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Induction of Flowering in Pineapple by Beta-Hydroxyethylhydrazine

Some years ago, Rodriguez demonstrated that ethylene could induce pineapple plants to flower (*1*). Subsequently, it was found that acetylene could also "force" differentiation of flower buds in the pineapple plant (*2*). With these exceptions, the chemical materials reported to induce flowering in the pineapple have had the chemical structure characteristic of plant growth regulators and have given positive results in the various tests for such properties (stimulation of cell elongation, initiation of roots, and so forth). Such flower-inducing materials as 2,4-dichlorophenoxyacetic acid, 1-naphthaleneacetic acid, and 2-naphthoxyacetic acid have a ring system nucleus, a double bond in the ring, a side chain containing a carboxyl group (or structure readily converted to it), and, presumably, the necessary spatial relationship between the ring and the carboxyl group (*3*). They are also active in the Went split-pea-stem test (*4*).

Beta-hydroxyethylhydrazine, H_2NN-HCH_2CH_2OH, gave no activity in the split-pea-stem test or in the *Avena* test and has none of the structures associated with plant-growth-regulator activity. However, it has induced early flowering in two tests at different seasons in the pineapple, *Ananas comosus,* variety smooth Cayenne (*5*). The chemical was applied in water by sprinkling can to 20 plants at each treatment rate in each test. Plants of test 1 were planted in May 1953; they were treated 18 May 1954, and the buds were counted 23 July 1954. Plants of test 2 were planted in October 1953; they were treated 15 Sptember 1954, and the buds were counted 15 November 1954 (Table 1).

Table 1. Induction of flowering in the pineapple with beta-hydroxyethylhydrazine.

Material	Concn. (%)	Plants with flower buds (No.)
Test 1		
Beta-hydroxy-ethylhydrazine	0.001	0
	0.01	0
	0.06	18
	0.12	20
Untreated control		1
Test 2		
Beta-hydroxy-ethylhydrazine	0.01	0
	0.06	1
	0.12	13
	0.23	18
Untreated control		0

It would be of interest to determine the effect of this chemical on long- or short-day plants undergoing inductive periods to ascertain whether it has the properties of an auxin antagonist.

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5. We wish to express our appreciation to the Olin Mathieson Chemical Corporation for providing the beta-hydroxyethylhydrazine and to Martha J. Kent for the split-pea-stem and *Avena* assays. This report is published with the approval of the director of the Pineapple Research Institute of Hawaii as technical paper No. 236.

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Effect of Stress on an Extinguished Fear Response

Gellhorn (*1*) has reported a series of studies that were made to determine whether convulsions induced by Metrazol, electroshock, or insulin hypoglycemia would effect the acquisition and/or extinction of a simple conditioned avoidance response. By means of a two-compartment box, a barrier, and shock, rats were taught a jumping response that enabled them to escape shock in one of the compartments. A bell was generally used as the conditioning stimulus, although in some cases a light was used. A number of Gellhorn's findings are pertinent to the present study. Gellhorn found that a conditioned avoidance response, extinguished by lack of reinforcement, could be reinstated following three to five convulsions that were induced by electroshock or Metrazol. Reinstatement lasted 5 to 10 days, after which a gradual decrease in the frequency of the response occurred. Gellhorn reported that with the use of insulin, only in the case of production of a coma state was the extinguished avoidance response reinstated. In this case, the recovered response lasted as long as several months without reinforcement. Evidence was presented that was interpreted as indicating that the coma state was the essential condition for reinstatement of the avoidance response.

The present study (*2*) reports the effect of stress induced by treadmill running on an extinguished conditioned fear response.

A modified Miller box (*3*) was used for the acquisition of a conditioned fear response. This apparatus consisted of a rectangular box, the over-all dimensions of which were 28 by 7½ by 11½ inches, that was divided into two equal chambers by a partition containing a manually operated vertical sliding door. One compartment, painted white, contained a charged grid floor and a lead weight that was suspended by a wire to the left of the intercompartment door. The second compartment was painted black; it contained no suspended weight and its grid floor was uncharged.

By means of standard conditioning procedure, ten male inbred albino rats were taught to escape shock in the white compartment by hitting the suspended weight with their forepaws. On performance of this act, the experimenter opened the sliding door and allowed the subject to escape to the black compartment. All animals were conditioned to hit the weight and escape to the black compartment immediately on placement in the white compartment, in the absence of shock.

After this habit had been acquired, the response was extinguished by lack of reinforcement. The subjects were then exposed to treadmill (*4*) running, with noninjurious shock as a motivator, for a 5-minute period daily for 1 week. Subsequently, the animals were replaced in the conditioning apparatus. Ten matched male albino rats used as controls were not subjected to the treadmill running.

We have previously reported (*5*) that some rats that were subjected to treadmill running became so highly agitated that they exhibited full pattern convulsions, including tonic, clonic, and comatose stages. In the present study, four of the ten experimental animals showed complete pattern fits on each exposure to the treadmill. The remaining six rats, although they were agitated, did not show seizures. It was found that after exposure to the treadmill running the extinguished fear response was reinstated in all experimental subjects. After a period of 1 week, the fear response of the six animals not showing the convulsions gradually disappeared; the fear response was not evidenced in any of these subjects 14 days following the treadmill experience. In the case of animals showing full pattern fits, the reinstated fear response continued to be manifested with regularity, and in the absence of reinforcement, for a period of 3 weeks following treadmill experience.

None of the controls showed spontaneous recovery of the extinguished habit during the 3-week period in which the experimentals evidenced reinstatement.

Despite the fact that the nature of the habit acquired by our subjects and the method used to induce the seizures differed from Gellhorn's, it seems apparent that convulsive states that include coma are most effective in the reinstatement of a conditioned fear response. However, in the case of reinstatement of the response used in this study, it seems that excitement produced by treadmill running does at least have a transient effect.

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22 July 1955

Relationship of Nitrogen Content of Hyaluronic Acid Preparations to Hyaluronidase Activity

Eighteen samples of hyaluronic acid were prepared (*1*) from various tissues by the methods of Harris and Harris (*2*), Tolksdorf *et al.* (*3*), Meyer (*4*), and Dorfman and Ott (*5*). Tissues employed were human umbilical cords, spleens, carcinomatous uteri, a portion of greater omentum invaded by carcinoma, a carcinomatous breast, pooled CBA mouse mammary tumors, pooled rat tumors (Walker 256), and pooled vitreous humor from beef eyes. Variations in the products from the same and different tissues are apparent in Table 1.

Turbidity was determined in the Coleman photonephelometer (Model 7) by a technique modified from that of Kass and Seastone (*6*). Aliquots (0.2 mg in 0.1 ml) of each of the hyaluronic acid preparations were placed in separate Wassermann tubes (13 by 100 mm). To each of these tubes was added 0.1 ml of acetate-buffered sodium chloride. After mixing, the tubes were incubated at 37°C for 15 minutes. Acidified albumin (7.0 ml) was added to each of the tubes, which were then incubated at room temperature for 20 minutes. The contents of each tube were poured into a cuvette and read in the photonephelometer adjusted to maximum sensitivity. To a second series of tubes containing the different substrates was added 0.1 ml of buffer solution containing 5 turbidity-reducing units of bull testis enzyme (Wyeth). This set of tubes was treated in the same manner as the controls. The effect of the given concentration of hyaluronidase on each of the hyaluronic acid preparations was determined by subsequent decrease in turbidity.

The percentage of nitrogen (duplicate samples of 1.0 mg) of the hyaluronic acid preparations was determined by the micro-Kjeldahl method as described by Kabat and Meyer (*7*).

Turbidities varied with different hyaluronic acid-hyaluronidase mixtures. A direct relationship was noted between the percentage of nitrogen of the hyaluronic acid preparations and the degree of activity of bull testis enzyme on the substrate. An enzyme activity ratio (EA ratio) was obtained for each substrate by dividing (i) the photonephelometer reading given by 0.2 of hyaluronic acid by (ii) the photonephelometer reading given by 0.2 mg of hyaluronic acid after it had been acted on by 5 turbidity reducing units of bull testis enzyme for 15 minutes.

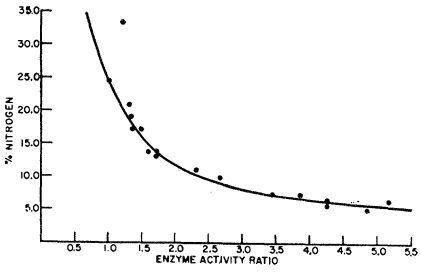

Fig. 1. Relationship of enzyme activity ratio of hyaluronic acid substrates to their nitrogen content.

Table 1 lists the laboratory number, tissue source of substrate, yield, EA ratio, and nitrogen content for each of the preparations. Hyaluronic acid preparations presenting low EA ratios are associated with high percentages of nitrogen and, inversely, those preparations yield-

Table 1. Hyaluronic acid preparations. Italic numbers in parentheses in column 1 refer to literature that describes the method of preparation.

Preparation	Tissue		Yield (g)	Galvanometer readings 0.2 mg substrate		EA ratio	Content (%N/mg)
	Source	Wt. (g)		No enzyme	5 TRU		
1 (*2*)	Human umbilical cord	100*	1.7	135	51	2.65	9.8
2 (*3, 5*)	Human umbilical cord	50*	18.0	34	8	4.25	6.5
3 (*3, 5*)	Human umbilical cord	2†	0.84	31	6	5.15	6.5
4 (*3, 5*)	Human umbilical cord	100*	4.75	92	19	4.85	5.1
5 (*3, 5*)	Cow carcinoma‡	89 → 4.5 →	0.05	135	101	1.33	19.1
6 (*3, 5*)	Cow carcinoma‡	89 → 5.0 →	0.28	81	19	4.25	5.9
7 (*3, 5*)	Human spleen (normal)	24* → 2.7 →	0.16	6	5	1.2	33.3
8 (*3, 5*)	5 human spleens	99* → 1.3 →	0.07	149	147	1.0	24.5
9 (*3, 5*)	Pooled rat tumors (Walker)	25*	2.0	111	87	1.3	20.8
10 (*3, 5*)	§	0.5	0.037	116	85	1.36	17.1
11 (*3, 5*)	§	0.5	0.033	90	61	1.47	17.1
12 (*3, 5*)	Human uterine adenocarcinoma	32* → 4.0 →	0.5	44	28	1.57	13.8
13 (*3, 5*)	Human uterine adenocarcinoma	20* → 5.6 →	0.5	60	4	15.0	14.1
14 (*3, 5*)	Human umbilical cord	90*	5.4	112	29	3.87	7.3
15 (*3*)	Human mammary adenocarcinoma	177‖	0.3	24	7	3.43	7.5
16 (*3*)	Human omental adenocarcinoma	398‖	3.8	36	21	1.71	13.2
17 (*3*)	CBA mouse mammary tumors (pooled)	46‖	0.34	27	16	1.7	13.8
18 (*4*)	Beef eye vitreous humor (pooled)	370‖	0.1	32	14	2.3	11.0

* Dry weight. † A portion of preparation 2 was reprocessed. ‡ Hereford cow squamous cell carcinoma collected by Frank X. Gassner of Colorado A & M College, Fort Collins. About 500 g (wet weight) yielded 89 g of substrate, which was reprocessed in two portions (preparations 5 and 6). § Preparation 9 was reprocessed in two 0.5-g amounts. Results recorded as preparations 10 and 11. ‖ Wet weight.