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Received November 28, 1960. P.S.E.B.M., 1961, v106.

Histochemistry LXIV. Succinic Dehydrogenase System in Isolated Mast Cells from the Rat.* (26338)

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Growing recognition of the important physiological role of mast cells prompted this study on a succinic dehydrogenase system which could reflect certain metabolic conditions in the cells as a function of the influence of various states and agents. Previously, in a study of mast cells isolated from peritoneal washings from the rat, *in vitro* effects on the cell morphology of agents, known or suspected of having physiological influences on these cells, were reported(1). This investigation is concerned with effects of age, environmental stress (cold), and subcutaneous injections of ACTH, *E. coli* endotoxin, histamine, serotonin, and compound 48/80 ("histamine liberator") on the activity of the enzyme system in the isolated cells.

Methods. Male albino rats, Sprague-Dawley (Holtzman, Madison, Wis.), were housed singly or in pairs in a constant climate room (25° ± 1°C, 30-50% relative humidity, controlled illumination, lights on 6 a.m. and off 6 p.m.) for at least 4 days prior to use. All rats, except those employed for study of age effects, were approximately 3 months old and weighed about 300 g. They received Purina Fox Chow and tap water *ad libitum,* and were killed instantly between 11:00 and 11:30 a.m. by a single blow on the head.

Suspensions of pure mast cells in Hanks' solution were obtained from peritoneal washings as described by Glick *et al.*(2) using a sucrose density gradient. After this work was started, Ficoll (Pharmacia, Uppsala, Sweden), a dextran that provides solutions of the required density with relatively low viscosity, became available, and its value for density gradient separations was shown(3). Mast cells isolated in a Ficoll gradient are not subjected to as great an osmotic strain and they retain their histamine which is lost to some degree during separation in sucrose (4). Comparison of the activity of the succinic dehydrogenase system in cells isolated in either medium showed no difference however (Table I). Apparently the enzyme in the mitochondria is unaffected or influenced to the same degree by either procedure, although greater morphological damage was observed in cells separated in the sucrose gradient.

After isolation, washing the cells in Hanks' solution had no morphological effect, but in a Versene-phosphate buffer (0.067 M phosphate, 5×10^{-4} M Versene), of the pH (7.7) required for optimal enzyme activity, the cells were all disrupted, resulting in marked increase in activity (Table I).

The activity of the succinic dehydrogenase

* This study was supported by research grants from N.I.H., U. S. Public Health Service.

† Data in this paper were presented in a thesis submitted by S. P. in partial fulfillment of the requirements of the Graduate School for the degree of Master of Science in Physiological Chemistry.

TABLE I. Influence of Isolation Technic on a Succinic Dehydrogenase System in Peritoneal Mast Cells from the Rat.

Technic	Enzyme activity (mμM INPT formazan/μg protein-nitrogen/hr) Mean*	S.D.	S.E.
Sucrose density gradient†	1.7	.20	.12
Ficoll " " †‡	1.7	.35	.20
Versene-phosphate wash†§	1.9	.28	.07
Hanks' solution wash§ (cells intact)	.3	.07	.04

* 3 experiments on separate rats.
† Cells disrupted by Versene-phosphate wash.
‡ 33.4% Ficoll dialyzed and concentrated to half vol (sp. gr. 1.11), density gradient made with 3 dilutions: 1.3, 2.0, 3.3 times, giving 1.07, 1.05, 1.02 sp. gr., respectively.
§ Cells isolated in Ficoll density gradient.

system was determined by modification of the method based on reduction of INPT [2-(p-iodophenyl)-3-(p-nitrophenyl)-5 phenyl tetrazolium chloride] (5). Optimal conditions for measurement of activity in mast cells were elaborated, and concentration of sample and digestion time were chosen to fall within range of a linear relationship with activity. The final procedure followed was: 1. Place 60 μl of buffer-substrate solution. (0.3% INPT in 0.033 M phosphate buffer, pH 7.7, and 0.33 M sodium succinate) in each of 3 reaction tubes (glass-stoppered, 200 μl capacity), and 60 μl of blank solution (buffer-substrate solution without the succinate) in each of another 3 tubes. 2. Add 20 μl of mast cell suspension to each of the tubes, stopper and place them in a 37°C bath for 1 hour. 3. Transfer tubes to boiling water for 1 min, centrifuge at 100 × g for 2 min, suck off supernatant fluid and dry residues (protected from light) overnight *in vacuo* over calcium chloride. 4. Dissolve formazan product in 50 μl of ethanol-tetrachloroethylene (3:1 by vol.) by mixing, letting stand for 1 hour in the dark and mixing again. 5. Cover tubes with Parafilm, centrifuge at 1000 × g for 1 min, transfer total supernatant fluids to 50 μl cuvettes, and measure absorbance at 505 mμ (Beckman DU spectrophotometer). 6. Calculate results from standard formazan curve.

The precision of the method was established by analysis of 10 samples of a given suspension of mast cells which gave a mean value of 1.67 mμM formazan/μg protein-nitrogen/hour, 0.065 std. dev., 0.021 std. error, 3.9% coefficient of variation.

Protein-nitrogen was determined by the bromsulfalein method (6).

Results. Although changes with age in number and morphology of peritoneal mast cells from the rat have been reported (7), no demonstrable effect of age on enzyme activity was found over the range 30-120 days (Table II).

In rats kept at 5°C for 1 week, increased numbers of tissue mast cells in a 1.5-2-month-old group and decreased numbers in an older (>1 year) group were observed (8). Prolonged exposure (2-4 weeks) at 2° or 6°C caused increased numbers in perivascular regions and decreased numbers, which gradually returned to initial values, in intervascular mesenteric areas of 300 g rats (9). In the 3-month-old, 300 g, rats used in this work, cold stress over short periods increased activity to a degree related to time of exposure, and injection of *E. coli* endotoxin also caused elevation. Therefore, it is not surprising that ACTH (15 mg/kg) increased activity with further increase at higher dose (25 mg/kg). But under the experimental conditions no change was observed with 10 mg/kg (Table III). With doses above 25 mg/kg, only disrupted mast cells could be isolated from the peritoneal washings. In all cases of elevated activity from treatments, a considerable proportion of disrupted cells were seen in the fractions isolated. These findings are in accord with evidence that stress conditions, ACTH stimulation of the

TABLE II. Effect of Age of Rat on a Succinic Dehydrogenase System in Peritoneal Mast Cells.*

Age (days)	No. analyses on separate rats	Enzyme activity (mμM INPT formazan/μg protein-nitrogen/hr) Mean	Std. dev.	Std. error
30	4	1.5	.17	.09
60	6	1.6	.19	.10
90	7	1.4	.21	.08
120	4	1.7	.16	.08

* Isolated in sucrose density gradient.

TABLE III. Influence of Treatments on a Succinic Dehydrogenase System in Peritoneal Mast Cells from the Rat.

Treatment*	Hours between inj. and killing	No. rats	Enzyme activity (mμM INPT formazan/μg protein-nitrogen/hr) Mean	Std. dev.	Std. error	p†
Control, 25°C		3	1.6	.07	.04	
4°C, 20 min.		4	2.1	.70	.35	>.2
4°C, 60 "		5	2.5	1.10	.49	>.1
4°C, 180 "		6	4.1	.95	.39	.01
Control	3	7	1.9	.33	.13	
10 mg ACTH/kg	3	9	1.9	.24	.08	.5
Control	3	4	1.2	.20	.10	
15 mg ACTH/kg	3	11	1.5	.49	.66	.01
Control	3	3	1.7	.19	.11	
25 mg ACTH/kg	3	6	2.7	.58	.24	.01
Control	2	4	1.2	.06	.02	
5 mg *E. coli* endotoxin‡/rat	2	8	2.6	.40	.14	.01
Control	1	4	1.4	.25	.13	
.5 mg 48/80§/kg	1	8	2.5	.62	.13	.01
Control	1	5	1.6	.26	.12	
1 mg histamine/rat	1	9	1.5	.30	.10	>.5
6 " " "	1	3	1.8	.48	.16	>.5
Control	1	5	1.7	.27	.12	
1 mg serotonin/rat	1	9	1.6	.40	.13	>.4
5 " " "	1	3	1.9	.20	.12	>.4

* Subcut. injections in 1 ml physiological saline solution, control injections of 1 ml of the saline solution. Cells isolated in sucrose density gradient.

† Significance of difference from control.

‡ Prepared and supplied by Dr. Wesley Spink, Dept. Medicine, Univ. Minnesota.

§ (Burroughs Wellcome) supplied by Dr. Edwin J. deBeer, Tuckahoe, N. Y.

adrenal, or adrenal cortical hormones cause disruption of mast cells under certain conditions (*e.g.*, 10, 11, 12). However, since all cells are disrupted by the Versene-phosphate buffer prior to measurement, the increased activities observed can not be ascribed to this factor. The increase in activity per unit protein-nitrogen may be due to an absolute increase in amount or state of activity of the enzyme system, to enhanced accessibility of enzyme to substrate because of a factor such as greater permeability of mitochondrial membranes, or to both. Evidence has been presented that mitochondria in mast cells are distinct from the granules containing histamine, heparin and serotonin(13).

Conceivably, loading the body with histamine or serotonin, both of which are synthesized in rat mast cells, might reduce their production with lowering of certain metabolic activities of the cells and decreased succinic dehydrogenase activity. The present work, admittedly not critical in settling this question, at least lends no support to the possibility, since injection of either histamine or serotonin had no significant effect on the enzyme activity as measured (Table III).

Summary. Isolation of mast cells from peritoneal washings of the rat in either a sucrose or Ficoll density gradient gave the same activity of a succinic dehydrogenase system in the cells. Disruption of isolated cells by washing them with a Versene-phosphate buffer (pH 7.7) greatly elevated the enzyme activity. Cold stress or subcutaneous injection of ACTH, *E. coli* endotoxin, or compound 48/80, increased activity per unit protein-nitrogen significantly, and independently of gross cell disruption, since all cells were disrupted for measurement. No effects on activity of age from 30 to 120 days, or of subcutaneous injections of histamine or serotonin were observed.

The authors are grateful to Dr. A. C. Parekh and Dr. L. J. Greenberg for suggestions and technical help.

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Received November 29, 1960. P.S.E.B.M., 1961, v106.

Effects of Pituitary Removal or Transplantation on Ovarian Ascorbic Acid Depletion in the Rat.* (26339)

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It is now accepted that luteinizing hormone of the pituitary (LH), when given intravenously to suitably prepared immature rats, will deplete ovarian ascorbic acid. This procedure forms the basis for a sensitive assay method for LH(1) and it has been reported that the other anterior lobe hormones are without effect on this response(1,2). Briefly, the assay animals are prepared by treating 25-26-day-old female rats with exogenous gonadotrophins to induce precocious ovulation and obtain animals with heavily luteinized ovaries. It has been shown previously that, following ovulation, such animals remain in a state of pseudopregnancy for 10-12 days as judged by vaginal smears, decidual reactions and the Hooker-Forbes test for plasma progesterone(3). Furthermore, it must be presumed that luteal function in these immature animals is promoted by pituitary luteotrophin secretion. It is not known, however, to what extent the ovarian response to LH (ascorbic acid depletion) may be conditioned or modified by other circulating pituitary hormones. It has been reported that a rapid loss of ovarian ascorbic acid follows hypophysectomy and that this is attended by a decreased sensitivity to LH (4). Since it has been shown that pituitary autografts in the kidney are capable of maintaining luteal function in the adult rat(5) it seemed of interest to study the response of ovarian ascorbic acid to LH in immature, pseudopregnant rats after hypophysectomy and hypophysectomy followed by grafting the pituitary under the renal capsule. In the latter situation it was anticipated that a continuous luteotrophin secretion by the graft would maintain the corpora lutea functional for extended periods of time. At the same time the ovaries of the autograft-bearing rats would be removed from any endogenous source of follicle stimulating hormone (FSH) or luteinizing hormone (LH) since it has been found that pituitary grafts in the kidney are incapable of secreting these hormones(5,6).

Methods. Holtzman female rats, 25 days of age, were injected (sbc) with 50 IU of pregnant mares' serum gonadotropin (Equinex).§ This was followed by an injection

* Supported in part by U.S.P.H.S. Research grant.

† This work was begun while the senior author was a Summer Medical Student Fellow under U.S.P.H.S. Fellowship Grant.

‡ The authors wish to acknowledge advice and help given by Dr. Lothar Salomon, of Biochemistry and Nutrition, and technical assistance of Mrs. Claudia Waldron.

§ Kindly supplied by Dr. John B. Jewell, Ayerst Laboratories, New York.