

ANNALES DE PARASITOLOGIE

HUMAINE ET COMPARÉE

Volume 61

1986

N° 4

© Masson, Paris, 1986.

Ann. Parasitol. Hum. Comp.,
1986, 61, n° 4, pp. 393-400.

MÉMOIRES ORIGINAUX

THE LEVELS OF TOTAL PROTEIN AND PROTEIN FRACTIONS IN THE SERUM OF RABBITS INFECTED WITH *EIMERIA STIEDAI*

M. GOMEZ-BAUTISTA*, M. V. GARCIA**, F. A. ROJO-VAZQUEZ***

SUMMARY. The total protein levels in rabbits infected with *Eimeria stiedai* together with the alterations in the different protein fractions over a period of 35 days were studied. It was observed that from the second week of infection onwards a progressive increase took place in total proteins; the electrophoretic study revealed that the hyperproteinemia is mainly due to the increase in γ -globulins accompanied by a decrease in the concentration of albumin and a increase in α and β -globulins. These protein alterations induce an important modification in the albumin/globulin ratio which decreases considerably.

Taux de protéines totales et de fractions protéiques dans le sérum de lapins infectés par *Eimeria stiedai*

RÉSUMÉ. On a étudié les niveaux de protéines totales dans le sérum des lapins infectés de *Eimeria stiedai* ainsi que les altérations manifestées dans les différentes fractions protéiques pendant une période de 35 jours. A partir de la 2^{de} semaine on a observé un accroissement progressif dans les protéines totales réfléchi dans l'étude électrophorétique, qui montrait que la hyperprotéïnémie est le résultat principalement de l'accroissement des γ -globulines accompagné d'une diminution des niveaux d'albumine et d'une augmentation des α et β -globulines. Ces altérations protéiques induisent une modification importante dans la proportion albumine/globulines qui descend significativement.

* Departamento de Parasitología, Facultad de Farmacia, Universidad de Salamanca, España.

** Departamento de Bioquímica, Facultad de Farmacia, Universidad de Salamanca, España.

*** Departamento de Patología infecciosa y parasitaria, Universidad Complutense, Madrid, España.

Accepté le 1^{er} août 1985.

Introduction

Hepatic coccidiosis is an important disease in rabbits caused by *Eimeria stiedai* infection. This protozoan parasitizes the epithelial cells of the bile ducts and though mortality is usually quite low, it is responsible for considerable economic losses to breeders.

Rabbits infected with *E. stiedai* exhibit substantial changes in biochemical parameters (Martine, Yvoré, 1974; Hein, 1977; Barriga, Arnoni, 1981; Gomez-Bautista *et al.*, 1985) which are the result of disturbances in liver function. Studies concerning the alterations in serum protein levels of rabbits infected with *E. stiedai* are rare. Barriga, Arnoni (1981) reported a non-significant but consistent hyperproteinemia on day 15 and hypoproteinemia from the 4th to the end of the 6th week of infection and their results were different to those observed by us in previous work (Gomez-Bautista, 1984) where it was observed that an important increase in serum protein levels appeared on day 23 after infection, preceded by a non-significant hypoproteinemia. With a view to obtaining new data, we were prompted to study the total proteins and the different protein fractions over a five-week infection period.

Materials and methods

A total of 30 New Zealand white rabbits free of coccidia were used in this work. The animals were randomly divided into 3 groups of 10, one group of uninfected controls (A) and two infected groups (B and C). The rabbits were housed individually in heat sterilized wire-floored metal cages and kept at 19-20 °C with thermostatic heating. Water and feed was administered "ad libitum".

At eight weeks of age the animals were inoculated with 10^4 oocysts of *E. stiedai* through a stomach tube. The total faecal output was collected daily and examined for oocysts of *E. stiedai* for confirmation of infection and of its absence in the controls.

The animals were bled, in all cases after a 12h fast, on days 0, 7, 14, 21, 28 and 35 after infection in groups A and B, which total proteins were determined, and on days 0, 17, 20, 23, 28 and 35 after infection in the animals of group C in which total proteins and protein fractions were determined. Approximately 1 ml of blood was collected from the central ear artery on each occasion. The blood was allowed to clot and the serum removed as soon as possible but always later than 4 h after collection. Total serum proteins were estimated by the Biuret reaction following the method described by Henry *et al.* (1957). A Spectronic 21 Bausch and Lomb Vis-Uv spectrophotometer was used.

Electrophoretic values were determined in bands of cellulose acetate (Cellogel) 2.5×17 cm, in sodium veronal (0.09M)- sodium diethylbarbiturate (8.24 g/l) buffer. Serum was applied with a macro-applicator. Migration time in a humid atmosphere was 60 min. at constant voltage (200 V).

The bands were stained with Amido block 10B (0.5 g in 45 ml of methanol +

45 ml of water + 10 ml of acetic acid) and destained, dehydrated and transparented in solution of methanol/cyclohexane/acetic acid (87 : 3 : 10). To complete the transparency procedure, the strips were placed on a glass plate for 2 h. Following these transparency and drying procedures the bands were read in a Gelman DCD-16 (UV) densitometer equipped with a graphic recorder. The percentage of each protein fraction is given directly by this apparatus.

Students's "t" test was used for statistical analysis of the results. Values are expressed as means ± standard deviation.

Results

In the control animals (group A) a mean total protein count of 6.5 g/100 ml was recorded over the 35 days of the study period (fig. 1). The serum protein levels in the rabbits infected with *E. stiedai* (group B) remained at basal levels until the second week of infection, after which the mean total protein count rose until values greater than 10 g/100 ml were reached during the 5 th week (fig. 1).

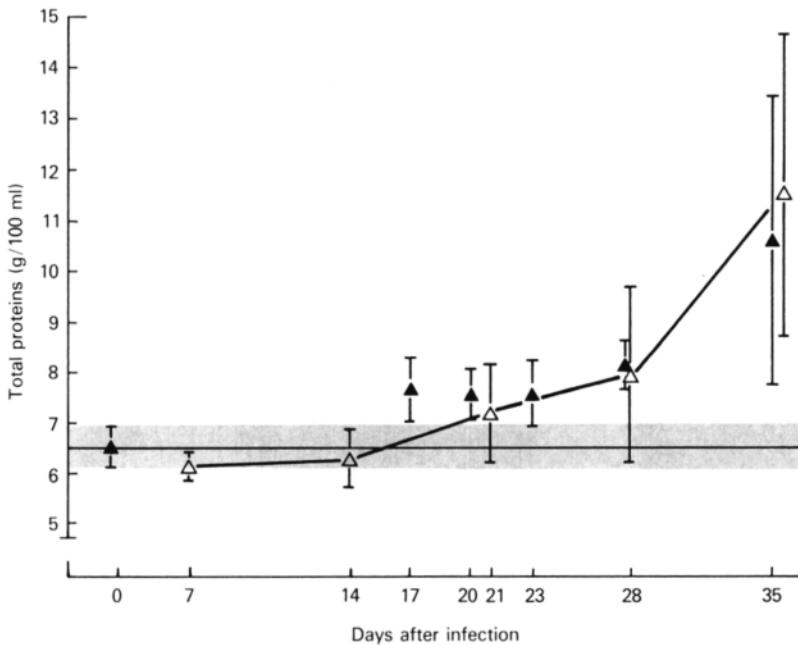


FIG. 1. — Proteinemia levels in rabbits infected with *Eimeria stiedai* (Δ, group B), (▲, group C) and uninfected controls (■).

Figures 2 through 7 show the electrophoretic migration pattern and the graphic register of the protein fractions in the animals of group C. The electrophoresis of the proteins allowed the obtention of 6 fractions : albumin, α-globulins, β₁-globulins,

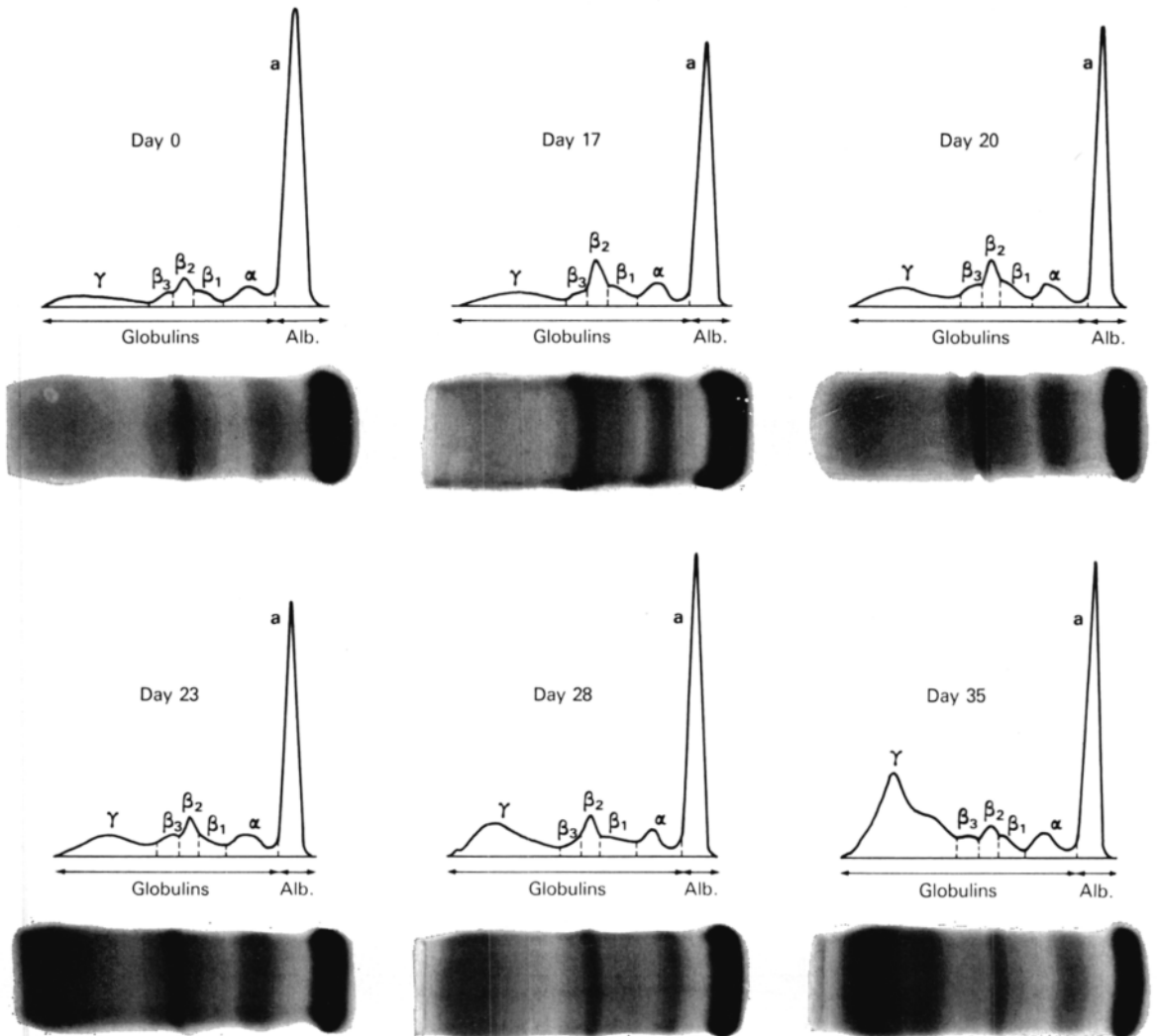


FIG. 2 to 7. — Scanned serum proteins of representative strips from rabbits, uninfected (day 0) and infected with *Eimeria stiedai* (Day 17, 20, 23, 28 and 35).

β_3 -globulins, β_2 -globulins and γ -globulins. The relative percentage of these fractions and its quantitative values are shown in *table I*, where it may be seen that the relative percentage of the serum protein fractions in rabbits before infection (day 0) was close to that reported by other authors for this species (Vaissaire *et al.*, 1976). *Table I* and *figure 8* show that on day 17 after infection the increase in proteins was due to an increase in globulins in the α , β_1 and β_2 fractions and that the progressive increase

in protein levels in the serum of infected animals from day 20 onwards was due mainly to the increase in the α , β_1 , β_2 and γ -globulins. The γ -globulins showed a very pronounced increase and on day 35 this fraction had become quantitatively the most important serum protein, even more than the albumin.

The relative percentage of the albumin fraction was seen to decrease until it reached 32.5 % at the end of the experimental period (*table I*). This represents only a small decrease in concentration between days 23 and 35 after infection (*table I*, *fig. 8*), though it reflects an important decrease in the albumin/globulin ratio which during the fifth week was approximately 1/3 of that found before infection (*table I*).

Oocysts of *E. stiedai* were first observed in the feces on day 14 in all infected animals; they reached their highest density in the faeces between days 21-28 after infection.

TABLE I. — Level of total protein and protein fractions in the serum of rabbits infected with *Eimeria stiedai*.

Day	No. of Anim.	Total Proteins (g/100 ml)	PROTEIN FRACTIONS						A/G ratio						
			Albumins		α -Globulins		β_1 -Globulins			β_2 -Globulins		β_3 -Globulins		γ -Globulins	
			(%)	(g/100 ml)	(%)	(g/100 ml)	(%)	(g/100 ml)		(%)	(g/100 ml)	(%)	(g/100 ml)	(%)	(g/100 ml)
0	5	6.5 ± 0.4	66.9 ± 2.3	4.70 ± 0.4	6.9 ± 0.4	0.48 ± 0.04	4.4 ± 0.5	0.31 ± 0.02	6.3 ± 0.6	0.45 ± 0.05	3.5 ± 0.3	0.25 ± 0.02	12.0 ± 2.0	0.84 ± 0.11	1.552
17	5	*7.7 ± 0.6	60.0 ± 1.4	4.65 ± 0.11	8.6 ± 0.9	*0.67 ± 0.07	6.4 ± 0.0	*0.50 ± 0.01	9.2 ± 0.9	*0.71 ± 0.07	3.5 ± 0.9	0.28 ± 0.08	12.3 ± 0.5	0.95 ± 0.04	1.503
20	5	*7.6 ± 0.5	59.3 ± 7.1	4.61 ± 0.62	9.2 ± 1.9	*0.72 ± 0.16	4.6 ± 1.1	0.36 ± 0.08	7.5 ± 1.0	*0.58 ± 0.06	4.7 ± 0.8	*0.36 ± 0.05	14.7 ± 3.7	*1.14 ± 0.27	1.458
23	7	*7.6 ± 0.6	53.1 ± 5.6	*4.05 ± 0.66	10.1 ± 0.8	*0.79 ± 0.10	4.8 ± 1.3	0.36 ± 0.08	8.0 ± 0.8	*0.60 ± 0.05	4.6 ± 1.0	*0.35 ± 0.08	19.1 ± 3.5	*1.44 ± 0.27	1.412
28	6	*8.2 ± 0.4	35.3 ± 14.1	*3.64 ± 0.65	9.3 ± 1.3	*0.76 ± 0.09	5.7 ± 1.4	*0.47 ± 0.12	7.9 ± 1.1	*0.64 ± 0.09	4.5 ± 1.4	*0.37 ± 0.11	26.5 ± 6.5	*2.15 ± 0.58	0.654
35	7	*10.7 ± 2.9	32.5 ± 3.8	*3.99 ± 0.60	6.7 ± 0.7	*0.87 ± 0.17	3.1 ± 0.3	*0.44 ± 0.09	5.3 ± 0.5	*0.66 ± 0.16	4.6 ± 0.5	*0.57 ± 0.13	47.4 ± 4.2	*5.86 ± 0.99	0.485

Results with asterisk are significantly different from uninfected controls ($p < 0.05$).

Discussion

The liver is the source of many of the proteins circulating in plasma. The hepatic cells are the sites of formation of α and β -globulins, albumin being the most important protein synthesized by the liver in quantitative terms ; it is thus not surprising that hepatobiliary diseases are often associated with striking changes in the concentration of these compounds. The effect of liver disease on the levels of serum proteins is complex and depends not only on its effect on protein synthesis but also on its effects on the volume and distribution of extracellular fluids, on the half-life of the individual proteins and on protein catabolism by various routes.

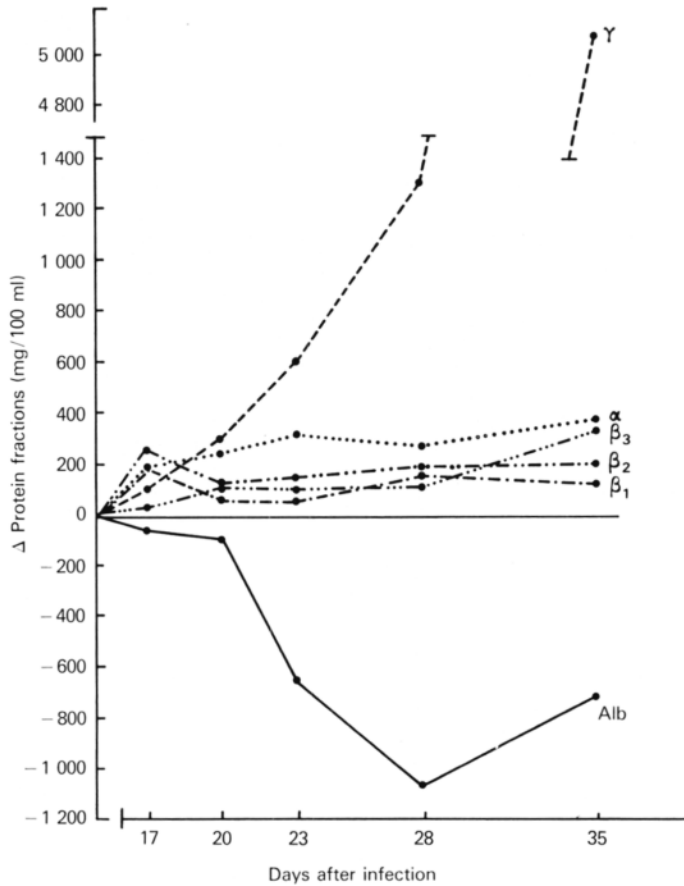


FIG. 8. — Increase in different protein fractions in the serum of rabbits infected with *Eimeria stiedai*.

Many reports have shown that protein-calorie deficiency induces a decrease in protein synthesis by the liver, though some studies have described an increase in the synthesis of intracellular proteins (Zern *et al.*, 1982). Anorexia in hepatic coccidiosis is restricted to the first four weeks of infection (Yvoré and Guillaume, 1976 ; Hein, 1977 ; Gomez-Bautista *et al.*, 1985) and inappetence may be a more important causative factor in the modifications in albumin and α and β -globulins observed in the serum of the infected animals, though it should also be remembered that in *E. stiedai* infection important disturbances take place in the utilization of the ration (Yvoré, Guillaume, 1976 ; Gomez-Bautista *et al.*, 1985) which would contribute to a state of protein deficit and consequently to the pattern of serum proteins observed in this work. Hypoalbuminemia in the presence of high levels of globulins in states of protein deficiency has been attributed to the use of aminoacids preferentially for the

production of globulins and other biologically more important proteins (Cohen, Hansen, 1962).

Serum albumin levels, however, do not reflect the degree of alteration in protein synthesis in liver disease. Thus, hepatic synthesis is only one of several factors affecting serum albumin and even when synthesis is markedly reduced the high half-life of albumin (20 days) may only induce minimum changes in serum albumin levels; hence, a reduction in the synthesis of albumin of 50 % can lead to a 20 % drop in serum levels (Price, Alberti, 1979). When synthesis is reduced serum albumin values also fall, though the fall is minimized because there is a reduction in the catabolic rate for albumin (McIntyre, 1983).

It is true that albumin synthesis falls in many liver diseases and low serum levels are usually recorded in such states but in many cases of hypoalbuminemia the absolute rate of synthesis may be normal or even increased when ascites is involved and the hepatic secretion of albumin is disturbed. In *E. stiedai* infection ascites of varying intensity is observed and it is thus not possible to affirm that the hypoalbuminemia corresponds to an reduction in albumin synthesis.

According to Bachman (1930) infection by *E. stiedai* causes no appreciable increase in the proportion of globulins and no changes in the albumin/globulin ratio. Our results are not in agreement with such findings and in infected rabbits a gradual variation of the albumin/globulin ratio could be seen whose main cause is the increase in globulins. The hyperproteinemia is mainly caused by the increase in γ -globulins and though changes in this protein fraction during liver disease may be due to a large number of factors, in this case it is clear that the production of antibodies must be the main causative factor. Our results are in agreement with the notion that in *E. stiedai* infected rabbits a slight increase in antibodies takes place over the first few weeks and that the highest titres are observed between days 30-60 after infection (Rose, 1972).

The alterations in serum proteins observed in our infected rabbits differ from those reported by Barriga, Arnoni (1981), who described results leading them to suspect that in *E. stiedai* infection "neither dietary nor immunological factors seem to have exerted a critical influence in the shape of the proteinemia curves".

In conclusion, the changes in the levels of serum proteins observed in infected animals seem to be associated with protein deficiency and to the increased production of antibodies. Consequently, it may be suggested that the alterations in serum proteins are mainly due to dietary, digestive/absorptive and immunological factors. However, other physiopathological aspects which could affect the protein synthesis machinery of the liver infected with *E. stiedai* and/or their catabolism and excretion cannot be precluded. It should be mentioned that in *Schistosoma mansoni* infection hypoalbuminemia is known to be associated with a decreased albumin mRNA (Zern *et al.*, 1983) and it is also known that animals infected with adult flukes are in a "hypercatabolic" state with respect to albumin (Dargie, Berry, 1979).

ACKNOWLEDGEMENTS. The authors should like to thank the "Instituto Nacional de Investigaciones Agrarias" (I.N.I.A.) for the technical assistance. Translation by N. Skinner.

REFERENCES

- BACHMAN G. W. Serological studies in experimental coccidiosis of rabbits. *Am. J. Hyg.*, 1930, 12, 624-640.
- BARRIGA O. O., ARNONI J. V. : Pathophysiology of hepatic coccidiosis in rabbits. *Vet. Parasitol.*, 1981, 8, 201-210.
- COHEN S., HANSEN J. D. L. : Metabolism of albumin and gamma-globulin in kwashiorkor. *Clin. Sci.*, 1962, 23, 351-359.
- DARGIE J. D., BERRY C. I. : The hypoalbuminaemia of ovine fascioliasis : the influence of protein intake on the albumin metabolism of infected and of pair-fed control sheep *Int. J. Parasitol.*, 1979, 9, 17-25.
- GOMEZ-BAUTISTA M. : Algunos aspectos de la infección por *Eimeria stiedai* (Lindemann, 1865) Kisskalt et Hartmann, 1907, en el conejo. *Tesis Doctoral*. Universidad de Salamanca, 1984, pp. 133.
- GOMEZ-BAUTISTA M., ROJO-VAZQUEZ F. A., ALUNDA J. M. : Aportaciones al conocimiento de la patología de la coccidiosis hepática en el conejo doméstico. *Med. Vet.*, 1985, 2, 11-21.
- HEIN B. : Zur Pathophysiologie der Gallengangscoccidiose des Kaninchens. Inaugural-Dissertation zur Erlangung des Doktorgrades bei dem Fachbereich Veterinärmedizin und Tierzucht der Justus-Liebig-Universität. Giessen. 1977, pp. 110.
- HENRY R. J., SOBEL C., BERKMAN S. : Interferences with biuret methods for serum proteins ; use of Benedict's qualitative glucose reagent as a biuret reagent. *Ann. Chem.*, 1957, 29, 1491.
- MARTINE G., YVORÉ P. : Aspects pathogéniques de la coccidiose hépatique du lapin domestique. *Cah. Med. Vet.*, 1974, 43, 147-158.
- MCINTYRE N. : The Liver. In : Williams, D. L., Marks V., Biochemistry in Clinical Practice. *The Pitman Press*, London, 1983, 139-154.
- PRICE C. P., ALBERTI K. G. M. M. : Biochemical assessment of liver function. In : Whight R., Alberti K. G. M. M., Karran S., Millwardsadler G. H. Liver and Biliary Disease : Pathophysiology-Diagnosis-Management. *WB Saunders Company Ltd*, London, 1979, 381-416.
- ROSE M. E. : Immune response to intracellular parasites. II. Coccidia. In Soulsby, E. J. L. Immunity to Animal Parasites. *Academic Press*, New York, 1972, 365-388.
- VAISSAIRE J., LABADIE J. P., RENAULT L., MAIRE Cl. : Électrophorèse des protéines sériques chez le lapin normal. *Rec. Med. Vet.*, 1976, 152, 249-252.
- YVORÉ P., GUILLAUME J. : The effects in the rabbit of hepatic coccidiosis on the digestibility of fat and energy. *Ann. Resch. Veter.*, 1976, 7, 343-348.
- ZERN M. A., SHAFRITZ D. A., SHIELDS D. : Hepatic protein synthesis and its regulation. In Arias I., Popper D., Schachter D., Schafritz D. A. The Liver : Biology and Pathobiology. *Raven Press*, New York, 1982, 103-121.
- ZERN M. A., SABER M. A., SHAFRITZ D. A. : Molecular Mechanisms for Changes in Hepatic Protein Synthesis Induced by Schistosomiasis Infection in Mice. *Biochemistry*, 1983, 22, 6072-6077.