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

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The serum concentrations of glucoproteins and \( \gamma \) 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 \( 7S \gamma_2 \) globulin and \( 19S \gamma_2 \) globulin, but not to \( 7S \gamma_1 \) 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 \( 37^\circ \) 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 \( \mu \)g).

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 (\( 1-\beta \) 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 \( \gamma \) 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

Fig. 1. Scheme of separation of serum protein fractions by electrophoresis in acrylamide gel and photograph of electrophorograms. 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) α2 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].