complement levels rise during ACTH treatment.

*Summary.* In 9 of 10 patients under treatment with ACTH or cortisone, the phagocytic activity of the neutrophilic leukocyte decreased. In the remaining case an increase was observed which was shown to be due to the development of specific antibody during treatment.

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## A Study of the Transfer of Serum Proteins into Tissue Injured by Tourniquet.* (18900)

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During the past 20 years, a large quantity of evidence has accumulated indicating that in traumatic shock there is a displacement of fluid from the blood into the tissue of the injured area. Blalock(1) reported that the loss of circulating volume could be accounted for by the gain in weight of the traumatized area. This observation has been supported by the work of Nickerson(2), Ashworth, Jester, and Lloyd(3), Swingle *et al.*(4), and others, and it has been concluded by most investigators in this field that the loss of plasma is the principal cause of the condition known as shock. Inasmuch as plasma is a complex substance consisting of several protein components as well as electrolytes and water, it is necessary to determine specifically the transfer of each of these substances in order to understand the role they play in the syndrome of shock.

This paper presents analytical data on the nature of the proteins found in injured and uninjured tissue and compares them with those found in serum.

*General procedure.* Partial ischemia in one hind leg of each of 6 anesthetized dogs was produced by binding with a tight tourniquet. The animals were anesthetized by intravenous injection of nembutal (0.6 cc of a 6% solution per kg body weight). The tourniquets were made tight by first wrapping about 10 feet of elastic bandage high up on the thigh, then a double loop of insulated wire which was drawn tight by twisting with pliers. This procedure produces neither complete nor reproducible degrees of ischemia, the variation being partially dependent on the size of the animal used. The bandage was left for 4 to 5 hours (Table I) and during the entire experiment small quantities of nembutal were administered when needed to keep the animal in deep anesthesia. Sometime during this period a relatively large quantity of the blue dye, T-1824, was injected via jugular vein. This was done for blood volume determinations and also to see whether dye-tagged albumin could be traced into the tissues. Rawson(5) has shown that 8 to 14 molecules of dye are bound by one molecule of albumin. In these experiments the number of moles of dye used was much less than the number of moles of albumin in the blood so that the albumin should be far from saturated and the binding relatively complete.

At about 1½ hours after release of the tourniquets the animals were exsanguinated and in one case (Dog No. 2) both femoral

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5. Rawson, R. A., *Am. J. Physiol.*, 1943, v138, 708.

TABLE I. General Data on Leg Injury and Dye Injection.

| Dog No. | Wt, kg | Dye inj, g | Time after application of tourniquet: Dye inj, min | Tourniquet removed, min | Exsanguinated, min |
|---|---|---|---|---|---|
| 1 | 10.7 | .0500 | 175 | 187 | 260 |
| 2 | 13.1 | .0550 | 235 | 245 | 330 |
| 3 | 8 | .0212 | 238 | 268 | 355 |
| 4 | 6.2 | .0212 | 257 | 299 | 360 |
| 5 | 14.5 | .0479 | 37 | 260 | 355 |
| 6 | 12 | .0479 | 230 | 255 | 360 |

arteries were cannulated and perfused with saline in an attempt to wash out the residual blood. About 500 g of flesh (not skin) was excised from each. Proteins were extracted from each by first adding a buffer 0.02 M with respect to sodium phosphate and 0.15 M with respect to NaCl and chopping and grinding in a mortar or by subjecting to the action of a Waring Blendor, then clarifying by centrifuging at 3000 r.p.m. The proteins extracted by the two procedures did not differ significantly except that the blendor extracted more protein from a given quantity of flesh and the ratio of the quantity of albumin to other components was slightly lower.

*Results. T-1824 dye in tissue.* Gross examination of the flesh of the injured and uninjured legs revealed, by its strong blue color, the presence of dye in the injured but not in the uninjured tissue. In every case there was this marked difference in the appearance of the 2 legs, and the color was strongest in the flesh immediately under the tourniquet. However, the dye could not be extracted by homogenizing the tissue in buffered saline but was centrifuged down at 3000 r.p.m. with the tissue debris.

*Serum proteins in tissue.* Electrophoretic analysis of the proteins extracted in saline-phosphate buffer at pH 7.4 from the injured and uninjured tissue revealed a marked difference (Fig. 1 and Table II). In the extract from the uninjured tissue the component having the mobility of albumin amounted to from 2.8% to 7.1% of the total pattern area, whereas in the extract from the injured tissue from 8.2% to 27.8% was albumin (other evidence of the identity of this albumin is given in the following section). Similar differences in injured and uninjured tissue extracts were found in mice(6). In the experiment where the dog legs were perfused the amount of extracted albumin in the uninjured flesh was hardly perceptible (only 2 or 3% of the total pattern area) whereas in the injured flesh it was about 17%.

In all of these extracts from injured legs of dogs as well as those of mice(6) the absence of $\alpha$- and $\beta$-globulins is very striking. It is impossible to determine the presence or absence of $\gamma$-globulin because of the proximity of its mobility to that of the normal tissue proteins but in the injuries dealt with here it appears that albumin is the only plasma protein that appreciably leaks through into the tissue after injury by tourniquet. The mobilities (Table II) of the albumins taken from the injured and uninjured legs appear, in some cases, to be significantly different, but the cause is not yet known.

*Immunochemical identification of albumin.* The methods of quantitative immunochemistry have provided additional criteria for the

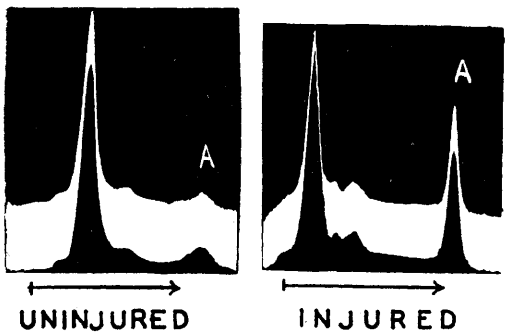


FIG. 1. Electrophoretic patterns of buffered saline extracts of tissues from contralateral uninjured leg and the leg injured by tourniquet of dog No. 4. (A = albumin).

6. Moore, D. H., and Fox, Jr., C. L., *Nature*, London, 1950, v165, 872.

TABLE II. Electrophoretic Comparison of Buffered Saline (pH 7.4) Extracts from Injured and Contralateral Uninjured Leg Tissues.

| Dog No. | Uninjured leg: Total pattern area, Arbitrary units | Uninjured leg: Albumin, % of total | Uninjured leg: Mob. alb., $-10^{-5}$ cm$^2$ sec$^{-1}$ volt$^{-1}$ | Injured leg: Total pattern area, Arbitrary units | Injured leg: Albumin, % of total | Injured leg: Mob. alb., $-10^{-5}$ cm$^2$ sec$^{-1}$ volt$^{-1}$ | Method of extraction |
|---|---|---|---|---|---|---|---|
| 2 | 154 | 5.8 | 7.1 | 152 | 22 | 5.8 | Mortar and pestle |
| 2 | 1265 | 2.8 | 7.1 | 1830 | 16.8 | 5.8 | Waring blendor |
| 3 | 240 | 4.2 | 6.1 | 315 | 22.2 | 6.2 | " " |
| 4 | 280 | 7.1 | 7 | 360 | 27.8 | 6 | " " |
| 5 | 715 | 3.5 | 6.8 | 735 | 8.2 | 6.4 | " " |
| 6 | 724 | 2.8 | 6.1 | 667 | 17.1 | 5.6 | " " |

identification of proteins(7). Therefore the quantitative precipitin reaction was used to compare the albumin obtained from serum with that from injured leg extract.

An antiserum to dog serum albumin (DSA) which had been prepared some years previously in the laboratory of Dr. Michael Heidelberger was used. The antiserum was diluted 1 to 8 with 0.15 M NaCl containing 1:10,000 merthiolate as a preservative. For purposes of the precipitin reaction, electrophoretically-separated samples of dog serum albumin (DSA) and injured dog's leg albumin (DLA) were used. The DSA was diluted to 120 and 124 $\mu$g nitrogen per ml (2 dilutions of the same preparation were made) and the DLA was diluted to 136 $\mu$g nitrogen per ml with 0.15 M NaCl and stored at these concentrations, all subsequent dilutions being made from these stocks. Final dilutions were made in saline containing normal rabbit serum and were prepared immediately prior to addition to the antiserum. The reaction mixtures in duplicate, with appropriate blanks, were allowed to stand from 48 to 72 hours at 0-5°C in the icebox. Nitrogen was determined by the Markham modification of the micro-Kjeldahl method(8).

The results are summarized in Table III. From these data it must be concluded that the 2 albumins behave identically with respect to the antiserum to one of them, and therefore are chemically either the same or very similar.

TABLE III. Addition of Dog Serum Albumin (DSA) and Albumin from Injured Leg (DLA) to 1.0 ml (1-8) Antiserum to Dog Serum Albumin.

| Preparation | Antigen N added, $\mu$g | Total N pptd, $\mu$g |
|---|---|---|
| DSA | 4.96* | 49* |
| DLA | 5.44* | 50* |
| DSA | 8.3 † | 61† |
| DLA | 8.2 | 62 |
| DSA | 10.3 | 75 |
| DLA | 10.9 | 72 |
| DSA | 12 | 82 |
| DSA | 16 † | 39†‡ |
| DLA | 16.3 | 37‡ |

* Double quantities were actually used.
† One and one-half times these quantities were used.
‡ Single determination.

*Ultracentrifugal identification of albumin.* Sedimentation constant determinations of albumin electrophoretically-separated from the serum and from tissue extract of the injured limb were carried out in an air-driven ultracentrifuge(9). The measured values which were made in the phosphate-saline buffer at temperatures between 20 and 30°C were corrected to the standard conditions of 20°C and a solvent of pure water.

The albumins from the serum and from the injured tissue were found to be indistinguishable, both having a sedimentation constant in the neighborhood of 4.5 S at concentrations of about 5 mg/ml.

*Discussion and conclusions.* The data presented here are believed to offer conclusive proof that a tight tourniquet applied to a hind leg of an anesthetized dog for a period of 4

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8. Markham, R., *Biochem. J.*, 1942, v36, 790.

9. Chiles, J. A., and Severinghaus, A. E., *Rev. Scient. Instruments*, 1940, v11, 71.

hours will, upon release, cause, as has been thought by many investigators, a transfer of serum albumin into the tissue affected by the tourniquet. A comparison of buffered saline extracts of tissue from injured and uninjured legs by means of electrophoresis revealed large quantities of plasma albumin in the injured tissue extracts in contrast to only traces in the contralateral uninjured tissue extracts. The albumin was further identified by immunochemical procedures and by its sedimentation constant in the ultracentrifuge. The fact that the albumin electrophoretically-separated from the tissue extracts reacted quantitatively with an anti-dog albumin rabbit serum in the identical manner as the albumin electrophoretically-separated from the dog serum would seem to indicate that the albumin in the injured tissue came from the serum.

The effect of the tourniquet seems to lower but not remove the barrier between the blood and the tissue because, of the plasma proteins, only albumin, which is the smallest molecule with the highest negative net charge, is transferred to any appreciable degree. The electrophoretic analyses also indicate that the loss of serum albumin is localized to the injured area. It is believed that the small amount of albumin (Table II) appearing in extracts from the uninjured legs comes from residual plasma in the capillaries. Perfusion of the legs with saline after exsanguination resulted in leaving in the control only a trace of material with the mobility of albumin. These data, therefore, support the idea that plasma albumin goes exclusively into the injured tissues.

*Summary* Electrophoretic, ultracentrifugal and immunochemical analyses of a protein extracted in buffered saline from tissues injured by the application of a tight tourniquet to one hind leg of the dogs indicate that large quantities of serum albumin enter the injured tissue. However, little or none of the $\alpha$- and $\beta$-globulins enters the tissue. The dye, T-1824, injected intravenously before release of the tourniquet appears in the tissue of the injured leg only.

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## Biologic Assay of Adrenal Cortex Hormones by the Method of Unstained Cell Counts.* (18901)

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Schrek(1,2) found that compounds B and F and extracts of the adrenal cortex had a direct cytocidal action on rabbit lymphocytes *in vitro*. Feldman(3) observed a direct toxic action on lymphocytes by compound A and by adrenal cortex extracts. In the present study, the lymphocytotoxic action is used for the biologic assay of the adrenal cortex hormones.

*Methods.* The reagents tested were compounds B, E and F, an extract of the adrenal cortex and chromatographic fractions of the extract. All of these reagents were kindly provided by Drs. William J. Haines and Marvin H. Kuizenga of the Upjohn Company. Various quantities of the reagents, dissolved in methyl alcohol, were distributed to small, sterile test tubes 100 x 13 mm. The alcohol was evaporated *in vacuo* at room temperature. The tubes with the dried reagent were stored

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2. Schrek, R., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 557.

3. Feldman, Joseph D., *Endocrinology*, 1950, v46, 552.