Technical Papers

The *in Vitro* Production of Cortisone by Mammalian Cells

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Recently favorable clinical results were obtained through the combined use of desoxycorticosterone acetate and ascorbic acid in the treatment of rheumatoid arthritis in Europe and America (*1, 2, 3, 4*). It was reasoned that oxidizing and reducing agents, especially in conjunction with the oxidation-reduction enzyme systems of the adrenal cortical cells, probably could synthesize cortisone from desoxycorticosterone or its precursors. Experiments were devised where the cortical tissue obtained from healthy animals was incubated in suitably enriched media with desoxycorticosterone. The steroids[2] were extracted from the medium and tested chemically by the paper chromatography method of Zaffaroni, Burton, and Keutman (*5*).

The culture medium used in these experiments contained equal amounts of Penassay broth (Difco) and Seneca hemoflagellate broth (*6*), adjusted to pH 7.5 with Na_2HPO_4. Fifty to 100 ml of this broth was put in 250-ml Erlenmeyer flasks and autoclaved. After cooling, human plasma was added to make 10% of the medium. To the first flask the following vitamins were added under sterile conditions: 1 ml of 10% ascorbic acid (100 mg), 2.5 ml of 1% thiamine hydrochloride (25 mg), and 0.5 ml 1% riboflavin, 1% pyridoxine, and 1% nicotinic acid (5 mg each). To the second flask 1 ml of 10% ascorbic acid (100 mg) was added; to the third flask, 5 units of insulin; to the fourth, all vitamins, plus 5 units insulin; to the fifth, 0.5 ml of 1% glutathione (5 mg); to the sixth, 5 mg glutathione and vitamins; to the seventh, 5 units insulin, 5 mg glutathione, and all vitamins. The eighth flask was control, containing only broth. To

TABLE 1

PRODUCTION OF CORTISONE FROM DESOXYCORTICOSTERONE BY VARIOUS TISSUES INCUBATED IN ENRICHED SENECA-PENASSAY BROTH

Flask No.	8	2	1	7	3	5	6	4
Tissue	DOC	DOC Vit. C	DOC Vit. C Vit. $B_1B_2B_6$ Nic. Ac.	DOC Vit. C Vit. $B_1B_2B_6$ Nic. Ac. insulin	DOC Insulin	DOC Glutathione	DOC Glutathione Vit. C Vit. $B_1B_2B_6$ Nic. Ac.	DOC Glutathione Vit. C Vit. $B_1B_2B_6$ Nic. Ac. insulin
Adrenal	+ or −	+	++	++++	+++	−	−	−
Liver				+ or ++				
Testis				+ or ++				
Kidney				+				
Ovary				− or +				
Cardiac muscle				−				
Striped muscle				−				
Spleen				−				
Lung				−				
Brain				−				
Thyroid				−				
Bone marrow				−				
Pancreas				−				
Placenta				−				
Prostate				−				

++++ = positive in over 75% of tissue or gland tested.
+++ = " in over 50% of " " " "
++ = " in 25 to 50% of " " " "
+ = " in about 25% of " " " "
+ or − = occasionally positive.
− = no production of glucocorticoid.

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[2] The steroids were obtained through the courtesy of Schering Corporation, Bloomfield, N. J.

all the flasks, 15 mg of desoxycorticosterone was added. Desoxycorticosterone in 150-mg quantity was dissolved in 33.4 ml propylene glycol and 16.6 ml distilled water. Autoclaving readily dissolved the steroid and sterilized it. Five ml was added to each flask.

The adrenal glands of cats, dogs, rats, guinea pigs, and chickens were removed under sterile technique while the animals were under ether anesthesia. Half a human adrenal from a case of Cushing syndrome removed by operation was also tested. Immediately following the removal, with the least lapse of time and manipulation, the glands were sliced with a sharp razor blade into 3 or 4 longitudinal slices, and placed in the flasks containing the medium and ingredients. To prevent bacterial contamination, 10,000 or 50,000 units of penicillin G were added. The flasks were incubated at 37° C, and every 48 hr the medium was replaced by freshly made broth and ingredients, on three occasions.

In another series of experiments, slices of the kidney, liver, ovary, testis, cardiac muscle, striped muscle, spleen, lung, brain, thyroid, bone marrow, and pancreas of the cat, dog, and rat, as well as human placenta and human prostate, were incubated in broth containing 15 mg desoxycorticosterone, 100 mg ascorbic acid, 25 mg thiamine, 5 mg pyridoxine, 5 mg riboflavin, 5 mg nicotinic acid, and 5 units of insulin. The broth in the flasks with the glandular tissue was kept sterile by the addition of penicillin G. The medium was changed every 48 hr on three occasions by substituting freshly made medium.

Fresh adrenals, kidneys, testis, and human placenta were separately tested for the presence of cortisone. The glandular tissue was ground up with glass sand in a mortar and extracted with either 20% trichloracetic acid or *N* HCl, or both, for 25 hr at 37° C. It was then extracted with ether, *N*/10, NaOH and then *N* HCl. This neutral extract was then tested for the presence of cortisone by paper chromatography.

Each sample of broth obtained from the culture flasks every 48 hr was tested for the presence of cortisone. Samples of the adrenal gland were removed at 24-hr intervals throughout the period of incubation and preserved in 10% formalin for histological studies. The broth was first adjusted to pH 1 with *N* HCl. The proteins were then precipitated with 20% trichloracetic acid. The supernatant and the precipitate were then extracted separately with ether, and then the extracts were combined, washed with *N*/10 NaOH and then *N* HCl, and finally evaporated to about 5 ml. The extracts were then tested individually for the presence of cortisone by the propylene-toluene chromatography method at the end of 72 hr, by spraying with 5% potassium iodide and 0.3% iodine solution.

The following results were obtained in these experiments:

1. Extracts of adrenals, liver, kidneys, testis, and human placenta were negative for cortisone. Table 1 shows the result of incubating various tissues with desoxycorticosterone, vitamins, and insulin.

2. Incubation of adrenal in media containing desoxycorticosterone, insulin, ascorbic acid, thiamine, pyridoxine, riboflavin, and nicotinic acid gave the most constant and potent paper chromatography test. Most of the positives were obtained in the second sample of broth. It was almost always negative in the first 48 hr of incubation. Positive results were also obtained in the third sample of broth.

3. Desoxycorticosterone plus adrenal gland and insulin gave the second highest positive; vitamin B complex was next, followed by ascorbic acid, although sometimes simple incubation of adrenals with desoxycorticosterone also yielded positive results.

4. When desoxycorticosterone and adrenal gland were incubated with glutathione, or with glutathione, insulin, ascorbic acid, and vitamin B complex, the results were consistently negative.

5. Positive chromatograms were obtained in a third of the experiments where the liver or testis was incubated with desoxycorticosterone, insulin, ascorbic acid and thiamine, riboflavin, pyridoxine, and nicotinic acid. The kidney and, to a lesser extent, the ovary also gave positive chromatograms.

6. All the other tissues tested gave negative results.

7. The adrenals of the cat and man (one case tested) gave the highest positives, followed by those of the dog, rat, and guinea pig, in the order cited, whereas the adrenal of the chicken was always negative.

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On the Detection of Intracranial Pathology by Ultrasound[1]

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This report deals with the initial progress of a long-range program on the application of ultrasonic techniques to medical problems (*1*). An immediate goal is the detection and localization of intracranial tumors and cerebral anatomic abnormalities.

There are two basic methods of using ultrasound for diagnosis. One uses echoes reflected from interfaces within an object, the other utilizes selective transmission through an object. In either method the useful range of vibration frequency appears to be of the order of a

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