

SERUM PROTEIN FRACTIONS IN BACTERIA-FREE GUINEA PIGS INVESTIGATED BY DISC ELECTROPHORESIS

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The serum concentrations of glucoproteins and γ globulins were much lower in bacteria-free guinea pigs than in controls. This difference was most marked when sera obtained from sterile animals were compared with those from normal animals aged 3 months.

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The serum globulin concentration in bacteriologically sterile animals, in the absence of a microflora, is considerably lower than in ordinary animals [19]. In bacteria-free guinea pigs this difference applies to 7S γ_2 globulin and 19S γ_2 globulin, but not to 7S γ_1 globulin [17].

The object of this investigation was to study the serum globulin concentrations in bacteria-free guinea pigs and to determine the glycoprotein composition of these sera.

EXPERIMENTAL METHOD

Bacteria-free guinea pigs were obtained by hysterectomy and subsequent growth in a flexible polyvinyl chloride incubator. Freedom from bacteria was tested after hysterectomy by examination of pieces of the uterus, fetal membranes, and placenta, and thereafter by examination of feces, urine, debris, remains of food and water, hair, and so on once or twice a week. Cultures were incubated at room temperature and at 37° under aerobic and anaerobic conditions (blood agar) for 3 weeks [4].

The animals were kept on autoclaved diet L-477.

One group of normal animals was fed with the same diet, and another group on normal diet (controls).

The total serum protein was determined with the SF-4A spectrophotometer at $\lambda=288\text{ m}\mu$. The sera were investigated by electrophoresis in acrylamide gel (disc electrophoresis) by Ornstein's method [16], with slight modifications. The quantity of protein applied during electrophoresis was the same in all experiments (30 μg).

Altogether 8 bacteria-free animals aged up to 96 days and 23 control guinea pigs of corresponding ages were used in the experiments.

EXPERIMENTAL RESULTS

Interpretation of the disc electrochromatograms was based on data in the literature [1, 5, 16]. With the exception of transferrin (1- β globulin) and albumin, every protein line in the disc electrochromatogram gives a positive PAS reaction [5].

The patterns discovered by the study of all the sera are illustrated in Fig. 1.

The largest number of fractions (14) was found in serum from ordinary guinea pigs aged 96 days. The content of γ globulins in sera from newborn guinea pigs sacrificed a few minutes after birth (without taking colostrum) was not less than in ordinary guinea pigs aged 2 months, a fact readily explained by the

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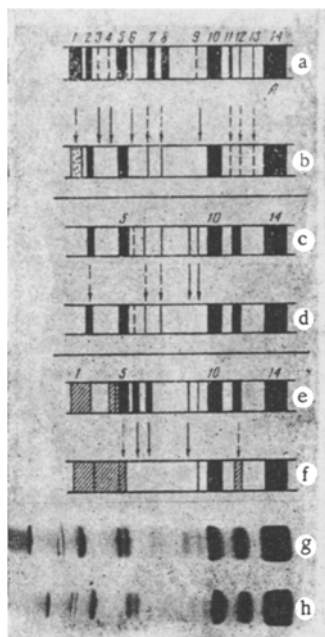


Fig. 1. Scheme of separation of serum protein fractions by electrophoresis in acrylamide gel and photograph of electrochromatograms. Arrows indicate fractions absent in experimental sera; broken arrows indicate fractions present in considerably reduced amounts. Principal fractions: 1) γ globulins; 2) β lipoproteins; 5) α_2 macroglobulin; 6-9) group of haptoglobins; 10) transferrin; 11) ceruloplasmin; 12-13) zone of "postalbumins"; 14) albumin; a) serum of ordinary and b) of bacteria-free guinea pigs aged 96 days; c) serum of ordinary and d) of bacteria-free guinea pigs aged 25 days; e) serum of ordinary guinea pig aged 57 days; f) serum of newborn guinea pig; g) serum of ordinary guinea pig, and h) of ordinary guinea pig kept on diet L-477. No significant difference can be seen between g and h.

G. A. Shapiro found that the blood cortisol level was considerably elevated in the bacteria-free guinea pigs grown by the writers. The adrenals of the bacteriologically sterile guinea pigs were considerably hypertrophied [4]. At the same time, glucocorticoids are known to reduce the serum glycoprotein concentration [10]. Consequently, the decrease in glycoprotein level in bacteria-free guinea pigs could be attributed to some degree to hypercorticism developing in these animals.

To elucidate the possible mechanism of the lowering of the serum glycoprotein level in the experimental animals, it is worth recalling two other phenomena: 1) the absence of a constant "physiological" inflammation in the intestinal mucous membrane of bacteria-free animals, such as is produced in ordinary animals by the normal intestinal microflora [11, 13], and 2) the increase in the serum sialoprotein concentration a few days after the beginning of inflammation [2, 3].

transplacental transmission of γ globulins in guinea pigs [18]. At the same time, some haptoglobin lines were completely absent from disc electrochromatograms of the sera of newborn guinea pigs. The same phenomenon has also been described for sera of newborn infants [1].

The content of glycoprotein fractions in the serum of ordinary guinea pigs increased with age, but no significant increase in the content of these fractions was found in the serum of bacteria-free guinea pigs. The older the bacteria-free and ordinary guinea pigs, the greater the difference, therefore, between the disc electrochromatograms of their sera. This applies also to the γ globulin zone on disc electrochromatograms of sera from bacteria-free animals. There are reports in the literature of a decrease in the γ globulin level in bacteria-free guinea pigs 2-3 months after birth [14, 15].

Regardless of age and sterility of the animals, besides the albumins there were always well-marked transferrin fractions on all disc electrochromatograms. A fifth fraction (α_2 macroglobulin) was also constantly present, except in the serum of newborn guinea pigs, where it was ill-defined.

The results show that in the absence of a normal microflora, not only is the process of globulin formation depressed, but more marked changes in the glycoprotein composition of the sera are also present.

These results are particularly interesting in the light of the immunobiological role of glycoproteins [1-3]. Of all the serum fractions in guinea pigs, it is the α glycoprotein which produces the greatest stimulation of phagocytic activity of the polymorphs [6].

It is also known that bacteria-free animals are more sensitive to bacterial infections [7], although they have no increased sensitivity to some virus infections [8]. Transferrin, the content of which, according to the results of these experiments, is not reduced, has also been shown to have antiviral activity [12].

The decrease in the serum glycoprotein concentration in bacteria-free guinea pigs in the present experiments is in agreement with observations indicating a reduced rate of cell renewal in bacteria-free animals [9].

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