EFFECT OF CORTICOTROPHIC HORMONE (ACTH) AND CORTISONE ACETATE ON THE PLASMA VOLUME AND THE CIRCULATING SERUM ALBUMIN AND GLOBULIN IN NORMAL DOGS*

BY

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The object of the present work was to examine how corticotrophic hormone (ACTH) and cortisone influence the plasma volume, the serum globulin and the serum albumin in the normal organism.

Apparently such an investigation in normal human subjects has only been made in one case (Mason et al., 1948); the subject, a woman aged 32 years, was given 1600 mg. of ACTH, distributed over 23½ days. The serum proteins — determined by chemical and electrophoretic fractionation — did not change during the period of observation. Otherwise a number of signs indicating an active function of the adrenal glands were observed.

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Similar experiments in normal animals have been made by Winter et al. (1950). In the treatment of normal rats with cortisone they found a fall in the alpha-globulin, a rise in the gamma-globulin (determined by chemical fractionation), and very slight changes in the albumin: globulin ratio.

It may be mentioned in this connection that in patients with Addison's disease low serum albumin and raised serum globulin values have been found in the electrophoretic analysis of the plasma (McCullagh & Lewis, 1945). In adrenalectomised rats a fall in the albumin and a rise in the globulin have been found (Levin & Leathem, 1942), and the same interference in dogs produced similar changes in the serum proteins (Hartman et al., 1942). The effect of these hormones in pathological conditions in man has been studied by a number of investigators (Hench et al., 1950; Thorn et al., 1949; Brochner-Mortensen et al., 1949). In the diseases examined (inter alia chronic polyarthritis, disseminated lupus erythematosus, dermatomyositis) there are, as a rule, increased serum globulin and decreased serum albumin values; the hormone treatment changes these concentrations in the normal direction. Special studies on the serum proteins have been made by Jager et al. (1951), and by Effersøe (1950). No satisfactory explanation of this effect has been given.

The effect of adrenal cortex hormones on a special serum globulin, the antibody-protein, was first examined by Dougherty, Chase & White (1945), and later by many other investigators. Dougherty et al. considered that they could demonstrate a rise in the concentration of antibody-protein in connection with the injection of these hormones. The authors who have repeated the experiments, using quantitative immuno-chemical methods, have been unable to confirm this. Bjørneboe, Fischel & Stoerk (1951) demonstrated that the antibody concentration in the blood of hyperimmunized rabbits fell during treatment with cortisone. It was shown by Teilum, Engbæk & Simonsen (1950) that there is a fall in the gamma-globulin and a rise in the alpha-globulin in hyperimmunized rabbits treated with cortisone.
It may be that the adrenal cortex hormones only exert their influence on the serum proteins in man under pathological conditions and in animals in a state of hyperimmunization. However, the above-mentioned observations of changes in the serum proteins in adrenalectomized animals seem to indicate that in the normal animal these hormones exert an effect on the ratio between the serum albumin and globulin.

We therefore considered that it might be of interest to examine the conditions in normal dogs. In order to follow any possible variations of the plasma volume simultaneously this was determined by means of the Evans Blue Dye method. In this way it was possible to observe variations, both in the serum albumin and serum globulin concentrations, and in the total quantities of circulating albumin and globulin.

**EXPERIMENTAL PLAN**

Two normal, healthy dogs were used for the experiments. They weighed 15 and 17.5 kg. respectively at the beginning of the experiment.

After a preliminary period of, 7 and 3 weeks respectively, the animals were given ACTH (the Danish preparation »ACTON« (the Frederiksborg chemiske Fabrikker (Drs. Vermehren)) for 29 days in doses rising from 15 to 160 mg.*) in the 24 hours, distributed in 4 intramuscular injections. After an interval of 26 days, when no hormone was administered, ACTH was given again for a period of 7 days, the dose being the same as during the first period. Following another interval of 10 days without any administration of hormone, cortisone acetate was administered in a dose of 50 mg. daily (as a single, daily intramuscular injection) for 10 days. The experiment was concluded with a period of 18 days during which no hormone was administered (Figs. 1 and 2).

During the entire experimental period, i. e. 150 and 122 days respectively, blood samples were, as a rule, taken once a week — a total of about 20 ml. of blood each time — a loss

*) 1 mg. = 1 international unit.
Dog Nr. 1 (Cleopatra) and Dog Nr. 2 (Josephine). The circulating albumin and globulin (g/kg), the weight (kg), the plasma volume (ml/kg) and the cell volume (haematocrite (per cent)) in two normal dogs before, during and after the administration of corticotrophic hormone (ACTH) and cortisone acetate.

Figs. 1 and 2.
of blood which must be said to be so trifling (about 1 or 2 per cent of the total volume of blood) that it cannot have interfered with the determinations of the blood volume. The dogs were always fasting when the blood samples were taken. Two days before the institution and 0, 2 and 18 days after the discontinuance of the treatment with cortisone about 10 ml. of blood were drawn in addition for the purpose of electrophoretic serum protein analysis, which was kindly undertaken by Dr. Niels Harboe, the University Institute of General Pathology.

METHODS

(a) The plasma volume (PV) was determined by the Evans Blue Dye method (T-1824) (Gregeresen, 1944). After a blank blood sample had been drawn, the contents of a glass ampoule with 2.500 mg. were injected intravenously, and 10 minutes later a blood sample was taken for photometry. As already mentioned, the dogs were always fasting and at rest during the determination. As the weight of the dogs varied during the experimental period, the plasma volume per kilo of the body weight was also calculated.

(b) The cell volume (haem.) was determined in undiluted heparinized blood by centrifuging of the blood until the cellular mass was translucent. The centrifugation lasted 30 minutes at 5000 r. p. m.

For each determination the mean of three determinations was used; glass tubes of a special construction were used (measuring 30 × 4 mm).

(c) The protein determinations in the serum were performed by means of the Kjeldahl analyses, with distillation and titration. We reckoned with a content of nitrogen of 16 per cent in the protein. The serum globulin was determined by salting out with 26 per cent Na₂SO₄ at 37° C.

(d) The circulating albumin and globulin were calculated in grammes on the basis of the PV and the albumin and globulin percentages. The circulating albumin and globulin were then calculated per kilo of the body weight.
The eosinophilic leukocytes were counted during the entire experimental period. H. C. Andersen's modification of v. Dungern's method was used (Andersen, 1943).

RESULTS

Our results are shown in Figs. 1 and 2 and in Table 1.

It appears that the weights of both dogs increased evenly during the whole experimental period, irrespective of the periods of hormone administration. The increase in weight was most marked in Dog 1 (Cleopatra).

Table 1.
The relative concentrations (per cent.) of albumin, alpha1-, alpha2-, beta1-, beta2- and gamma-globulin in two normal dogs before, immediately (0 and 2 days) after and 18 days after administration of cortisone for 10 days (see figs. 1 and 2).

<table>
<thead>
<tr>
<th></th>
<th>Dog 1 (Cleopatra)</th>
<th>Dog 2 (Josephine)</th>
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<tbody>
<tr>
<td>Dates</td>
<td>May</td>
<td>June</td>
</tr>
<tr>
<td>21st</td>
<td>40.5</td>
<td>43.5</td>
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<tr>
<td>1st</td>
<td>13.5</td>
<td>9.5</td>
</tr>
<tr>
<td>20th</td>
<td>10.0</td>
<td>19.0</td>
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The plasma volume of the same dog increased evenly during the whole experimental period, corresponding to the great increase in its weight (from 17.5 to 27.0 kg). The plasma volume per kg. was practically unchanged during the whole experiment, irrespective of the period of hormone administration. In Dog 2 (Josephine) both the total plasma volume and
the plasma volume per kilo remained fairly constant and independent of the hormone administration.

In both dogs the haematocrite-values showed a tendency to slightly lower values during the periods when ACTH and cortisone were administered.

With regard to the values of circulating albumin and globulin per kg. of body weight, these remained fairly constant in both dogs during the experimental period, and there were no typical changes during the periods, when ACTH and cortisone were administered.

The results of the electrophoretic analyses of the serum proteins are shown in Table 1. This shows the relative concentrations (in per cent) of albumin, alpha$_1$-, alpha$_2$-, beta$_1$, beta$_2$- and gamma-globulin in blood taken, respectively, 2 days before the institution and 0, 2 and 18 days after the discontinuance of the treatment with cortisone. 18 days after the discontinuance of the treatment with cortisone there was a continued rise in the albumin concentration in No. 1, but a fall in No. 2. The concentrations of alpha$_2$-globulin fell, whilst the gamma-globulin concentrations showed a rise in both animals (though only a very slight rise in No. 1). Unquestionable reversible changes in the electrophoretically determined protein fractions were observed in connection with the cortisone treatment only in the alpha$_2$-globulin fraction.

The number of eosinophilic leukocytes in the blood of these two dogs showed great fluctuations. In one dog (No. 1, Cleopatra) there seemed to be a tendency to lower values during the treatment with hormones, in the other animal (No. 2, Josephine) there was no definite relation between the treatment with hormones and eosinopenia. It may be added that the hormonal preparations used have proved very effective in human subjects.

DISCUSSION

In the course of treatment with ACTH and cortisone no change in the plasma volume was found in these two normal
dogs. The serum albumin and serum globulin, determined by means of sodium sulphate fractionation, showed no definite changes in connection with the treatment with hormones, and this applies both to the relative concentrations in the serum and to the total amounts of circulating proteins. Electrophoretic examinations showed slight rises in the albumin and alpha₂-globulin concentrations and a very slight fall in the alpha₁-, beta₂- and gamma-globulin concentration in connection with cortisone treatment. Reversible changes in connection with the treatment were found only in the alpha₂-globulin fraction, and in the case of this fraction only does it appear possible that the changes are associated with the effect of cortisone. Whether the changes in the albumin fraction and the gamma-globulin fraction are of the same nature as the changes seen under pathological conditions, cannot be ascertained on the basis of the present material.

It is possible that the dosage of ACTH and cortisone employed in these experiments, though high, is not high enough to be effective in dogs. The results of the eosinophil counts suggest this explanation.

SUMMARY

The effect of ACTH and cortisone on the plasma volume and circulating albumin and globulin (determined by means of chemical fractionation) in the serum, was investigated in two normal dogs. These values showed no definite change with the hormone treatment.

Electrophoretic examinations showed slight rises in the albumin and the alpha₂-globulin concentration and quite a small fall in the alpha₁-, beta₂- and gamma-globulin concentrations in connection with the administration of cortisone. Only the changes in the alpha₂-globulin fraction and the gamma-globulin fraction subsided again after the discontinuance of cortisone, and the changes can only be considered unquestionable as far as the alpha₂-globulin fraction is concerned. Continued investigations by means of electrophoretic analysis.
of the plasma proteins will show whether these slight changes
are real or accidental.

It is possible that in dogs a relatively high dosage of these
hormones, higher than that employed in these experiments, is
necessary to get maximal effect on the serum proteins.

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