

of bean seedlings as described earlier (1). After treatment of the bean seedlings, the remainder of the eluate was diluted with an additional 9 ml of water and tested for corn root curvatures as described above. If the active compound was found to occur on each of two adjacent strips of the chromatogram, its movement was calculated midway between the two strips. Indoleacetic acid was located on the chromatograms with the ferric chloride-perchloric acid reagent (2). The R_f values obtained (Table 1) indicate that the compounds causing root curvatures and bean malformations are identical and have R_f values substantially different from those obtained for IAA (3).

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References and Notes

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3. This report is journal paper No. 1267 of the Purdue Agricultural Experiment Station. I am indebted to Mrs. Nancy Hung and Mrs. Hikmet Egilmez for technical assistance.

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Enzymatic Action of Rabbit Serum on Cortisone Acetate and Hydrocortisone Acetate

Enzymatic activity upon corticosteroids has been reported for tissues (1). The only known report, to date, on the enzymatic effect of serum per se is a paper by H. H. Wotiz *et al.* (2) on the metabolism of testosterone by human serum. We wish to report (3) the presence of an esterase in normal rabbit serum which is absent from normal human serum and which removes the acetate

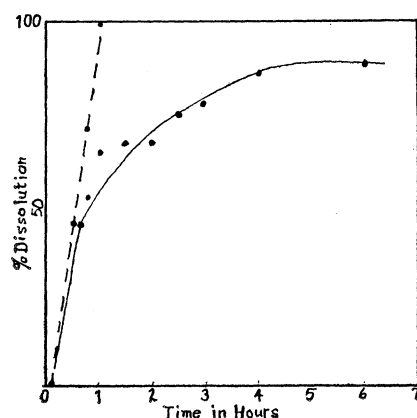


Fig. 1. Percentage dissolution of CA (dashed line) and HCA (solid line) in 10 percent normal rabbit serum in saline (NRS).

group from cortisone acetate and to a lesser degree from hydrocortisone acetate.

Following the initial studies in this laboratory on the effect of cortisone acetate upon the susceptibility of HeLa cells to poliovirus (4), the observation was made that in cultures maintained in Eagle's medium (5) with 10 percent human serum the cortisone acetate remained in particulate form, while in those cultures in which rabbit serum was substituted for the human serum the compound soon went into solution.

To investigate this phenomenon quantitatively, three sets of reaction tubes were initiated with 0.9 percent saline (SAL), 10 percent normal human serum in saline (NHS), or 10 percent normal rabbit serum in saline (NRS). To the tubes of each set were added either cortisone acetate (CA) (6), hydrocortisone acetate (HCA) (6), or hydrocortisone free alcohol (H-OH) (6), each resulting in a final concentration of 0.25 mg/ml. All tubes were incubated at 37°C. The hydrocortisone free alcohol was immediately soluble in all three reaction mixtures. The optical density of the tubes containing the steroid acetates was determined immediately and at intervals thereafter. Readings were made on the Bausch and Lomb Spectronic 20 with a wavelength setting of 560 mμ and were corrected with respect to homologous blanks. Figure 1 shows the percentage dissolution of the steroids with time.

Fresh rabbit serum was used in the experiments presented. Considerable activity could still be demonstrated, however, in serum stored at 4°C for 1 month. The heat lability of this enzyme is shown in the experiment summarized in Table 1. Fresh rabbit serum was heated at 56°C for 30 minutes. Comparable tubes were set up with NRS made with heated and unheated serum. Either HCA or CA was added at a final concentration of 0.25 mg/ml. Optical density values were followed with a Coleman, Jr. spectrophotometer with a wavelength setting of 560 mμ.

When a pH indicator such as phenol red was incorporated with the reaction mixture, it was seen that a pH drop from 7.4 to 6.8 occurred during the reaction period, indicating the formation of an acid. This did not occur in either the 0.9 percent saline or the 10 percent normal human saline reaction mixtures.

Samples of the NRS cortisone acetate reaction mixture were analyzed by the Merck Sharp & Dohme Research Laboratories. Their paper strip data showed that cortisone free alcohol was essentially the only form of cortisone present in the final reaction mixtures (7).

We conclude that normal rabbit serum contains an esterase capable of splitting CA into the free alcohol and

Table 1. Optical density readings on Coleman, Jr. spectrophotometer. Normal rabbit serum unheated versus normal rabbit serum heated. All readings were corrected to a saline NRS blank of constant zero reading.

Time (hr)	CA, not heated	CA, heated	HCA, not heated	HCA, heated
0.00	0.32	0.31	0.20	0.20
0.25	0.28	0.31	0.18	0.20
0.50	0.22	0.31	0.17	0.20
0.75	0.12	0.31	0.097	0.20
1.00	0.00	0.31	0.097	0.20
2.00	0.00	0.31	0.097	0.20
3.00	0.00	0.31	0.097	0.20
24.0	0.00	0.31	0.097	0.20

acetic acid. Rabbit serum also has esterase activity toward hydrocortisone acetate. Human serum did not exhibit either esterase activity in the 24-hour test period.

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Pericentral Cortical Projections to Motor and Sensory Nuclei

In an experimental neuroanatomical study in the cat (1) in which the Nauta-Gygax silver impregnation technique (2), was used, some of the corticobulbar fibers were found to be distributed (i) to the spinal trigeminal complex and the adjacent lateral tegmentum up to the level of the isthmus and (ii) to the region of the nuclei cuneatus and gracilis. No corticofugal fibers were distributed to the motor nuclei. Moreover, lesions within the limits of the cat's "motor cortex" (3) revealed that the projection to the spinal trigeminal complex and the adja-