present work indicates that hydrated tricalcium phosphate also is an efficient adsorbent of hemocyanin, and its general use in the purification of hemocyanins from their natural sources is suggested.

*Résumé*. On a purifié l'hémocyanine de l'hémolymphe du Buccin, *Murex trunculus*, principalement par adsorption au phosphate de calcium. On propose l'emploi général de cette méthode de purification des hémocyanines.

W. H. Bannister, J. V. Bannister, and H. Micallef

*Evans Laboratories, Royal University of Malta, Valletta (Malta), May 12, 1966.*

STUDIORUM PROGRESSUS

## Timidity and Metabolic Elimination Patterns in Audiogenic-Seizure Susceptible and Resistant Female Rats

Inconsistencies exist concerning the correlations of timidity to emotionality and to audiogenic-seizure susceptibility. It has been reported that emotional rats are non-aggressive and are more timid in the home cage and in the presence of the investigator[1,2]. On the other hand, others found no relationship between timidity, or fearfulness and emotionality[3,4].

Whereas, Martin and Hall[5] and Billingslea[1] concluded that non-emotional rats are more susceptible to audiogenic-seizures, Griffiths[6] conversely obtained results opposed to the hypothesis that non-emotionality and low activity are directly related to seizure-susceptibility. Lindzey[7], likewise, reported a positive relationship between seizure-susceptibility and emotionality.

This investigation sought to clarify the relationship between timidity and audiogenic-seizure susceptibility as well as to study the daily metabolic elimination processes of susceptible versus resistant rats. It is of interest that increases in fecal elimination and urination responses by rats and mice during short time intervals (3–5 min) in open-field enclosures have been reported to be valid measures of emotionality[4,5].

59 female Wistar rats averaging 75 g were tested for susceptibility to audiogenic seizures and convulsions. All rats were subjected to alarm bell stimuli for a maximum of 5 min on 4 occasions during a 2 week preliminary trial and acclimatization period. Intervals of 3–4 days elapsed between the successive auditory-stress trials. Whenever convulsive attacks occurred, the auditory stimulus was stopped immediately to minimize possible stress effects induced by the convulsive process. The mixed-noise levels attained a range of 115–120 decibels depending on the loci of the animals. All test and control rats were housed, tested and stimulated singly in $16 \times 18 \times 11$ in. metal cages. It should be recognized that isolation per se can produce behavioral, metabolic and endocrinal alterations[8].

At the time of the stimulus, the alarm bell was placed centrally on the wire-mesh ceiling of the metal cage. Locomotor and behavioral activities of each rat were observed prior to, during, and for a 5 min post-noise period. The onset and frequency of wild running, tonic and/or clonic convulsions were used as criteria for grouping of the susceptible rats according to the frequency and severity of the audiogenic-seizures and convulsive attacks.

Upon completion of the pre-screening and classification procedures, body weight, food consumption, dry fecal weights and fecal pellet counts were recorded weekly during the following 2 week rest and study period. 24 h urine collections were initiated on the 5th day. As a prerequisite for timidity determinations, all animals were deprived of food and water during the urine collection period. Subsequently, the animals were then transferred to a timidity testing cage similar in size to its home cage.

The degree of timidity of the seizure-susceptible and resistant rats was determined by modifications of Bindra and Thompson[3] and Hunt and Otis[4] procedures. Timidity has been assayed by the amount of time required for a hungry rat to leave its cage and to seize food pellets. The testing cage was located on an illuminated table top with a dish of Purina fox chow 12 inches in front of the cage. Each animal was allowed to acclimatize in the closed cage for 15 min, then the cage door was opened to permit free access to the food supply. The activity and behavior of each animal was then recorded for a 20 min period, in particular, grooming and corner-to-corner exploratory activities, as well as the time needed for the rodent to place both forefeet out of the cage, to leave the cage and to reach the food dish. Arbitrary scores of 1200 sec were assigned to animals which did not attain these goals.

While realizing the possibility of errors due to subjective evaluations, to facilitate the determinations of gradations in the response patterns of the animals, all audiogenic-seizure susceptible rats were grouped according to the frequency and severity of the wild-running seizures and convulsive attacks. Group I, for example, contained the most susceptible animals, each rat having circled wildly and suffered a convulsive attack at each of the 4 auditory-stress trials. Group II consisted of animals displaying running seizures at each of the 4 trials but only 2–3 convulsions, and group III, the least susceptible, contained rats exhibiting 1–4 seizures and either none or at most 2 convulsions during the 4 trials. The control group consisted of animals showing complete resistance to the auditory-stress stimuli. None of these rats exhibited signs of wild-running seizures or convulsions.

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At the completion of the 2-week observation period, all rats were autopsied for endocrine and associated organ weight evaluations[9].

To facilitate evaluation of the data, the results are listed in the Tables in terms of the most susceptible group and combinations involving orders of lesser susceptibility (i.e. susceptible group I vs combined groups I and II vs combined groups I, II and III vs resistant control group).

Analyses by Student's *t*-test procedures[10] revealed no significant differences in the initial body weight and final body weight of the seizure-susceptible vs resistant rats. Although moderate increases were observed in the respective body weight gains of the audiogenic-seizure susceptible rats during the 2-week study period, none of these changes was statistically significant. Similarly, no significant alterations were observed in the food consumption of the susceptible vs resistant animals.

Table I presents the urine volumes, fecal data and the ratios of fecal weight eliminated/food consumed. In general, the results indicated significant and/or marked increases in the urine excretion and fecal elimination processes of the susceptible rats as indicated by the differences in fecal pellet numbers and fecal weights excreted. In most instances, the degree of the alterations tended to be greatest and more pronounced in the highly susceptible group I animals and appeared to diminish as the population size increased by addition of the less susceptible groups. In the few cases where values were not significantly increased in the susceptible groups, the *P* values obtained were low (urine volumes: group I and II; fecal weights: group I).

Analyses further revealed significant and/or marked increases in the ratios of the fecal weights per g of food consumed for the 2-week period. These changes suggest interference and inefficiency in the digestive processes of the susceptible animals. Again, inclusion of the less susceptible animals diminished the differences between the resistant and seizure-prone rats.

Table II presents the time and animal frequency data estimating the degree of timidity and non-aggressiveness of the seizure-susceptible and resistant rats. The goals listed were the time elapsed for the animals to place both forefeet outside the cage, to leave the cage and to reach the food. It is of interest that none of the most susceptible

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Table I. Urine volume, total number of fecal pellets and fecal weight elimination differences between audiogenic-seizure susceptible and resistant female rats

| | N | 5th day Urine volume (ml) | *P*-value | 2 weeks No. of fecal pellets | *P*-value | Fecal weight (g) | *P*-value | Ratio fecal weight/food consumed (g) | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| Audiogenic-seizure susceptible rats | | | | | | | | | |
| Group I ± S.E. | 6 | 5.2 ± 1.7 | 0.04 | 742.6 ± 46.6 | < 0.01 | 57.0 ± 2.8 | 0.13 | 0.2880 ± 0.0264 | < 0.01 |
| Group I and II ± S.E. | 12 | 4.3 ± 0.8 | 0.14 | 738.0 ± 26.6 | < 0.001 | 57.3 ± 1.6 | 0.03 | 0.2757 ± 0.0137 | 0.04 |
| Group I, II, and III ± S.E. | 29 | 4.5 ± 0.5 | < 0.001 | 691.7 ± 16.8 | 0.02 | 55.5 ± 1.0 | 0.05 | 0.2641 ± 0.0073 | 0.10 |
| Audiogenic-seizure resistant rats | | | | | | | | | |
| Control ± S.E. | 30 | 3.3 ± 0.3 | | 640.4 ± 12.3 | | 52.1 ± 1.2 | | 0.2484 ± 0.0057 | |

Table II. Time and frequency results of timidity differences between audiogenic-seizure susceptible and resistant female rats

| | N | Period of time in sec to: Putting 2 legs out | *P*-value | Leaving cage | *P*-value | Reaching food | *P*-value | Frequency of animals Putting 2 legs out | *P*-value | Leaving cage | *P*-value | Reaching food | *P*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Audiogenic-seizure susceptible rats | | | | | | | | | | | | | |
| Group I ± S.E. | 6 | 1200.0 ± 0.0 | 0.03 | 1200.0 ± 0.0 | 0.10 | 1200.0 ± 0.0 | 0.11 | 0/6 | – | 0/6 | – | 0/6 | – |
| Group I and II ± S.E. | 12 | 1026.4 ± 117.3 | 0.07 | 1124.6 ± 75.5 | 0.09 | 1125.8 ± 74.3 | 0.12 | 2/12 | 0.01 | 1/12 | 0.02 | 1/12 | 0.02 |
| Group I, II, and III ± S.E. | 29 | 871.1 ± 91.1 | 0.24 | 990.4 ± 76.7 | 0.30 | 1013.3 ± 69.4 | 0.38 | 10/29 | 0.15 | 7/29 | 0.29 | 6/29 | 0.18 |
| Audiogenic-seizure resistant rats | | | | | | | | | | | | | |
| Control ± S.E. | 30 | 714.7 ± 90.7 | | 865.8 ± 86.8 | | 920.9 ± 74.3 | | 16/30 | | 11/30 | | 11/30 | |

rats (group I) ever attempted to put both feet outside the cage. As one included the less susceptible groups, progressive decreases were noted with regard to the differences between the time periods and in the levels of significance between the various combined-susceptible vs resistant group data.

Chi-square frequency analyses[10] of the combined group I and II vs resistant rats demonstrated significant decreases in the number of susceptible animals which either failed to place their both forefeet outside the cage, to leave the cage or to reach the food.

In evaluating the data, the time and frequency analyses clearly demonstrated evidence of increased timidity in the more highly seizure-susceptible animals. The degree of timidity revealed a certain relationship to the grade of susceptibility exhibited by the various groups. Generally, observations of the locomotor and exploratory activity of the susceptible vs resistant rats yielded impressions of a decrease in the locomotor activity of the susceptible animals. This was exemplified by a general reduction in corner-to-corner exploratory and locomotor movements. On the other hand, impressions were noted of an increase in grooming and face and body-washing activities by the seizure-susceptible rats. It is of interest that SCOTT[11] reported less activity and fewer active animals in adult rats made fearful by shock treatments during infancy. YERKES[12] similarly observed that timid rats are more inhibited in the maze.

Although this investigation did not include open-field emotional elimination studies, the findings do present evidence of significant increases in the total daily metabolic elimination rates of the susceptible female rats. Further experimentation is needed to determine whether a positive correlation would exist between emotional defecation and normal elimination processes in the susceptible rats. However, as with timidity, the alterations in fecal and urinary elimination data revealed increases which varied with the degree of audiogenic-seizure susceptibility. Examination of the timidity and body waste data appeared to demonstrate a positive relationship between the 2 parameters with group I animals showing the greatest changes in both categories.

It is of interest that the most significant alterations in fecal weight eliminated per g of food intake were likewise presented by group I rats and secondarily by group II animals. The changes produced in these ratios would seemingly suggest possible interference or impairment in the metabolic, digestive and food absorption processes of the audiogenic-seizure susceptible rats. Further investigations are therefore needed to determine whether normal secretions of digestive juices, or absorption or motility of the digestive tract were affected. Certainly, it has been well authenticated that acute emotional states and anxiety situations are unfavorable to normal secretions of digestive enzymes and inhibit contractions of the stomach and intestines[13]. GRACE et al.[14] also reported that anger and hostility caused hyperfunction of the colon which included increased motor activity, mucous secretion and blood flow. Numerous investigators have reported increased defecation or urination resulting from sudden emotional and stressful stimulation of animals and man[13,15,16]. However, the present findings of heightened fecal elimination and increased timidity probably represent chronic manifestations of audiogenic-seizure susceptibility and may be assumed to have a hormonal basis or relationship.

It is of interest that MOYER[17] conjectured that secretions of the adrenal cortex stimulated by fear reactions tend to have a facilitating effect on the autonomic nervous system, increasing fecal production. FEUER and BROADHURST[18] also reported that strains of rats selectively bred for a high rate of emotional defecation have a lower level of thyroid hormone in the gland and in the blood. They postulated that the high defecating strain response to emotional stimuli chronically depresses TSH secretion, consequently reducing thyroid activity.

Significantly, subsequent autopsy of the seizure-susceptible and resistant animals revealed that the most susceptible group showed the highest and most significant increase in relative adrenal weight accompanied by a corresponding significant decrease in the thymus weight[9]. In addition, $O_2$ consumption decreases of the intact rats were accompanied by pronounced thyroid weight decreases in the most susceptible group ($P = 0.09$), thus revealing marked suggestions of hypothyroidism. Functionally, the adrenal-thyroid results agreed with and paralleled observations of MOYER[17] and FEUER and BROADHURST[18] in providing a partial basis for the increased fecal excretion rates noted in the audiogenic-seizure susceptible animals. Behaviorally, WOODBURY et al.[19] reported that hyperthyroidism increased and hypothyroidism decreased aggressiveness and emotional excitability of laboratory animals.

In conclusion, the present data demonstrate a positive relationship between timidity and seizure-susceptibility in female rats. Evidence is also presented of increased urine and fecal elimination output which vary with the degree of susceptibility to audiogenic-seizures. Changes in fecal weight eliminated per g of food intake ratios suggest impairments and abnormalities in digestive system functions and food metabolism processes. The various elimination and timidity data agree with evidence of heightened adrenocortical activity and suggestions of hypothyroidal function in the audiogenic-seizure susceptible animals.

*Résumé*. Les résultats ont démontré un certain rapport entre la timidité et la tendance aux crises convulsives audiogènes chez les rates. L'augmentation de la quantité des urines et des matières fécales fut aussi notée dans les groupes d'animaux sensibles. L'augmentation du poids des matières fécales en fonction du gramme d'aliments ingérés suggère un trouble et une déficience des fonctions digestives et du métabolisme alimentaire chez les animaux à tendances convulsives.

A. S. WELTMAN and A. M. SACKLER

*Laboratories for Therapeutic Research, Research Institute of the Brooklyn College of Pharmacy, Long Island University, Brooklyn (New York, USA),*
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