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CLINICAL RESULTS OBTAINED IN CATTLE AND SWINE BY MEANS OF BIOLOGICAL IMMUNOSTIMULATORS*

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Abstract—The conditioned infections due to opportunistic organisms, can be controlled by biological immunostimulators. The POLI-IF (Newcastle virus plus endotoxin of *E. coli* and Freund's incomplete adjuvant) rapidly induces the aspecific immunity. Given twice with 7–10 days interval in between, on occasion of a programmed stress (weaning, transport, crowding) it proved its efficacy in artificially suckled calves and in weaning piglets. The field trials, carried out on 2,782 treated calves in comparison with 2,909 untreated controls and on 4,387 piglets in comparison with 4,461 untreated controls, revealed statistically significant differences for $P < 0.005$ among the groups of treated and control animals. The immunostimulator reduced the incidence of the disease, dead and discarded animals, as well as it shortened the mean duration of the disease in single heads. Though the way the POLI-IF acts is not perfectly known so far, yet it increases the serum bactericidal activity and the circulating leukocytes, while it induces high levels of IFN. In our opinion the activity of the POLI-IF is bound to 3 factors at least: activation of the complementary fractions, mobilization of the immunocompetent cells, induction of IFN.

Key words: Natural immunity, non-specific immunity, disease resistance, natural resistance, interferon inducers, immunization, immunostimulation, complement activation

Résumé—Les infections conditionnées peuvent être maîtrisées par l'emploi d'immunostimulants biologiques capables d'induire une immunité non spécifique. Le POLI-IF (NDV, endotoxine de *E. coli*, adjuvant incomplet de Freund) injecté deux fois à un intervalle de 7–10 jours, à l'occasion d'un stress programmé (sevrage, transport, "crowding"), a contrôlé les infections conditionnées de veaux allaités artificiellement et de porcelets au sevrage. Les épreuves sur le terrain effectuées sur 2782 veaux traités avec POLI-IF à côté de 2909 non traités des mêmes élevages, et sur 4387 porcelets traités à côté de 4461 non traités, ont montré des différences statistiquement significatives pour $P < 0.005$ entre les groupes traités et les groupes témoins. L'immunostimulant réduit remarquablement l'incidence des animaux touchés, morts et écartés et abrège la durée moyenne de la maladie de chaque animal. Bien que les modalités de son action ne soient pas parfaitement connues, le POLI-IF accroît l'activité bactéricide du sérum, le nombre des leucocytes circulants et induit des niveaux élevés d'interféron. Cela fait supposer que l'activité de cet immunostimulant est liée à trois facteurs: activation des fractions complémentaires, mobilisation des cellules immunocompétentes, induction d'IFN.

Mots-clés: Immunité naturelle, immunité non-spécifique, résistance à la maladie, résistance naturelle, inducteurs d'interféron, immunisation, immunostimulation, activation du complément

INTRODUCTION

The regular application of valid means of specific defence has markedly lowered the rate of the main infectious diseases in humans and animals. Several infections have been eradicated through active and passive immunization, chemotherapeutics, antibiotics. The

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Table 1. Syndromes from opportunistic micro-organisms not referable to classical infections

Syndromes	More frequent opportunistics	Favouring factors	Species involved
Conditioned or multifactorial infections	Single or associated viruses, bacteria, protozoa, mycetes	Stress, crowding, Immunodeficiencies	Any species in intensive farming
Chronic, progressive or persistent infections	<i>Retroviridae</i> , <i>Reoviridae</i> , <i>Papovaviridae</i> <i>Herpesviridae</i>	Stress immunodeficiencies	Cattle, sheep, swine, mink, dog, fowl
Feed infections	<i>Salmonellae</i> , <i>Colibacilli</i> , <i>Staphylococci</i> , <i>Clostridia</i> , <i>Listeriae</i> , <i>Mycetes</i>	Contaminated or badly stored feeds; managerial mistakes	Cattle, swine, fowl, mink, dog, cat

effect of other infections has been limited with measures of veterinary policy, improved hygiene, disinfections, deratizations and anti-parasitic treatments. Nevertheless, the problems of infectious pathology have increased. Other diseases once not considered as infectious have appeared, their identification through Koch's principles and eradication by means of the widely known prophylactic measures being rather impossible. It is a matter of enzootic infections involving complex relations among organisms, animals, environment, feeding, farming techniques. The various syndromes can be classified according to Table 1.

A. *Conditioned infections* mostly encountered in intensive breedings are supported by opportunistic organisms that act singly or associated. Their development depends upon different conditioning factors.

B. *Chronic, progressive or persistent infections* that are increasingly known as virological and immunological diagnostic techniques are improved. They are slow, latent, tolerated, hidden infections and virus-associated tumours.

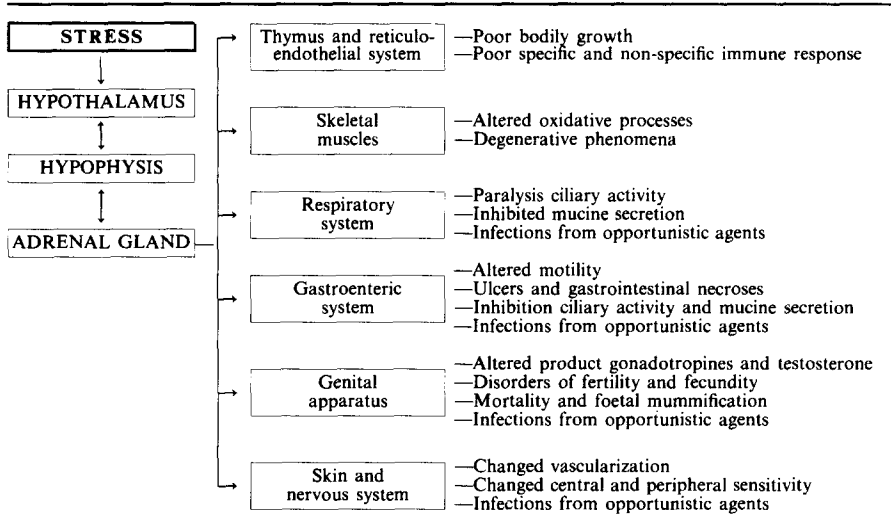
C. *Alimentary toxoinfections* supported by a number of bacteria and mycetes. The increasing spreading of such syndromes often depends upon long-term storage of fodders.

The control of conditioned syndromes in cattle and swine from intensive breedings is dealt with in this paper. The conditioned infections are supported by opportunistic organisms and non-microbial factors, according to the scheme at Table 2.

Table 2. Factors favouring the onset of the conditioned infections

CONDITIONED OR MULTIFACTORIAL INFECTION		
Microbial factors	Non-microbial factors	
<i>Opportunistic agents</i>	<i>Endogenous</i>	<i>Exogenous</i>
Viruses	Poor colostrum assumption	Unfavourable microclimate
Bacteria	Immunosuppression	Inadequate feeding
Protozoa	Transport stress	Crowding
Mycetes	Cooling	Change of sheds
	Persistent diseases	Poor hygiene

Table 3. Hypothetical scheme of stress-derived actions and reactions seen in the different body systems



All these factors induce pathological changes, schematized in Table 3, which favour the establishment opportunistic organisms.

Some forms of stress, though unavoidable, can be foreseen, programmed and ruled. Transport, crowding, changes in diet and sheds, seasonal climatic variations, if foreseen, should not cause serious damage to farmers. Owing to this, treatment with immunostimulators in problem-herds were started in order to check the possible action of the opportunistic agents with stimulation of the non-specific natural immunity, with criteria analogous to those of German authors [13, 14, 15, 16].

MATERIALS AND METHODS

Immunostimulator

The biological immunostimulators employed (POLI-IF) is a mixture in anaparts of: Inactivated, purified and concentrated Newcastle virus, corresponding to 10^{10} EID₅₀/ml. Raw endotoxin of *E. coli* extracted from a suspension of 3.10^9 bacteria/ml. Incomplete Freund's adjuvant. POLI-IF has been previously used [8, 9].

Animals and treatments

Albino mice, Swiss, of 20–25 g. Albino guinea-pigs, Pirbright strain, of 450–500 g. Albino rabbits, New Zealand, of 2300–2500 g. Newborn piglets, of 5-week age, cross-breeds Large White × Landrace. Artificially suckled calves, of 2 to 8-week age, from different breeds such as Marchigiana, Charolaise, Italian Friesian or their crossbreeds. *In safety tests* the rodents received 2.0 ml/kg live weight (l.wt.) of POLI-IF s.c. or i.m.; piglets had 1.0/5 kg l.wt.; calves 1.0/30 kg l.wt. *In efficacy tests* piglets of 5-week age received 2 injections of 0.5 ml POLI-IF s.c., at intervals of 10 days. The calves of 2 to 8-week age were injected twice 1.0 ml POLI-IF s.c. at intervals of 10 days.

Techniques

The efficacy of POLI-IF in laboratory has been evaluated indirectly, as the experimental infection with opportunistic agents is rather unrealizable. Tests have been carried out on piglets of 5 weeks, of which 14 treated and 6 controls and 18 calves of 4 weeks, artificially suckled (12 treated and 6 controls). The treatment with POLI-IF has been carried out twice at intervals of 10 days. In injected animals and in untreated controls the following remarks have been made:

Daily clinical examination for 4 weeks following the first injection;

Hemochromocytometric examinations prior to both injections with POLI-IF and then for 7 consecutive days. The values of non-injected animals belonging to the same groups have been held as normal;

Determination of the circulating interferon (IFN) rate, by means of inhibition of the vaccinia virus grown on pig renal cells, line PK15 or on calf renal cells. The titration has been carried out with a microplate technique on animal serum prior to each injection of POLI-IF and then 12, 24, 48, 72 and 96 h after treatment with POLI-IF.

Determination of the serum bactericidal activity (SBA) according to Koller *et al.* [10], already employed by Dorn *et al.* [4, 5] and Galassi *et al.* [6] in calves and piglets, respectively. The *E. coli* strain "Abbotstown" 0149:K88 ac:H19, phase S, was used for the evaluation of the SBA. The serum was obtained from animals through natural coagulation of the blood drawn before treatment and 12, 24, 48, 72 and 96 h after treatment with POLI-IF.

The efficacy of POLI-IF in the field has been tested in problem-herds. Nearly 50% of the animals has been treated on programmed stresses (transport, crowding, changes in diets and sheds etc.). At the onset of the conditioned syndrome the following operation has been done:

Daily clinical examination of animals for duration, severity and issue of the disease, also in connection with a possible therapy.

Taking of a blood sample on a significant percentage of animals both in the acute and in the infection phases of convalescence to identify the involved opportunistic agents serologically.

Collections of pathological material from animals with acute infection or from the dead ones, for pathological and microbiological examinations, as to discover the opportunistic agents.

Mathematical and statistical data processing to ascertain the variability of parameters in treated groups and in untreated controls. Reckoning of the chi square to check the significance of the possible differences between treated and control animals.

RESULTS

The safety tests of the POLI-IF, carried out s.c. or i.m. on laboratory rodents had a positive issue: no general reactions were ascertained and local reactions were persistent small granulomas. Calves exhibited a small granuloma at the injection point and in some animals a mild hyperthermia and anorexia were seen within 24 h post injection.

The reactions in pigs were similar to those seen in calves but the percentage of animals with general consequence was higher and ranged 10%.

The efficacy tests in laboratory, performed on calves and piglets provided homogeneous

results. The mean values together with the deviation have been reported in graphs:

At the clinical examination local and general reactions previously described in the safety tests have been remarked.

Hematologically, no remarkable modifications of the erythrocytes and hemoglobin rate were seen whereas in POLI-IF treated animals, *a marked increase in the number of leukocytes* was checked. In Figs 1 and 2 the mean values and the mean square deviation seen in calves and piglets are reported. With the horizontal lines the field is shown where the normal values of controls are framed. Following the injection the increase of leukocytes reaches the peak within 12 h in piglets and in 48 h in calves. In such points the circulating leukocytes number is redoubled. Within 48 h following the peak the values get back to normal again. At the 2nd injection the increase of circulating leukocytes is rapid and the peak, in both animal species, is reached 24 h later. The values of the 2nd peak are higher than those of the previous one and even the duration of the phenomenon is longer. Within 72 h the circulating leukocytes in piglets get back to normal while in calves they are still double in number after 4 days from injection.

The rate of circulating interferons is reported in Figs 3 and 4 for calves and piglets, respectively. The sudden appearance of circulating IFN is seen after POLI-IF injection and peaks are reached between 12 and 24 h, with mean values of about 100 U/ml. After the 2nd injection the phenomenon takes place again, but the peak shows higher mean values,

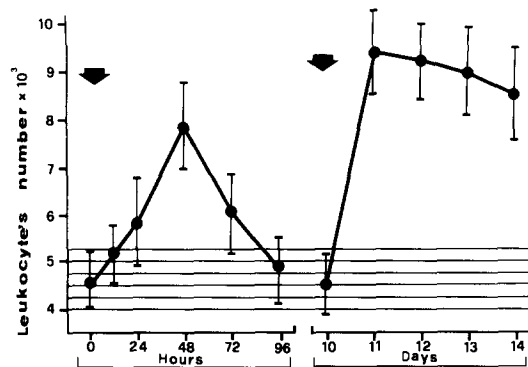


Fig. 1. Variation in the circulating leukocytes following POLI-IF injection in calves.

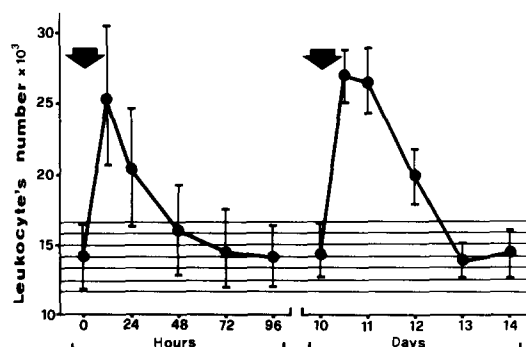


Fig. 2. Variation in the circulating leukocytes following POLI-IF injection in pigs.

nearly 140 U/ml and 260 U/ml in calves and piglets, respectively. Even the IFN, after the 2nd injection still remain in circulation for 24 h more than the first one.

The *serum bactericidal activity* (SBA) is reported in Figs 5 and 6 for calves and piglets, respectively. The SBA has been followed only after the 1st injection for 4 days. In fact, we considered that the immunostimulator contains raw toxin of *E. coli* and the technique to find the SBA is based on the inhibiting activity of the serum towards a colibacillus.

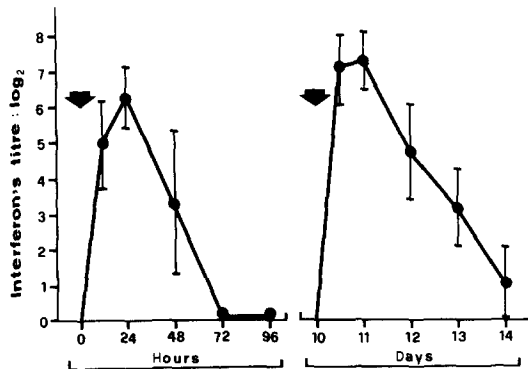


Fig. 3. Increase in IFN checked in calves after treatment with POLI-IF.

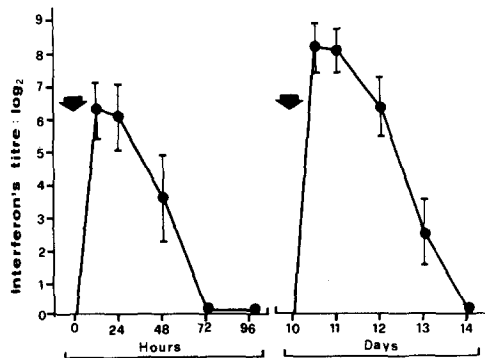


Fig. 4. Increase in IFN checked in pigs after treatment with POLI-IF.

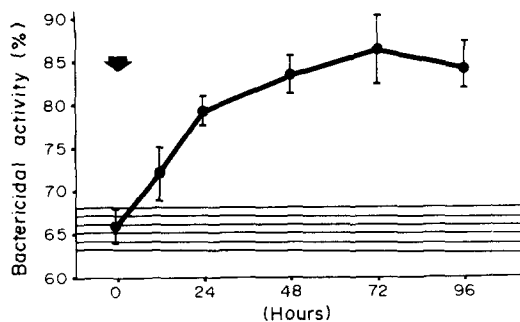


Fig. 5. Increase of the bactericidal activity in blood serum of calves after treatment with POLI-IF.

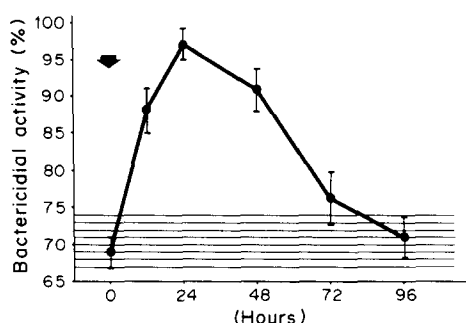


Fig. 6. Increase of the bactericidal activity in blood serum of piglets after treatment with POLI-IF.

In such conditions the titration performed 5 days post injection could have also shown specific antibodies whose values summed to the specific ones would increase the SBA titres.

In control calves the SBA has constant values, ranging between 63 and 68% (in Fig. 5 the values are reported in the form of horizontal lines). In injected animals the percent values rapidly increase and reached the peak within 72 h, with mean values of 86% and the SBA remains on such values for at least 24 h. In piglets the mean values of controls are constant around 70% (see horizontal lines in Fig. 6). After POLI-IF injection a rapid increase of SBA is seen, the peak being reached within 24 h, up to mean values of 97%. Subsequently, the activity rapidly decreases and 96 h post injection the values are already back to normal.

The efficacy tests in the field, carried out in problem-herds allowed us to check the practical value of the immunostimulator [7, 8, 9].

In calves suckled artificially the conditioning factors that favoured the action of the opportunistic agents were: poor assumption of colostrum, sudden passing to the artificial suckling, crowding in sheds with unproper microclimate. In these breedings, infections generally affect both digestive and respiratory systems and within 1–2 weeks they rapidly involve most animals. Within 2 to 3 weeks, if properly treated, the same syndromes decline and gradually disappear.

Losses are to be ascribed to lack of weight increases, high mortality, large number of discarded heads. The results obtained with the POLI-IF in 16 problem-herds with artificially suckled calves are reported in Table 4. The first injection of the immunostimulator has been carried out on 50% of the animals as soon as they entered the breeding, that is when transport, crowding and the beginning of the artificial suckling already had a marked stressing action. The 2nd injection, in the previously treated subjects, carried out 7–10 days from the first one, generally matched the beginning of the

Table 4. POLI-IF field trials in problem-herds of 2–8 week artificially suckled calves

	Treated with POLI-IF	Untreated controls
Herd animals	2,782	2,909
Conditioned infections	608 (21.8%)	1,931 (66.4%)
Mean disease duration: days	8.7	18.2
Dead	47 (1.7%)	249 (8.6%)
Discarded	38 (1.4%)	176 (6.1%)

conditioned pathology in the most debilitated calves. The effects were the same in all breedings, although the index of the affected animals shows differences in the breedings. As a whole, the results are positive and can be schematized as follows:

The group of calves treated with POLI-IF had 2782 animals. Among these the conditioned infections affected 608 animals (21.8%). The agents in the different foci were IBR, PI₃, Adeno- and Rotaviruses, *E. coli*, *S. dublin*, *Pasteurellae* and *Mycoplasma*. In the same breeding two or more opportunistic agents were always present at the same time. The reaction to the conventional therapy, though different from focus, was generally good, so that the mean duration of the disease slightly exceeded a week (8.7 days). Forty animals died (1.4%) and the discarded ones with chronic lesions or with a poor weight increase were 38 (1.4%). As a whole, losses due to mortality and discarding were 3.1% in treated groups.

The groups of untreated control calves consisting of 2909 animals, even if belonging to the POLI-IF treated breedings, had even more severe damages. The conditioned infections affected 1931 animals (66.4%); the opportunistic agents involved were the same and even these animals were often attacked by 2 or more species of organisms at the same time. The reaction to therapy often resulted unsatisfactorily so that the mean duration of the disease was nearly three weeks (18.2 days). The mortality involved 249 heads (8.6%) while the discarded ones were 176 (6.1%). As a whole, animal losses in control groups were 14.7%. The differences, among control and treated groups were statistically significant: $P < 0.005$.

In piglets at weaning the main stressing factors were transport, crowding, the sudden replacement of the maternal milk in the diet, the change of the environmental microclimate. Even in these animals the opportunistic organisms mainly involve digestive and respiratory systems within 7–10 days. The spread of the syndrome is rapid and the duration of the disease in the breeding is nearly 3 weeks, provided that the therapy is appropriate. Losses are due to a marked mortality, discard of irrecoverable animals owing to their chronic lesions, lack of weight increase, expensive mass- or individual therapy.

In Table 5 are summarized the results obtained in different weaning cycles of 4 problem-herds, where nearly 50% of animals were treated with POLI-IF during their transport from delivery rooms to weaning sites. The remaining 50% consisted of untreated controls. After 7–10 days the POLI-IF was injected once again to treated animals. The results, even if slightly different from a cycle to another, can be summarized as follows.

The groups of piglets treated with POLI-IF consisting of 4,387 animals had 264 heads affected with conditioned infections, the rate being 6.1%. The opportunistic agents were Rotavirus, Coronavirus-like, *E. coli*, Streptococci, *Campylobacter* spp., *Bordetella bronchiseptica*, *Haemophilus* spp., *Pasteurellae*, *Mycoplasmas*, *Coccidia*, *Trichomonas*. In most cases, in the same group of animals, more opportunistic agents were seen, contemporaneously.

Table 5. POLI-IF field trials on pigs from problem-herds with conditioned infections at weaning

	Treated with POLI-IF	Untreated controls
Piglets at weaning (4–5-week old)	4,387	4,461
Affected with conditioned infections	264 (6.1%)	1,828 (40.9%)
Mean disease duration: days	6.3	47.8
Dead before 10 weeks	52 (1.2%)	396 (8.8%)
Discarded before 10 weeks	13 (0.3%)	49 (1.1%)

However, the response of animals to the ordinary therapy was generally good. The duration of the disease in animal groups was nearly a week (6.3 days) and only 52 heads died, with a rate of 1.2%. The discarded animals were 13 (0.3%). Losses among treated animals were 65 (1.5%).

The groups of untreated control piglets, are 4,461 heads. Among these the subjects with conditioned infections were 1,828 (40.9%).

The opportunistic agents seen were the same of the POLI-IF treated groups. However, in spite of the same therapy, the incidence of the various syndromes was much more severe. The duration of the disease, in single animals, markedly exceeded 2 weeks (17.8 days); the individuals that died were 396 (8.8%); the discarded heads were 49 (1.1%). As a whole, losses among animals of control groups were 9.9%).

The statistical differences among control groups and those of treated ones are rather significant: $P < 0.005$.

DISCUSSION

In the last years a number of substances have been discovered, such as synthetic products, inorganic compounds, substances of natural origin capable of acting on the immune response, specific or aspecific [11]. On the ground of these researches new therapies have been suggested in order to modify, stimulate or weaken the immune response, according to the needs. In the veterinary field too the experimentation on modulators, depressors and stimulators of the immunity has been carried out since long, yet the products available are limited in number and often wrongly used, owing to a poor technical information. However, the needs in farms of profit animals are more and more urgent and the research in this field has to be deepened. There are some difficulties for the evaluation of activities that modify the immunologic reaction of the single animal or of the group (herd or stock-farming).

Nowadays, we have few elements to give useful information either for choosing the immunostimulator or for the evaluation of its efficacy. The elements of evaluation are extremely limited when the activity of drugs acting on an aspecific immunity is intended to be determined. In such cases the evaluation has to be done indirectly, once seen the difficulty in the determination of the experimental infections with opportunistic organisms.

The stimulation of the aspecific immunity by means of biologicals has caught the attention of a number of research workers such as Cancellotti and Galassi [1], Mallick *et al.* [12] and May [13]. It does not surprise that a biological product as the POLI-IF has provided good results in the field for the control of the conditioned infections. In these syndromes the vaccines against a limited number of agents often have an unsatisfactory issue. The stress factors favour opportunistic agents, that exert their pathogenic activity in an unpredictable way and as it often happens, under variable microbial associations. In such circumstances the specific cell-mediated immunity, provoked by the immunostimulators, provides the means for a valid defence.

Our research cannot define the way such a defence develops, yet the reliefs performed may provide an explanation of general character. The first evident datum is that the aspecific immunity is evoked within a short time: 12–24 h after injection the peaks of the immunostimulator activity are reached (increase of circulating leukocytes, rate of IFN and SBA).

This makes us suppose that, in the case of latent conditioned syndromes, the rapid use of immunostimulators could be helpful. In addition, it is to be stressed that each parameter we checked may constitute a significant index of the reactivity degree of the animal. In fact, each parameter means an accrual of activities bound to the cell-mediated aspecific immunity.

The increase of circulating leukocytes shows the mobilization of the immuno-competent cells. Further research will be able to establish the relationships among the different types of circulating cells, but it is clear that the mobilization allows them to carry out rapidly the meeting, the recognition, the possible neutralization and destruction of the infecting agent. It will be also possible to codify the specific antibodies whose preparation will be performed later.

The increase of circulating IFN does not show only the rapid realization of the antiviral defence. The IFN system has shown its capability to influence other defensive mechanisms [2, 3]: it can modulate the immune responses, inhibit the growth of tumours, influence the expression of the membrane antigens, start the phagocytosis, enhance the cytotoxicity of the lymphocytes, mime the action of some hormones.

The increase of the SBA is the expression of a series of actions among which worthwhile quoting is the activation of the complementary fractions [17]. Complex biological phenomena are bound to this activation namely: opsonization, phagocytosis, bacterial and viral lyses, acute inflammatory process.

To conclude, it can be stated that the biological immunostimulators, as the POLI-IF, exert a marked activity in the control of the conditioned infections of calves and piglets. The ways through which the activity develops deserves further investigation; however, they seem to depend upon three phenomena: the activation of the complementary fractions, the leukocyte mobilization, the interferon induction.

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