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## Effect on Joint Permeability of Adrenal Cortex Extract,* ACTH, Cortisone Hydroxy-phenyl Cinchoninic Acid and Hyaluronidase. (19535)

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Recent experimental and clinical studies have indicated a functional relationship between the mesenchymal tissues, the anterior pituitary gland and the adrenal cortex. The sequence of events in this relationship indicates that under conditions of stress, augmented adrenocorticotropic hormone (ACTH) production stimulates the adrenal cortex to release corticoid hormones. The corticoid hormones influence, in some way, the integrity of the connective tissues or ground substance. However, the changes in function and structure of the mesenchymal tissues which occur under the influence of these hormones are still not completely understood. It has been postulated that the mechanism of action of ACTH and cortisone is related in part to inhibition of the enzyme hyaluronidase and to a resulting decrease of membrane permeability(1-4). Furthermore, it has been hypothesized that the above mentioned hormones may exert their beneficial effects in the collagen diseases in part at least through this action on permeability(5,6).

In an attempt to determine whether joint permeability of rabbits is altered by injection of these hormones and related substances, a method was used which is similar to that reported by Seifter *et al.*(4). It consists of injecting phenolsulfonephthalein solution (PSP) into the knee joint of a rabbit under nembutal anesthesia and determining the rate of its excretion in the urine. The following procedure was carried out:

The bladder of the anesthetized rabbit was washed with fresh 10 cc portions of tap water until the sample withdrawn was clear. After waiting 10 minutes the bladder was washed again; a 10 cc portion of water was then introduced and withdrawn from the bladder to be used as the blank specimen in the photocolorimetric determination of PSP. Twenty-five hundredths cc (1500 $\mu$g) of PSP was then injected into the knee joint of the rabbit. Care was taken to ensure that the PSP was injected into the joint cavity and that hemorrhage and undue trauma were not produced. After 5 minutes the bladder was washed and a sample taken in the described manner. Similar samples were obtained at 10-minute intervals throughout the remainder of the experiment, which lasted 135 minutes. The time of appearance of PSP in the urine was noted, and the concentration of dye measured in each sample, using a Lumetron photoelectric colorimeter. After the above control studies were performed separate series

* We are indebted to Dr. David Klein, Wilson Laboratories, Chicago, for the cortical adrenal extract.

TABLE I. Route and Time of Administration, Number and Total Dose of Substances Used.

| Drug | Animal No. | Route | Time of admin. before dye, hr | No. of doses | Total dose |
|---|---|---|---|---|---|
| Cortisone | 1 | I.M. | 1 | 1 | 10 |
| | 2 | " | 18, 12, 1 | 3 | 15 } mg/kg |
| | 3 | " | 19, 1 | 2 | 20 |
| | 4 | " | 44, 25, 17, 2 | 4 | 25 |
| ACE | 1 | " | 1.5 | 1 | 1 |
| | 2 | " | 4, 2 | 2 | 2 } cc |
| | 3 | I.V. | .75 | 1 | 1 |
| | 4 | I.M. & I.V. | 15, 1 | 2 | 2 |
| HPC | 1 | Oral | 24, 2 | 2 | 200 |
| | 2 | " | 24, 2 | 2 | 200 } mg/kg |
| | 3 | " | 24, 2 | 2 | 200 |
| ACTH | 1 | I.P. | 12, 1 | 2 | 4 |
| | 2 | " | 48, 24, 12, 1 | 4 | 8 } mg/kg |
| | 3 | " | 30, 18, 12, 6 | 4 | 12 |
| | 4 | " | 30, 18, 12, 6 | 4 | 14 |
| Hyaluronidase | 1 | Into joint | With PSP | 1 | 100 |
| | 2 | " " | " " | 1 | 100 } TRU* |
| | 3 | " " | " " | 1 | 100 |
| ACTH + hyaluronidase | 1 | I.P. : joint | 24,† 18, 13, 7, 1 | 5 | 38 mg : 100 TRU |
| | 2 | " " | 25, 19, 13, 7, 1 | 5 | |
| | 3 | " " | 25, 19, 13, 7, 1 | 5 | 40 mg : 100 TRU |
| | 4 | " " | 25, 19, 13, 7, 1 | 5 | |

* TRU = Turbidity reducing units.
† ACTH injection times; hyaluronidase was given with PSP.

of experiments were conducted to determine the influence of hyaluronidase, of ACE (Adrenal cortical extract, prepared by Wilson Laboratories), of 11-dehydro-17-hydroxy-corticosterone (Cortone Acetate, Merck) of adrenocorticotropic hormone (ACTH, Armour) and of 2 hydroxy-phenyl cinchoninic acid (HPC)(7,8) upon the permeability of the rabbit's synovial membrane to PSP, as revealed by the rate of excretion of this substance in the urine after injection into the knee joint. Cortisone, ACE, and ACTH were given intramuscularly, intraperitoneally, or intravenously at varying intervals before the dye was injected. Because of its insolubility in water, HPC (Lilly) was administered in a suspension by stomach tube before PSP was given. Hyaluronidase was injected directly into the knee joint. The substances were administered in the dosage and at the intervals shown in Table I.

*Results.* The results are shown graphically in Fig. 1-5. The mean values for PSP excretion by control animals are labeled on all the graphs. In all 10 control rabbits, the dye appeared within 5 minutes and reached a maximum concentration in either the 25th or 35th minute sample. The 135th minute samples contained an average of 18 $\gamma$ ($\mu$g) of PSP. The average of the total dose excreted by the controls in the 135 minutes was 65% with a range of 57 to 77% (Fig. 1). Although the standard error (SE) has little statistical significance when computed on such a small series of animals, the $\pm 2$ SE is used here to indicate the scatter of the control values.

The mean rate of PSP excretion in the cortisone-treated group is indicated in Fig. 1. The mean total excretion of PSP in this series was 65% with a range of 61-72%. It will be observed that the mean total excretion by these animals was influenced little, if at all, by the administration of cortisone.

Whitehead and Darley(9) had obtained suggestive clinical results in a series of 8 cases of rheumatoid arthritis, with an extract of adrenal cortex prepared by the method of Swingle and Pfiffner(10). Accordingly, we studied the effect of ACE prepared by their method on the permeability of the knee joint to PSP. The adrenal cortex extract used in this series of experiments was prepared for us by Dr. David Klein, of Wilson Laboratories, Chicago, and was adjusted in strength so that

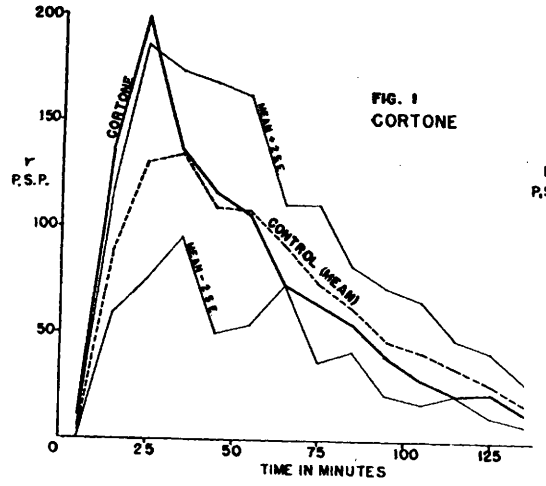
FIG. 1
CORTONE
CORTONE
MEAN ± S.E.
CONTROL (MEAN)
MEAN ± S.E.
γ P.S.P.
200
150
100
50
0
25
50
75
100
125
TIME IN MINUTES

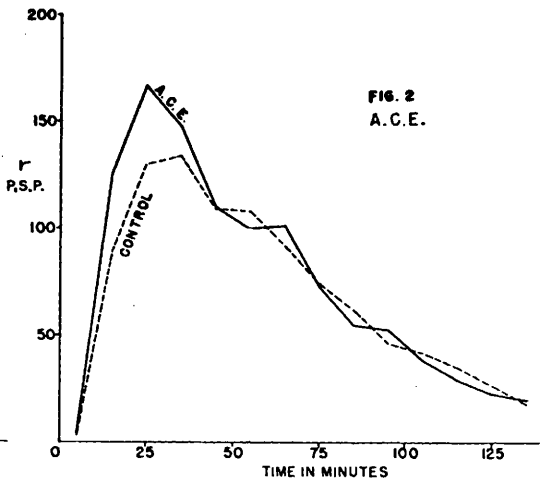
FIG. 2
A.C.E.
A.C.E.
CONTROL
γ P.S.P.
200
150
100
50
0
25
50
75
100
125
TIME IN MINUTES

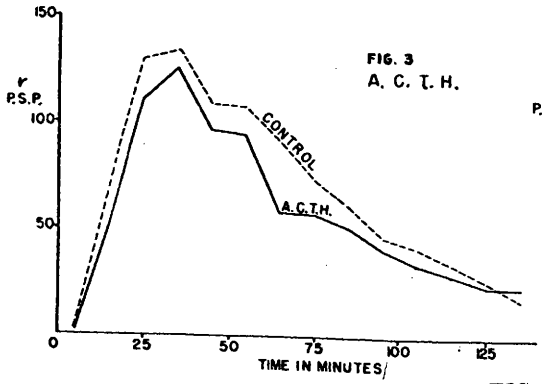
FIG. 3
A. C. T. H.
CONTROL
A.C.T.H.
γ P.S.P.
150
100
50
0
25
50
75
100
125
TIME IN MINUTES

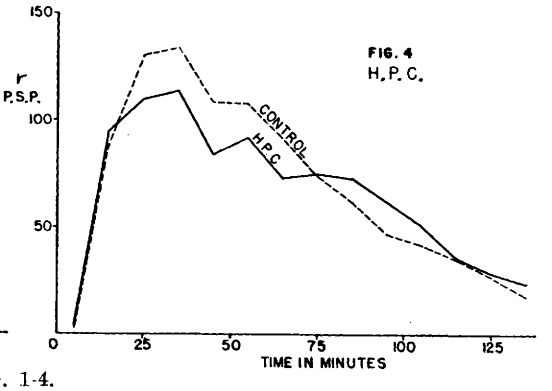
FIG. 4
H.P.C.
CONTROL
H.P.C.
γ P.S.P.
150
100
50
0
25
50
75
100
125
TIME IN MINUTES

FIG. 1-4.

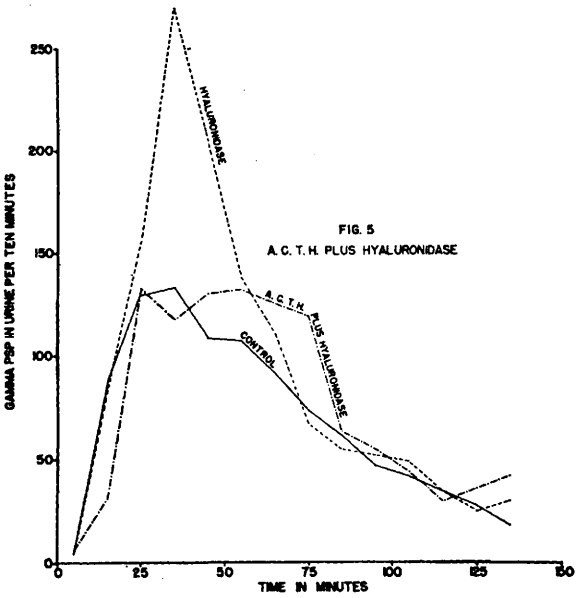
FIG. 5
A.C.T.H. PLUS HYALURONIDASE
HYALURONIDASE
A.C.T.H. PLUS HYALURONIDASE
CONTROL
GAMMA PSP IN URINE PER TEN MINUTES
250
200
150
100
50
0
25
50
75
100
125
150
TIME IN MINUTES

FIG. 5.

1 cc of extract was equivalent to 30 g of beef adrenal. We are greatly indebted to Dr. Klein for providing this product. The average excretion of PSP in the 4 animals treated with ACE is indicated in Fig. 2. The mean total PSP excretion was 62% with a range of from 59 to 68%. These results, therefore, provide little evidence that ACE had any influence on the permeability of the joint membrane and/or the kidney to PSP.

The influence of ACTH on the excretion of PSP is shown in Fig. 3. The mean total excretion was 54% with a range of from 45 to 61%. Of all the substances used in these experiments only ACTH may have had a slight depressant effect upon either joint or kidney permeability.

The results of the administration of HPC 100 mg/kg administered orally 24 and 2 hours prior to the PSP injection are shown in Fig. 4. The mean total excretion of PSP was 61%; range 57-64%.

Each of 3 rabbits was given 100 turbidity reducing units (TRU) of hyaluronidase (Alidase, Searle) dissolved in the 0.25 cc of PSP. This mixture was injected into the synovial capsule of the knee joint. The results of this experiment are illustrated in Fig. 5. The total excretion of PSP averaged 85%; range, 82-86%; this was a marked increase over control values. Apparently there is a latent period of approximately 25 minutes before the enzyme becomes effective, since the initial portions of the PSP excretion curves of the control and enzyme treated animals are practically superimposable for that period.

The effect of the combined administration of hyaluronidase and ACTH on the excretion of PSP is shown in Fig. 5. The ACTH was given intraperitoneally in the dose and at the intervals shown in Table I. The average excretion of PSP was 71% (range 69-74%). In these experiments ACTH appeared to counteract the effect of hyaluronidase upon the rate of excretion of PSP.

*Discussion.* The results secured in the present work indicate that normal joint permeability as determined by the PSP test is not decreased appreciably by adrenal cortex extract, cortisone acetate, ACTH, or HPC. The results obtained are confirmatory of those reported by several other investigators (2,11,15) using different technics. It seems possible to say unequivocally that adrenal cortex extract, ACTH or cortisone do not alter the permeability of the *normal* joint membrane or any other tissue that has been tested. On the contrary when permeability has been increased by hyaluronidase administration(3,4) or by inflammation(11) then the 11-oxysteroid hormones and ACTH tend to reduce the permeability toward the normal.

Whether permeability of the joint membrane to PSP reflects in any accurate fashion the normal state of this tissue is of course open to question. That ACTH or cortisone do not alter the permeability of the kidney to PSP has been determined by Paul *et al.*(12).

The possibility that a reduction in permeability is an important common denominator in the anti-inflammatory action exercised by cortisone and indirectly by ACTH has been emphasized by several writers(13-15). Moon *et al.*(14) observed that diapedesis, capillary hemorrhage, edema, hyperemia, and leucocytic infiltration induced by thermal injury were much less marked in cortisone treated animals. The resistance of the capillary walls is known to be increased by cortisone, and this may be a common factor in the reduction of each of the features in the inflammatory reaction. Whether the effect of these hormones is primarily on permeability or whether other factors are also prominently concerned in the anti-inflammatory action still remains undetermined.

*Summary and conclusions.* 1. Permeability studies were conducted on rabbits by injection of PSP into the joint and recovery of the dye from the urine. 2. Cortisone, ACTH, adrenal cortex extract and hydroxyphenylcinchoninic acid (HPC) given to normal rabbits failed to cause any significant alteration of synovial permeability. 3. ACTH given parenterally exhibited a significant antihyaluronidase effect. 4. We agree that the permeability of the joint membrane to PSP is not necessarily a measure of its permeability to normal body substances.

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## Amino Acids of Turnip Yellow Mosaic Virus.*† (19536)

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A study has been undertaken in our laboratory of the adsorptive properties of the recently described crystalline turnip yellow mosaic virus(1) and of the nucleic acid derived therefrom. A complete analysis of the nucleic acid moiety of the virus has been published(2). The present communication contains the analysis of the protein prepared from the virus.

*Experimental.* The protein was liberated from the virus by treatment with 35% ethanol at room temperature at a neutral pH(1). The precipitated protein was washed free of nucleic acid. Suitable quantities of the protein were hydrolyzed and analyzed for amino acids by microbiological and paper chromatographic procedures which have been described in detail in a previous communication(3).

*Results. Chromatographic findings.* A typical 2-dimensional chromatogram prepared from a volume of acid hydrolysate corresponding to 1 mg of the original protein is shown in Fig. 1. Eighteen ninhydrin-reactive substances were detected. In addition to the usually occurring amino acids small amounts of constituents which have been *tentatively* identified as glucosamine (spot 2), α-amino-n-butyric acid (spot 6), and γ-aminobutyric acid (spot 11) were also observed. It is not certain whether the latter 3 substances are present as impurities or are actually constituents of the virus protein. Tryptophan was not detected because of destruction during acid hydrolysis, and the phenylalanine spot was obscured by the large spot produced by the leucines. The most noteworthy finding on the chromatograms were the large quantities of proline, serine, and threonine. The proportion of threonine was greater than that found in any protein previously examined in our

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