TABLE I.—PERCENTAGES OF VARIOUS GLYCOSIDES IN THE SEEDS OF *D. grandiflora*

Glycosides	Absorbance[a]	mcg.[b] in 50 µl.	mg. in 5 ml.	Gm. in 100 Gm. Drug
Digitalinum verum	0.115	84	8.4	0.0168
Purpurea glycoside B	0.101	60	6.0	0.0120
Purpurea glycoside A	0.205	230	23.0	0.0460
Strospesid	0.147	134	13.4	0.0268

[a] Average of five determinations. [b] These amounts were calculated from the relationship between the concentration and absorbance which were given in previous paper (1).

it was thought that it could also be used in the separation of digitalis glycosides. After a few experiments with different solvent systems, this material was at least as effective as silica gel G. Figure 4 shows that the pigments were so well separated from the glycosides that it was possible to obtain pure glycosides by using the same powder in a column. The solvent system used was isopropyl ether:methanol (10:3) (by volume). Raymond reagent was sprayed on the plates to develop the spots. The R_f values were: purpurea glycoside B, 0.037; purpurea glycoside A, 0.12; strospesid, 0.48; and digitalinum verum, 0.9.

Quantitative Estimation.—The standard curves were prepared as described previously (1). The same reagents and technique were used.

Fifty microliters of the chloroformic solution of the crude drug was applied to the paper. After development, the areas on the paper corresponding to the native glycosides were cut out and extracted with MeOH. The colors were developed and measured as described previously. The results are shown in Table I.

CONCLUSION

This study showed that only primary glycosides are present in the seeds of *D. grandiflora*. This eliminates the time factor on the degradation of the glycosides and makes the previous findings on the seeds of *D. ferruginea* more interesting. A successful separation was obtained by thin-layer and paper chromatography, the colorimetric determination showed a rather high yield of the total glycosides.

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Effect of Aspartic Acid Salts on Exhaustion Produced by Sleep Deprivation

By ROBERT A. LEVITT and WILSE B. WEBB

Male rats were maintained on a constantly moving wheel in a study of prolonged sleep deprivation. Animals in three age groups were used, and aspartic acid salts were given to half the animals in each age group. The results showed that aspartic acid salts significantly inhibited performance of rats in this situation. Also, the same negative relationship between age and exhaustion time as that found by Webb and Agnew (2) was obtained.

A DELAY IN exhaustion of rats in a swim test as a result of potassium and magnesium aspartate administration has been reported by Rosen *et al.* (1). Webb and Agnew (2) reported a relationship between exhaustion rates and age of rats using a continuous activity wheel. The latter experimenters suggested that sleep deprivation rather than fatigue may have been the critical exhaustion variable involved.

The present investigation duplicated the Webb and Agnew procedure with the addition of aspartate experimental groups.[1] It was reasoned that the more adequately controlled activity output may furnish additional data regarding the action of aspartates. Further, because aspartates are presumably metabolized in the muscle cells, the results may clarify the role of activity *per se* in the exhaustion times reported by Webb and Agnew and hypothesized as sleep deprivation effects.

METHOD

Forty male Lashley-hooded rats from the University of Florida colony were used: Group I, 12 animals, 150 days old (weight range 300–440 Gm.); Group II, 16 animals, 175 days old (weight range 310–460 Gm.); Group III, 12 animals, 235 days old (weight range 370–490 Gm.). Each group was divided into two equal groups matched by weight and assigned to the control or experimental

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[1] The aspartic acid salts used in this study were supplied by Wyeth Laboratories, Inc., Philadelphia, Pa.

condition randomly. Group I-E received 250 mg. each of potassium and magnesium aspartate every 12 hours starting within the first hour of the experiment. The drug was administered suspended in 5 ml. distilled water directly into the stomach with a No. 18 catheter and syringe. (The potassium, but not the magnesium aspartate, would dissolve in the quantity of water used; for this reason only potassium aspartate was administered to groups II-E and III-E.) Group II-E received 200 mg. of potassium aspartate intraperitoneally in 1 ml. distilled water every 12 hours. Group III-E received 50 mg. of potassium aspartate i.p. every 12 hours in 0.5 ml. of distilled water. Each control group received an equal amount of distilled water administered by the same route as its experimental group.

The rats were placed in individual 5.5 × 9.5-in. cubicles, on wheels, two-thirds submerged in water, which rotated at a constant speed of approximately 2 r.p.m. Food trays were placed in each cubicle so that the animals could feed at any time. The animals remained on these wheels continuously except when they were removed twice a day for the drug administration. The total distance covered by an animal during the day was 0.7 mile. The rats, when exhausted, fell from the wheel into the water and were unable to remount the wheel. Animals were removed from the experiment when they fell into the water after being replaced on the wheel three times during a 15-minute period. This procedure is the same as that employed by Webb and Agnew.

RESULTS

Figure 1 shows for the experimental and control groups at each age the mean hours at which the criterion of exhaustion was reached. Data for only 34 animals are included in the analysis because six did not learn to walk on the wheel (one in Group I-C, two in Group I-E, one in Group III-C, and two in Group III-E). Three animals were removed from the wheel at 136 hours at they seemed to be interminable walkers (one in Group I-C, one in Group II-E, and one in Group II-C).

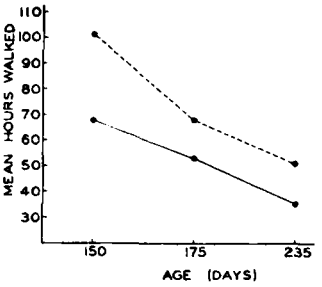

Fig. 1.—Mean hours until exhaustion for the control and experimental groups at three ages. Key: ●---●, control; ●—●, experimental.

DISCUSSION

An analysis of variance resulted in a significant age difference ($p < .01$) and a significant drug effect ($p < .05$).

First it should be recognized that the design is flawed by the changed dosage procedures in the three age groups. Despite this flaw, the consistency of the results across all three conditions supports certain general conclusions.

The previously reported relationship between age and exhaustion time was confirmed for both the experimental and the control groups. On the other hand, the results of the aspartate group (*a*) showed no differential age effect (when compared with the control groups) and (*b*) were contrary to the results reported by Rosen *et al.* (1).

The latter finding suggests a differential action of aspartic acid in a chronic exhaustion situation such as used here in contrast to the acute exhaustion procedure involved in the swim test. It is possible that aspartic acid may result in an overexpenditure of energy in a low requirement situation which reduces the possibility of resisting terminal exhaustion.

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Mineral Acid Salts of Lidocaine

By HENRY M. KOEHLER and JOHN J. HEFFERREN

Some physicochemical properties of the hydrobromide, hydrochloride, nitrate, perchlorate, phosphate, and sulfate salts of lidocaine are reported.

All local anesthetic agents with one or two exceptions are marketed as hydrochloride salts. Since these agents are generally available in aqueous or glycol solutions or ointments for parenteral or topical administration, physical properties such as hygroscopicity are not so important as those of a drug normally formulated as a tablet or capsule. For example, aqueous solutions of lidocaine hydrochloride for pharmaceutical dosage forms are prepared by adding lidocaine base U.S.P. XVI (1) to a slight molar excess of dilute hydrochloric acid, rather than by dissolving the hydrochloride salt in water (2). In his dissertation about the synthesis and characteristics of anilide-type local anesthetics, Löfgren listed the melting points of four salts of lidocaine and the solubility of the hydrochloride salt in the common organic solvents (3). Except for this work, there is little or no published information on the hydrochloride (4) or other salts of lidocaine. This, coupled with the general feeling that lidocaine hydrochloride was difficult to prepare and somewhat hard to handle (5) led to the preparation and study of the mineral acid salts of lidocaine.

The hydrobromide and hydrochloride salts were

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