ance of a component not normally demonstrated, and (3) in the mitochondrial fraction. It is suggested that these changes represent an adaptation to work demands placed upon the heart under experimental conditions.

1. Sobel, H., Graboyes, S., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 725.
2. Sobel, H., Myers, Sara M., Marmorston, J., *Circulation Research,* 1955, v3, 454.
3. Sobel, H., Myers, Sara, M., Cohen, F., *Arch. Biochem. and Biophys.,*

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Brain Excitability in Relation to Age and Hypophysectomy. (24453)

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A type of brain excitability may be determined by measuring minimal electroshock threshold (EST) of rats. A number of variables have been reported to change this response: body weight, nutritional factors, endocrine imbalances, etc.(1-5). The undefined significance of body weight and of other variables occurring in chronic experiments makes it difficult to obtain, by EST measurement, an exact perspective of the role of the endocrine system on sensitivity of the central nervous system, and this may account for disparities in conclusions(2-5). In an attempt to explain the interrelationship of EST with body, brain, and adrenal weights, and age in intact growing rats, and in early and late hypophysectomized rats the following experiments were performed.

Materials and methods. Two hundred and eight, male Sprague-Dawley rats were used. Body weight and EST measurements were made on each of 10 groups of 10 intact rats, on each of 8 groups of 10 early hypophysectomized rats, and on each of 2 groups of 9 and 2 groups of 10 late hypophysectomized rats. Intact animals were exposed to electrical stimulation only once at 10 different time intervals between 23 and 95 days of age; the early hypophysectomized rats at 8 intervals between 25 and 137 days of age; and the late hypophysectomized rats at 4 intervals between age 70 and 137 days. Electroshock was produced by a 60-cycle stimulator with a 0.16 second duration through corneal electrodes. Tissues were removed and weighed 30 minutes after electroshocking. Coefficients of correlation and regression between EST and the other factors examined (organ and body weights and age) were statistically evaluated.

The results are summarized in Table I. Intact animals showed an increase in body, brain, liver, and adrenal weights; also EST increased with age. Early hypophysectomized rats (operated at 23 days) exhibited an initial drop in body weight followed by an increase. Brain and adrenal weights decreased; whereas liver weights and EST increased, although less rapidly than in intact animals. Late hypophysectomy (operation at 67 days) suppressed growth; body and brain weights remained steady, but adrenal and liver weights decreased. The adrenals appeared to atrophy whereas EST continued to increase parallel to rate of increase observed in the intact animal.

Discussion. In intact, growing animals, absolute value of EST increases with age and consequently with body, and other organ weights. In the hypophysectomized animals, *in which body and brain weight increase is inhibited,* EST still increases with time. When EST values were plotted against age, straight lines resulted; their coefficients of regression have been calculated by standard statistical methods. At 95% confidence intervals, there was no significant difference between the coefficients for intact (0.362) and late hypophysectomized rats (0.324). On the other hand, this coefficient was significantly different (0.220) for early hypophysectomized animals. Age appears to be most directly related to EST in both hypophysectomized and normal animals. Instead, if EST

TABLE I. Body, Brain, Liver, and Adrenal Weight and EST Means in Rats after Exposure to Single Shock.

Age (days)	No. of rats	Body wt, g ± S.D.		Brain wt, mg ± S.D.		Liver, mg ± S.D.		Adrenals (2), mg ± S.D.		EST, ma ± S.D.		EST, ma/100 g body wt
						Intact						
23	10	51.3	3.3	1320	33	2413	375	21.5	1.4			
25	"	50.2	2.5	1347	43	2275	148	15.7	1.6			
28	"	65.9	3.2	1383*						16.7	.1	25.2
39	"	128.4	6.7	1524	96	6895	644	26.6	2.5			
53	"	210.3	10.9	1557	170	12859	540	32.1	5.8	20.8	.5	9.75
67	"	264.1	15.0	1660	57	13486	1004	38.6	3.9	21.1	.6	8.55
70	"	272.6	17.9	1577	270	13823	1001	40.1	4.2	24.1	.8	8.9
81	"	284.4	10.2	1568	15	13765	1500	43.5	5.6	29.7	.5	10.5
95	"	315.3	20.0	1582	42	14497	1625	49.0	6.2			
						Early hypophysectomized (23 days)						
25	10	48.5	4.1	1424	97	2302	233	14.5	.4			
28	"	57.3	5.6	1436*						16.3	.2	28.2
39	"	69.0	7.4	1484	54	3228	386	8.1	1.7			
53	"	68.9	5.2	1476	124	3504	407	8.6	2.8	18.6	.5	27.
70	"	73.3	4.1	1450	77	3371	397	8.3	2.0	19.2	.7	26.
81	"	70.1	2.9	1481	77	3708	255	9.6	1.5	21.3	.5	30.
95	"	76.2	8.6	1478	84	3484	461	7.9	.9	21.4	.9	28.
137	"	91.1	7.5	1463	63	4263	733	9.5	2.3	27.8	1.1	30.
						Late hypophysectomized (67 days)						
70	9	220.2	17.0	1545	89	10333	793	31.9	4.4	21.4	.9	9.7
81	10	207.2	19.1	1581	78	8401	618	18.1	4.0	26.0	1.4	12.5
95	"	209.3	18.3	1579	63	9089	1178	14.2	1.8	32.6	1.9	15.6
137	9	215.0	14.7	1539	157	9085	1420	15.1	1.0	42.3	2.7	19.7

* Interpolated values.

is represented in terms of body weight, the values obtained would be distributed in disorder, as would be expected if one assumed that the electric current applied through the corneal electrodes acted in a closed system, involving the CNS and not the total body mass. In normal animals, there is a possibility that the mass of the brain may be one of the factors responsible for EST increase. However, the EST-brain weight relationship is certainly not a direct one (Table I). It cannot be excluded that EST may be influenced by brain weight as well as age. The first factor could act through a mass effect on general uptake of the current; the second (age) through a more specific cellular variation, which would be influenced by the endocrine system. If brain weight influenced EST, then the values obtained in hypophysectomized rats (in which this weight did not significantly change) represented a higher relative threshold in comparison to the controls. The EST-Age correlation coefficient following early hypophysectomy leads to the conclusion that, when the animal is more affected by the hormonal deficiency, as in young, growing animals, the threshold is more susceptible to reduction. This may be related to some fundamental process involving neurons, parallel to the general lack of development.

The present findings, showing gradual increase of EST in the nonoperated animal as time increases, would be sufficient to account for the difference of threshold before and after hypophysectomy; this observation in the past(2,3) has been interpreted as a hypoexcitability induced by the deficiency status. In a general way, inability to change one set of variables (*e.g.*, hormonal influences of hypophysectomy) and maintain the others (body and organ weights or age) in the same condition as the controls, makes interpretation of the findings obtained on the convulsive threshold under the influence of endocrine deficiencies more difficult than has been assumed.

Summary. An investigation was carried out in Sprague-Dawley, male rats to determine relationship of total body, brain, adrenal, and liver weights, and age on EST in intact growing, and in early and late hypophysectomized rats. 1. EST is progressively

increased in relation to age in intact growing, and in early and late hypophysectomized rats. 2. Statistical analysis of values obtained shows that regression coefficients of Age vs. EST of intact growing and of late hypophysectomized rats are almost identical, whereas those of early hypophysectomized rats are lower. It is possible that early hypophysectomized rats become more sensitive to the stimulus than intact or late hypophysectomized animals. 3. The conclusion is reached that other variables, particularly age, have to be considered in seeking information of the effect of deficiency state itself on EST, or more generally on central nervous system sensitivity. On this basis, many previous conclusions require revision.

1. Davenport, V., Davenport, H., *Nutrition,* 1949, v36, 139.

2. Woodbury, D., *J. Pharmacol. Exp. Therap.,* 1952, v105, 27.

3. De Salva, D., Hendley, C., Ercoli, N., *Arch. Int. Pharmacodyn. Therap.,* 1954, v100, 35.

4. Ercoli, N., De Salva, S., *Ibid.* 1956, 108, 62.

5. De Salva, S., Evans, R., Johnson, C., Schaur, W., *Fed. Proc.,* 1958, v17, 1430.

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Studies in Histochemistry LIII. Microbiological Assay in Quantitative Histo- and Cytochemistry.* (24454)

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Quantitative chemical analysis of histologically or cytologically defined samples often requires micro technics to deal with the small quantities involved. Microbiological assay, the method of choice for certain B vitamins etc., has a remarkably great sensitivity which is well suited to the exacting requirements of histo- and cytochemical studies. Nevertheless, this analytical approach has been surprisingly neglected, and in fact only an occasional micro adaptation for any purpose has been made, *e.g.*,(1-4). Bioautography offers a sensitive means of detection but without the degree of quantitation afforded by the usual microbiological assay. Excellent comprehensive reviews of these technics have just appeared(5,6). Recent instrumental developments have made feasible the extension of microbiological assay to a scale requiring 500 to 1000 times less sample without loss in accuracy or precision. In this communication such a technic will be described with application to measurement of biotin, liberated from bound form by acid hydrolysis, and pantothenate, liberated by enzyme hydrolysis.

Materials and methods. The micro equipment employed has been described(7). Pyrex culture tubes 28 mm long, 4 mm inner diameter, with sleeve-type rubber serum-bottle stoppers were used. Constriction pipettes were employed for all volumes under 100 μl and mixing in the tubes was carried out by "buzzing." A constant temperature water bath with a plastic plate containing holes, 6 mm diameter, to hold the culture tubes served as the micro incubator. The plate was suspended 15 mm under the water surface so that the tubes were submerged but the tops of the stoppers were not. An ordinary well-controlled incubator can be used instead. A micro centrifuge (Microchemical Specialties Co., Berkeley) was also used. Growth was determined turbidimetrically at 650 mμ with a Beckman DU spectrophotometer. Lowry-Bessey micro cuvettes were employed for larger volumes (100 μl) and Glick-Grunbaum inserts were used with these cuvettes for small volumes (6 μl). *Lactobacillus arabinosus,* strain 17-5, with "double strength"

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