Neotetrazolium in Determination of Succinic Dehydrogenase Activity in the Ovary.* (19545)

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Tetrazolium salts as indicators of dehydrogenase activity in living cells are recent additions to the histochemical armamentarium(1). The compound used in the present study, pp′ diphenylene bis 2-(3,5 diphenyl tetrazolium chloride) (neotetrazolium (NT)) is pale yellow in solution. It is converted to insoluble blue to black formazan at the site of active metabolic processes(2). The reduction of the tetrazolium salt by tissue slices appears to reflect the reductase or dehydrogenase activity of tissues and has been used to study such enzyme activity(3). If an excess of succinate is furnished as a substrate for the reaction, much of the formazan deposition is probably related to the presence of succinic dehydrogenase in the cells(4). Shelton and Schneider(5) found NT more useful than certain other tetrazolium compounds in localization of dehydrogenase activity. They felt that tetrazolium salts cannot be used for the demonstration of endogenous dehydrogenase activity in fresh tissue slices, but sites of succinic dehydrogenase activity can be localized in tissue sections. Using an homogenization method, Meyer and McShan(6) found a high level of succinic dehydrogenase in the corpora lutea of rat ovary during pregnancy. They felt that this high enzyme activity is correlated with function and growth of the corpus.

It seemed of interest to determine if formazan deposition takes place at sites of growth in the ovary. Blocks of ovaries of rabbits undergoing Friedman tests(7) were processed in neotetrazolium. It was felt that these tissues were associated with histologically demonstrable areas of rapid tissue proliferation. They should form a suitable basis for additional evaluation of the technic. In this study maximum formazan deposition corresponded well to sites of maximum growth.

Materials and methods. Nine virgin female rabbits were injected with urine from pregnant women and 9 with urine from nonpregnant women using a standard routine for the Friedman test(8), except that the second injection was given at a 24-hour interval. Forty-eight hours after the first injection fresh blocks from each ovary (about 3 mm in thickness) were separately placed in flasks containing 4.5 ml of 1% NT in 0.1 M phosphate in normal saline buffered to pH 7.4. To each flask, 0.5 ml of 0.3 M sodium succinate was added. Incubation with gentle agitation at 37°C for one hour followed. The blocks were then fixed in neutralized 10% formalin. Frozen sections (15 μ) from these blocks were mounted in gum arabic.

Results. At laparotomy, the ovaries of rabbits injected with urine from pregnant women had hemorrhagic follicles(7). The ovaries of rabbits injected with urine from nonpregnant women had cystic non-hemorrhagic follicles.

After incubation in NT all blocks acquired a deep purple color(2). In the sections, deposition of black or brown pigment was found. A light deposition was seen through the stroma in all ovaries. Heavily stained groups of interstitial cells were present in all ovaries. These were more concentrated and more prominent in Friedman negative ovaries (Fig. 1A). In all ovaries follicular cells around the ova had a strong staining reaction. The actual ova contained little or no pigment. The cystic follicles of the Friedman negative ovaries showed an intense deposition of fine pigment granules in the granulosa cells. The cell layers of the corpora hemorrhagica in the Friedman positive ovaries presented a much more prominent deposition of black pigment with larger granules than seen elsewhere (Fig. 1B).

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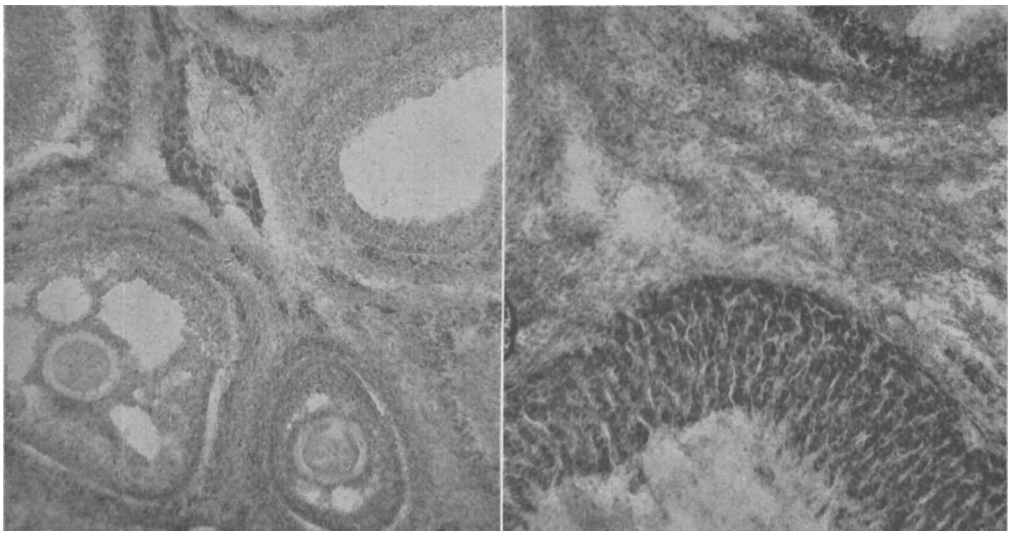

FIG. 1. Ovary of rabbit after incubation for 1 hr at 37°C in saline containing .9% neotetrazolium (NT) and .03 M sodium succinate. Reduced NT (formazan) deposited in cells. (A) Section from Friedman negative ovary with formazan granules outlining follicular cells, interstitial cells, and granulosa cells of cystic follicles (magnification 90 ×). (B) Section of Friedman positive ovary showing heavier deposition of formazan in wall of corpus hemorrhagicum (90 ×).

Occasionally cell layers adjacent to some of the largest hemorrhagic follicles had little or no pigment deposition. The larger cells of the corpora lutea contained finer and less prominent pigment deposition than seen in the corpora hemorrhagica.

Discussion. In general sites of formazan deposition correlated well with anticipated areas of tissue proliferation. Growth rate and metabolic rate are not parallel, but a rapidly proliferating tissue must be undergoing active metabolic processes. If NT is converted to formazan at the site of active metabolic processes(2), follicle cells of rabbit ovaries under the influence of follicle stimulating hormone in the urine of nonpregnant women should show moderate formazan deposition. In the present study this formazan deposition was found. Since the corpora hemorrhagica and corpora lutea of rabbit ovaries under the influence of urine from pregnant women become even larger, a much more active tissue proliferation was present. This was accompanied by the heavy deposition of larger pigment granules in the cell layers of the corpora hemorrhagica and corpora lutea. In this connection there is said to be an excellent correlation between the oxygen consumption of tissue as measured by the conventional Warburg technic and formazan deposition(1). The occasional failure of cell layers adjacent to some of the largest hemorrhages to show formazan deposition may result from: 1. Chemical interference by the blood in the hemorrhagic follicles. 2. Temporary diminution in dehydrogenase activity of adjacent tissue once hemorrhage has occurred.

It is interesting to consider the patterns of formazan deposition in relation to tissue growth in this series with analogous studies regarding human tumors(9) and growth of plant tissues(10).

Summary. Ovaries from rabbits injected with urine from pregnant and nonpregnant women were incubated in neotetrazolium with succinate. The granulosa cell layers of the cystic follicles in the ovaries of rabbits receiving urine from nonpregnant women showed moderate formazan deposition. The cell layers of the corpora hemorrhagica and corpora lutea in the ovaries of rabbits receiving urine from pregnant women showed much heavier deposition of larger formazan granules. In general, the pattern and degree of formazan deposition corresponded to areas of cellular proliferation.

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Absence of Eosinopenic Response to ACTH Administration by the Aerosol Route in Normal Subjects.* (19546)

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A number of investigators(1-5) have clearly demonstrated that the administration of ACTH in human subjects with unimpaired adrenal function produces a significant eosinopenia. This response has been employed as an index of adrenocortical function and in the assessment of the adrenocortical response to various forms of stress. In all instances, the hormone was administered either by the intramuscular or the intravenous route. The purpose of the present investigation was to study the effect of ACTH by the aerosol route upon the circulating eosinophils of normal individuals.

Plan of study. Six subjects (5 ♂ and one ♀), with ages ranging from 25 to 72, were selected. Two were free from any disease, one was affected with chronic bronchial asthma, but had been free from attacks for many months at the time of the experiment, and 3 had miscellaneous conditions (Table I). The entire group presented no signs of endocrine dysfunction, and all were permitted to carry on their usual routine during the period of investigation. An eosinophil count was taken on each of the subjects at 10 A.M., one hour after breakfast. This was considered the initial or control count. In order to check the accuracy of the procedure, several of the counts were repeated by another observer whose results agreed. The Henneman modification(6) of the Randolph method was employed in the following manner: 2 cc of venous blood were drawn and placed in an oxalate-containing tube. A white-cell pipette was then filled to the 1.0 mark with oxalated blood. Phloxine-propylene diluent was next drawn up to the 11 mark, and the pipette mechanically shaken for about 2 minutes. Both sides of 2 Max Levy double counting chambers were filled, and the eosinophils in 4 ruled areas (comprising 16 sq mm each) were counted after the expiration of one-half hour, to allow for settling. The total of the 4 areas was then multiplied by a factor, 0.78. Following the control count, 25 mg of ACTH dissolved in 1 cc of distilled water were injected intramuscularly and an eosinophil count was taken 4 hours later. After an elapse of several days to insure complete dissipation of the drug, control eosinophil counts were again taken as described above, and immediately afterwards, 25 mg of ACTH dissolved in 1 cc of distilled water were given to each subject by the aerosol route. In 3 instances a De Vilbis No. 40 hand nebulizer was used, and in the remaining 3, a Vaponefrin nebulizer, at-

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