EFFECT OF PARA-AMINOBENZOIC ACID ON THE METABOLISM OF CORTISONE IN LIVER TISSUE*

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In previous reports we have presented clinical evidence of the synergistic action of para-aminobenzoic acid and cortisone in the treatment of rheumatoid arthritis[6,7,8]. These clinical studies have been confirmed by others[2,4,10]. Our original hypothesis was based on the known interference of para-aminobenzoic acid with the inactivation of female sex hormone by liver tissue *in vitro*[1]. The similarity in structure of cortisone and female sex hormone suggested to us that both might pursue a similar metabolic pathway in the body. Since at that time nothing was known of the inactivation of cortisone in the body we resorted to clinical trial. The results which were obtained were sufficiently encouraging to warrant the use of this method for continued treatment of rheumatoid arthritis.

Since then convincing proof of the inactivation of cortisone largely by liver tissue has been produced[3,5]. We have adapted the method of Schneider and Horstmann[5] to test the synergistic action of para-aminobenzoic acid and cortisone *in vitro*.

Method. We have followed the procedure outlined by Schneider and Horstmann** and have modified it only by adding sodium para-aminobenzoate to the test vessels prior to incubation. Sodium para-aminobenzoate was used in concentrations ranging from 5 to 50 mg. per test vessel. Suitable controls in accordance with the original method were employed. After incubation our procedure followed the protein precipitation and extraction procedures and column chromatography of the original method without deviation. Two analytic procedures were employed namely ultraviolet spectrophotometry over the range of 220 to 260 mu and the Porter-Silber assay.

Results. A total of 31 satisfactory experiments were performed with the above method. An illustrative experiment employing rat liver slices is reported in Table 1. A similar experiment employing surviving human liver slices is shown in Table 2. Only ultraviolet spectroscopic results obtained are reported since we found that side chain destruction was too marked to give measurable results with the Porter-Silber reaction.

As these experiments illustrate, almost complete destruction of cortisone acetate occurred in the control vessels which did not contain sodium para-aminobenzoate while very marked preservation of the conjugated unsaturated system of the cortisone molecule was present in the vessels containing sodium para-aminobenzoate. Thus 86.9% of the cortisone present was reduced by rat liver slices after 3 hours of in-

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cubation while in the presence of from 5 to 50 mg. of sodium para-aminobenzoate per vessel only 22.7% was so reduced. Using surviving human liver slices 82.7% of the cortisone is degraded after 3 hours of incubation while in the presence of similar quantities of sodium para-aminobenzoate 17.4% of cortisone was destroyed. Quantities of sodium para-aminobenzoate less than 5 mg. per vessel were not effective in inhibiting cortisone degradation to a significant degree. All experiments performed produced very similar results.

Discussion. These experiments confirm the findings of other investigators that Compound E is rapidly metabolized by liver tissue under the conditions experimentally imposed. This metabolic degradation is markedly interfered with when para-aminobenzoic acid or its salts are added to the system. The para-aminobenzoic acid appears to inhibit the degradation of the conjugated unsaturated system of the Compound E molecule far more than the degradation of the side chain.

TABLE 1.—INCUBATION OF COMPOUND E AND PABA WITH RAT LIVER SLICES

Vessel No.	*Ultraviolet Spectroscopy*		
	At 240 mu. D	*Average* D	*Per Cent Reduction*
1—(Bp)	0	0	
2—(Cp_1)	0.140		
3—(Cp_2)	0.134	0.137	
4—(E_1)	0.022		
5—(E_2)	0.014	0.018	86.9
6—(Ep_1)	0.110		
7—(Ep_2)	0.102	0.106	22.7

Blank vessel (Bp) contained 15 mg. of PABA and 210 ± 2 mg. of rat liver slices in buffered solution. Control vessels (Cp_1 and Cp_2) contained in addition 840 gamma of Compound E worked up without incubation. Vessels (E_1 and E_2) containing the measured amount of Compound E in the tissue buffered solution were incubated for 3 hours in an oxygen atmosphere. Vessels (Ep_1 and Ep_2) contained in addition to the measured amount of tissue and Cortisone 15 mg. of PABA in buffered solution. These vessels were treated in the same manner as vessels (E_1 and E_2).

TABLE 2.—INCUBATION OF COMPOUND E AND PABA WITH HUMAN LIVER SLICES

Vessel No.	*Ultraviolet Spectroscopy*		
	At 240 mu. D	*Average* D	*Per Cent Reduction*
1—(Bp)	0	0	
2—(Cp_1)	0.130		
3—(Cp_2)	0.125	0.127	
4—(E_1)	0.025		
5—(E_2)	0.020	0.022	82.7
6—(Ep_1)	0.110		
7—(Ep_2)	0.100	0.105	17.4

Experiment outlined in this table was identical with the one shown in Table 1 except that in this run human liver slices were substituted for those of the rat.

One can only speculate at this time as to the exact nature of the enzymatic systems involved. Indirect clinical evidence employing tetraethylthiuram disulphide[9] would tend to support the impression that several known hepatic oxidative enzymes are blocked. Tetraethylthiuram disulphide does not lend itself to *in vitro* experiment because of its marked insolubility in physiologic fluids but clinically has been shown to exert a similar synergistic action with cortisone when administered to patients with rheumatoid arthritis. Further attempts to investigate the metabolic pathways involved in the degradation of cortisone are now in progress.

The experimental *in vitro* findings above reported are proof of the validity of our original hypothesis and serve to support and explain our clinical results with combined cortisone and para-aminobenzoic acid therapy of rheumatoid arthritis.

Summary. 1. The metabolism of cortisone in surviving rat and human liver slices has been studied and previously reported findings have been confirmed.

2. Para-aminobenzoic acid has been shown to interfere markedly with the rapid reduction of the unsaturated conjugated system of the cortisone molecule while permitting more rapid degradation of the side chain.

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