

IMMUNOGLOBULIN PROTEOSYNTHESIS IN ORGAN CULTURES FROM NEWBORN PIGLETS

J. HAMPL, J. FRANZ, J. ŠTĚPÁNEK, A. HOLUB, J. MENŠÍK

Veterinary Research Institute, 621 32 Brno

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Abstract

HAMPL J., J. FRANZ, J. ŠTĚPÁNEK, A. HOLUB, J. MENŠÍK: *Immunoglobulin Proteosynthesis in Organ Cultures from Newborn Piglets*. Acta vet. Brno 49, 1980: 67–74.

The synthesis of total protein including immunoglobulins by piglet ileum, jejunum, mesenteric lymph node, spleen, lung, trachea, salivary gland and nasal mucosa was studied by affinity chromatography using tissue fragments obtained from 9 colostrum-deprived, hysterectomy-derived and 9 conventional colostrum-fed piglets and grown in culture medium enriched with ^{14}C -labelled amino acids. Newly synthesized protein and immunoglobulins IgG, IgA and IgM were demonstrated in all the organs under study in the two groups as early as 2 days after birth.

Immunoglobulins IgG, IgM, IgA, total protein.

Newborn pigs, like the young of other animal species, are not immunologically competent at birth. Therefore only very low immunoglobulin concentrations can be demonstrated in their blood before ingestion of colostrum (Bourne, Curtis, Johnson and Collings 1974; Franěk, Říha and Šterzl 1961; Franěk and Říha 1964; Kim, Bradley and Watson 1966; Menšík and Franz 1969; Porter, 1969, Prokešová and Rejnek 1973; Setcavage and Kim 1976; Wellmann and Reblin 1972), with the exception of piglets stimulated antigenically *in utero* (Avrameas and Thernynck 1969; Franz, Menšík and Pokorný 1971; Menšík and Franz 1969; Wellmann and Reblin 1972). The resistance of piglets during early postnatal life depends practically on passive acquisition of immunoglobulins and specific antibodies through ingestion of colostrum, with this process being restricted to the first two days after birth. Passively acquired antibodies circulating in the body fluids are then eliminated depending on the biological half-lives of individual immunoglobulin classes (Courtis and Bourne 1973; Martinsson 1975; Prokešová and Rejnek 1973) and the resultant lack of circulating antibodies is gradually made up for by active antibody production.

The present study was designated to assess the involvement of individual organs of newborn pigs in the production of proteins and immunoglobulins IgG, IgA, and IgM during the first five weeks after birth using tissue fragments grown in culture medium enriched with ^{14}C -labelled amino acids.

Materials and Methods

Experimental Animals

Nine conventional sow-reared piglets and nine hysterectomy-derived piglets reared in incubators under specific-pathogen-free conditions were employed. Three animals were killed from each group at 2, 10 and 35 days of age. Organ cultures were prepared from the following organs: ileum, jejunum, spleen, lung, salivary gland, nasal mucosa, trachea and mesenteric lymph node.

Cultivation of Organ Fragments

The tissues were minced into 1- to 2-mm fragments, washed in several changes of Trowel's medium and started on plastic lattice in petri dishes containing 1.2 ml of culture medium which was essentially that of Trowel supplemented with 1 per cent inactivated foetal calf serum and

penicillin and streptomycin and containing 185 kBq (5 uCi) of ^{14}C lysine and ^{14}C isoleucine/ml, with the concentration of non-labelled lysine and isoleucine in the medium being ten times reduced. The organ cultures were incubated in an atmosphere of 95 per cent O_2 and 5 per cent CO_2 at 37°C for 24 hours.

Protein Synthesis

After cultivation, the tissue fragments were frozen, thawed, homogenized and then separated from the culture medium by centrifugation at 3500 r. p. m. for 15 minutes. Nonincorporated labelled amino acids were removed by dialysis of the culture medium against phosphate buffered saline for 48 hours. Volume was adjusted to 1.5 ml by pressure dialysis. The amount of newly synthesized total protein was determined in an aliquot of the dialyzed medium by precipitation with 10 per cent trichloroacetic acid. The precipitate was then dissolved in NCS solubilizer (Nuclear Chicago) for 24 hours and its radioactivity was measured by liquid scintillation and expressed as counts per minute per gram (wet weight) of tissue.

Preparation of Antisera

Antisera against pig immunoglobulins were prepared by immunizing rabbits with immunoelectrophoretically pure pig immunoglobulins IgG, IgM and IgA. The rabbit antisera were saturated with insolubilized nonspecific Ig according to Avrameas and Thernynck (1969). The specificity of these antisera was ascertained by immunoelectrophoresis and double diffusion in agar gel.

Affinity Chromatography

Specific immunosorbents for isolation of pig IgG, IgA and IgM were prepared by coupling monovalent rabbit antisera to CNBr-activated Sepharose 4B (Axén, Porath and Ernback 1967). Four ml of antiserum was precipitated with 40 per cent saturated ammonium sulphate. The antibodies were dialyzed against the coupling buffer and then coupled to 5 g of wet CNBr-activated Sepharose 4B; the remaining reactive sites were blocked with 1 M ethanolamine. The gel thus obtained was packed into small chromatographic columns and checked for specificity before use.

The newly synthesized immunoglobulins IgG, IgM and IgA were determined by affinity chromatography according to Svenerholm and Holmgren (1977). The samples were run through the columns. Nonspecific proteins were washed out with starting buffer, pH 7. Elution of specifically bound material was carried out with glycine buffer, pH 3. A portion of each immunoglobulin eluate was mixed with the solubilizer and allowed to solubilize for 2 hours. The radioactivity was measured by liquid scintillation and the amounts of newly synthesized immunoglobulins IgG, IgA and IgM were determined as counts per minute per gram of tissue.

Results

Total Protein Synthesis

The amounts of total protein synthesized by the organs under study were higher in hysterectomy-derived, colostrum-deprived animals. In both experimental groups, the magnitude of protein synthesis was highest from nasal mucosa and lowest from trachea and jejunum (Fig. 1–3). There were no substantial changes in the rate of protein synthesis during the observation period.

Immunoglobulin Synthesis

In conventionally reared piglets the synthesis of IgG by the organs under study was demonstrated as early as two days after birth in amounts exceeding considerably those of either IgA or IgM. The IgG values then decreased with age in all the organs except jejunum and trachea (Fig. 4–6).

On the other hand, the amounts of IgG synthesized by the organs of hysterectomy-derived, colostrum-deprived piglets were much lower than in conventional piglets at 2 days after birth, but increased thereafter, with the exception of trachea, approaching the values in conventional animals.

The synthesis of IgM by the organs of piglets of both experimental groups

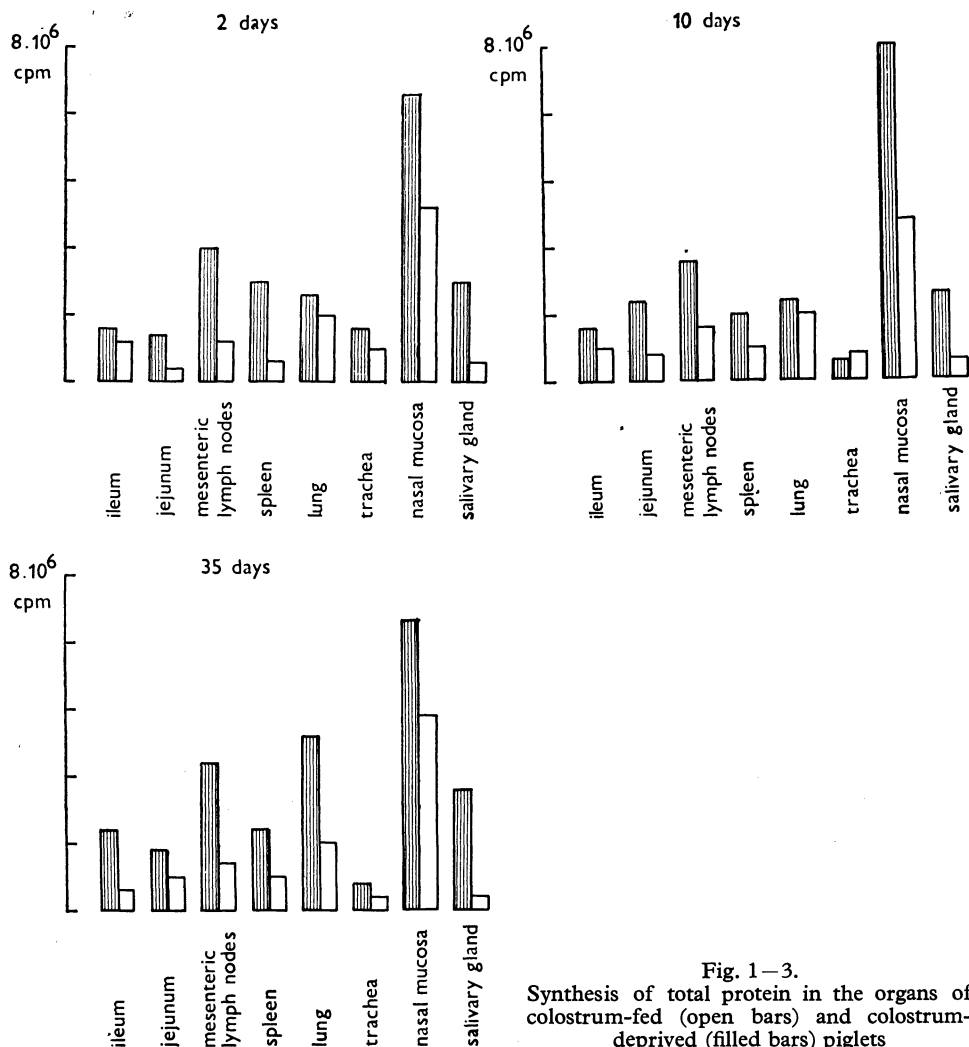


Fig. 1-3. Synthesis of total protein in the organs of colostrum-fed (open bars) and colostrum-deprived (filled bars) piglets

was also demonstrated as early as two days after birth, but its amount was lower and the differences between colostrum-deprived and conventional piglets were less marked, with the exception of ileum, than in the synthesis of IgG. The rate of IgM synthesis during the observation period changed little in conventional piglets, with a decrease being recorded only for ileum, and rose by the 35th day after birth in colostrum-deprived piglets (Fig. 7-9).

The synthesis of IgA, like that of IgM and IgG, was demonstrated in the organs of both colostrum-deprived and conventional piglets as early as two days after birth. Its amounts were similar to those of IgM. In conventional piglets, the magnitude of IgA synthesis was higher from ileum than from the other organs and decreased during the observation period similarly to the synthesis of IgM. The IgA synthesis from the remaining organs of both colostrum-deprived and con-

ventional animals changed little during the observation period, with increases being recorded only for the nasal mucosa and salivary gland of conventional piglets (Fig. 10–12).

Discussion

Numerous attempts have been made to determine the onset of immunoglobulin synthesis in newborn piglets on the basis of demonstration of immunoglobulin-synthesizing cells (Allen and Porter 1973; Bradley, Bourne and Brown 1976; Brown and Bourne 1976). Our objective was a quantitative assessment of protein and immunoglobulin synthesis in the organs of newborn pigs using tissue fragments grown *in vitro* and following the procedure that proved useful in similar studies (Lai, McClelland and Van Furth 1976; Lee, Hand and Cantey 1974; Svennerholm and Holmgren 1977; Stakeberg, Gustafson and Schersten 1974).

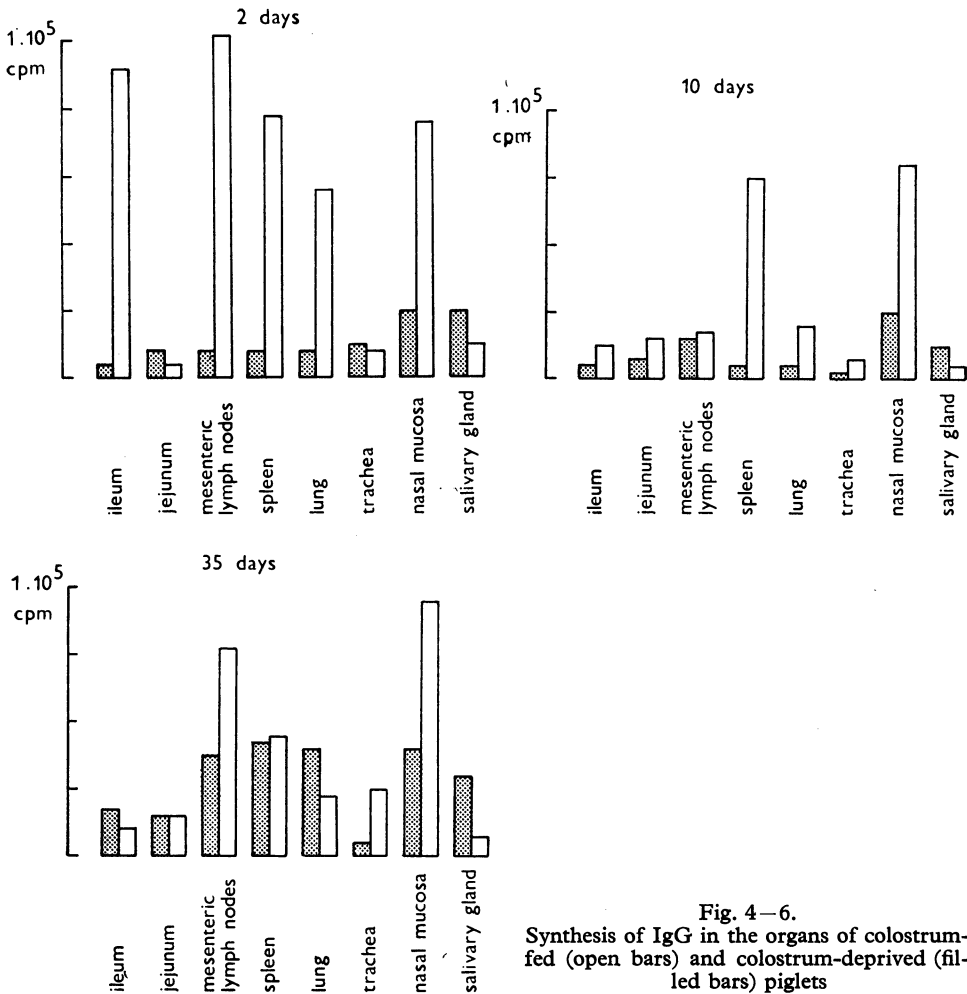


Fig. 4–6. Synthesis of IgG in the organs of colostrum-fed (open bars) and colostrum-deprived (filled bars) piglets

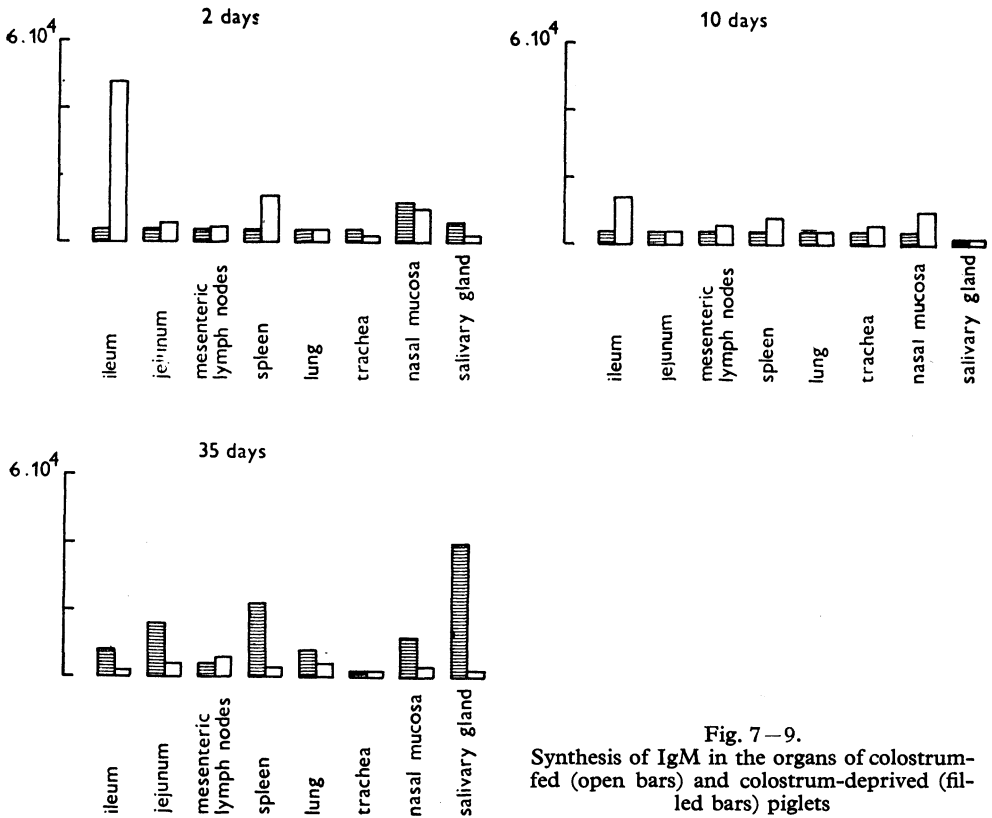


Fig. 7-9. Synthesis of IgM in the organs of colostrum-fed (open bars) and colostrum-deprived (filled bars) piglets

An observation of particular interest is the finding that the synthesis of total protein by tissue cultures maintained under constant conditions was higher in colostrum-deprived than in conventional piglets. On the other hand, the amount of immunoglobulin, particularly IgG, synthesized by the tissues was higher in colostrum-fed than in colostrum-deprived piglets.

These findings are reminiscent of the observations of Brown and Bourne (1976) who using peroxidase-conjugated antisera failed to demonstrate immunoglobulin-containing cells in intestine, spleen and mesenteric lymph node of newborn pigs before ingestion of colostrum, but found immunoglobulin-synthesizing cell populations in the organs from piglets that ingested colostrum. In our view, the demonstration of IgG, IgA and IgM synthesis in colostrum-deprived piglets in the present study is due to the sensitivity of the method employed.

Prokešová and Rejnek (1973) demonstrated the formation of IgM, IgG and IgA by individual lymphoid cells in newborn pigs by means of autoradiography. The possibility of immunoglobulin synthesis in foetal pig cells was suggested by Chapman et al. (1974), Jarošková (1977) and Prokešová (1977). Using autoradiography, the last-named investigator demonstrated immunoglobulin-synthesizing cells in foetal pigs aged 44 days. Allen and Porter (1973) using immunofluorescence demonstrated the presence of IgA secretory component in the intestinal mucosa (crypt epithelial cells) of foetal pigs aged 80 days. The capacity

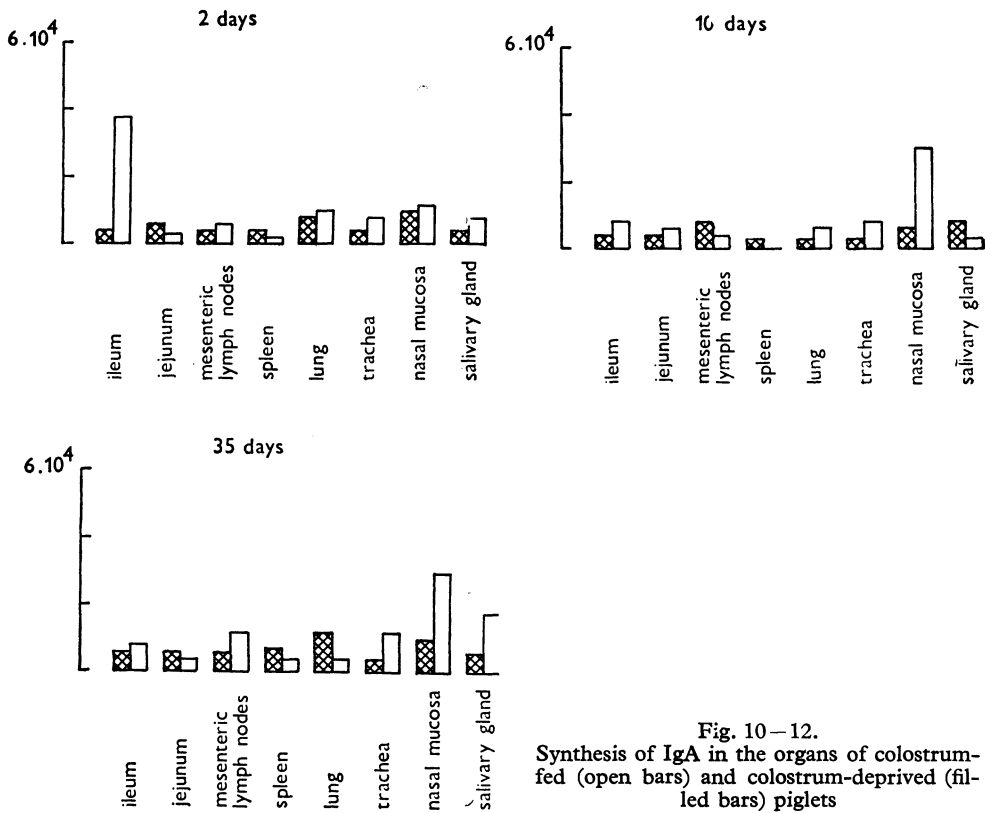


Fig. 10-12. Synthesis of IgA in the organs of colostrum-fed (open bars) and colostrum-deprived (filled bars) piglets

of foetal pigs to produce specific antibody, particularly against viral antigens, has been demonstrated (Bourne, Curtis, Johnson and Collings 1974; Franz, Menšík and Pokorný 1971; Menšík and Franz 1969).

The incidence of immunoglobulin-synthesizing cells in the respiratory tract of the pig was a subject of studies by Bradley et al. (1976) who using peroxidase-conjugated antiserum failed to find IgG, IgA and IgM synthesizing cells in colostrum-deprived newborn pigs, but found them in the respiratory tract of piglets on the 6th and 7th days after ingestion of colostrum. The views on the distribution of newly synthesized immunoglobulin of various classes have been rather conflicting (Allen and Porter 1973; Prokešová 1977). They seem to depend on the organ examined, the method of detection and, last but not least, on the binding capacity of the antiserum. Our results suggest that the bulk of the newly synthesized immunoglobulin was IgG, particularly in colostrum-fed piglets. The method employed in the present study is apparently more sensitive than immunofluorescence and peroxidase methods and may prove useful as an *in vitro* test of the immunoglobulin-synthesizing capacity of the lymphatic tissue of central lymphatic organs or the submucosal lymphatic tissue of the respiratory and intestinal tract. The results reported here indicate a stimulatory effect of colostrum manifested by increased synthesis of immunoglobulins in both peripheral and central lymphatic tissue.

Proteosyntéza imunoglobulinů v orgánových kulturách u novorozeneých selat

Kultivaci orgánových fragmentů v médiu obsahujícím ^{14}C značené aminokyseliny a následnou afinitní chromatografií byla sledována schopnost syntézy bílkoviny včetně imunoglobulinů. V orgánech (ileum, jejunum, mezenterální mízní uzlina, slezina, plíce, slinná žláza, nosní sliznice) bezkolostrálních SPF a kolostrem krmených selat byla prokázána syntéza bílkoviny a imunoglobulinů IgG, IgA a IgM již ve 2. dni života.

Протеосинтез иммуноглобулинов в органокультурах новорожденных поросят

Автор путем культивирования органофрагментов в среде содержащей ^{14}C меченые аминокислоты и путем следующей аффинитной хроматографии исследовал способность синтеза белка включая иммуноглобулины. В органах (подвздошная и тощая кишки, лимфатический брыжеечный узел, селезенка, легкие, слюнные железы, слизистая оболочка носа) лишенных молозива и специфических патогенов и выкармливаемых молозивом поросят синтез белка и иммуноглобулинов IgG, IgA и IgM был установлен уже на второй день жизни.

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