrier, and acted in an unknown manner over the fetal small intestine epithelium, making it more absorptive. However, if metyrapone crossed the placenta and inhibited the fetal adrenal, a delay in parturition, similar to the described by Coggings and First (1977), may have occurred. This was not observed in this study.

The onset of antibody synthesis by the newborn is delayed by the presence of immunoglobulin in circulation (Klobasa et al. 1981). The production of IgG by A and C piglets was apparently sufficient to override the normal rate of catabolism by day 16, while M pigs were still lowering their IgG concentration by day 21.

The maximum concentration of porcine IgG accrued by C pigs in this study was lower than the values reported by others (Curtis and Bourne 1971; Klobasa et al. 1981). However, in the C pigs the sum of the concentration of bovine and porcine IgG was comparable to the concentration reported by these authors. This indicates that the amount of bovine IgG absorbed may have substituted part of the porcine IgG which could have been absorbed by the small intestine of the piglets.

From this experiment it can be concluded that adrenal function in the pregnant sow is affected by ACTH and metyrapone although the dosage of metyrapone used did not alter serum levels of cortisol or corticosterone. Piglets from sows treated with ACTH during late gestation had a slight increase in absorptive capacity compared to the controls. However, those piglets from sows treated with metyrapone during late gestation had a substantial increase in their IgG absorptive capacity.

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