phatase and mucin was studied in the vaginal epithelium of ovariectomized mice and in castrates injected with estrogen and progesterone. In the untreated castrate the vaginal epithelium contains negligible quantities of all these cellular constituents. Injection of estrogen, and estrogen with progesterone concurrently, results in a marked increase in both phosphatase and ribonucleic acid. Mucification occurs only when the two hormones act simultaneously. Progesterone alone has no effect on any of the substances studied.

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Age and Susceptibility to Convulsions.*

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Although the great susceptibility of children to convulsive disorders is well established, little experimental work has been done on the relationship of age to convulsive reactivity. Froehlich and Mirsky[1] showed that 75 to 100% of 7- to 17-day-old rats convulse when injected with 0.5 to 1.0 mg acid fuchsin per gm subcutaneously. Adult rats however did not show convulsions even after injection with 3 mg per g, but these animals show a high incidence of convulsions after treatment with theophylline. The latter drug seems to increase the permeability of the blood-brain barrier according to experiments of Froehlich and Zak mentioned in this paper (apparently unpublished). This interpretation is supported by Aird[2,3] and collaborators who found that the percentage of convulsions induced by several convulsive drugs (strychnine, picrotoxin and cocaine) decreased after pretreatment with Brilliant Vital Red or Trypan Red and that the amount of cocaine present in the brain was greatly reduced by the dye although the concentration of cocaine in the blood remained unchanged. Moreover, experiments of Behnsen[4] on the mouse and Dölter and Kruse[5] on human infants have given evidence of a greater permeability of the blood-brain barrier at an early than at a later age. For this reason experiments involving parenteral injection of convulsive drugs cannot be used for the investigation of the question of whether the convulsive reactivity of the brain itself depends on age.

The proper method for studying this question seems to be the application of electrical stimuli adequate to produce convulsions. No experimental studies on normal material seem to have been reported but observations by Watterson[6] on schizophrenics suggest that the duration of convulsive threshold current increases with age (investigated for the interval of 19 to 49 years). In view of the fact that this study was limited to clinical cases in which the disease process may have contributed to the convulsive reactivity and since the younger age group which is known to be particularly susceptible to convulsions could obviously not be included it was decided to investigate the effect of electrically induced convulsions on rats of varying age.

Method. The experiments were performed on 24 hour fasted Sprague-Dawley rats†

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1 Froehlich, A., and Mirsky, I. A., *Arch. Neurol. Psychiat.*, 1942, **47**, 30.

2 Aird, R. B., *Arch. Neurol. Psychiat.*, 1939, **42**, 700.

3 Aird, R. B., and Strait, L., *Arch. Neurol. Psychiat.*, 1944, **51**, 54.

4 Behnsen, G., *Anat. Anzeiger*, 1926, **61**; *Erg. H.*, 179, and *Z. Zellforschung*, 1927, **4**, 515.

5 Dölter and Kruse cf. Gellhorn, E., *Das Permeabilitätsproblem, Berlin* (Springer), 1929.

6 Watterson, D. J., *Neurol. Neurosurg. Psychiat.*, 1945, **8**, 121.

TABLE I.
Age and Convulsive Reaction to Electroshock.

No. of rats	Average Wt, g	Average Age, wks	No. of convulsant rats ++++*	+++	++	+	±	% of convulsant rats
			I. Current of 24 ma for 0.8 sec.					
10	52	4	10	0	0	0	0	100
27	72	7	26	1	0	0	0	100
29	120	10	15	8	4	2	0	100
9	164	18	1	0	3	1	4	44
30	244	24	0	0	1	5	24	20
18	282	50	0	0	0	1	17	6
			II. Current of 27 ma for 0.8 sec.					
37	250	37	5	4	0	3	25	32
24	342	58	1	0	0	1	22	8
15	360	76	0	0	0	1	14	7
			III. Current of 29 ma for 0.8 sec.					
12	228	25	3	3	2	3	1	92
11	233	68	1	2	1	1	6	46

* ++++ = tonic-clonic convulsions.
+++ = clonic convulsions > 10 sec.
++ = " " 6 to 10 sec.
+ = " " 2 to 5 sec.
± = no convulsions.

varying in age between several weeks to more than 2 years. A shock of 0.4 second duration was applied to both ears with a stimulator‡ which delivered a 60 cycle square stimulus. The output current to the animal is independent of the resistance of the animal and the electrodes. It can be applied for any interval by a manual switch or an electronic photo timer. The stimulating current is derived from one-half of the 700 volt secondary of a conventional radio power transformer applied to the animal in series with the plate of a 6F6 radio tube whose screen voltage is held constant by a voltage regulator tube. Since the plate current of a pentode with a constant screen voltage is independent of its voltage over a wide range, the current through the animal is independent of its resistance or the contact resistance of the electrodes. No electrode paste need be used. The waveform approaches a square wave since the 6F6 tube rectifies the 60 cycle sine wave and because of its constant current characteristics the current rises almost instantly to the predetermined value and remains there for approximately 1/120 sec. when it drops rapidly to zero. The current is measured by placing a d.c. milliammeter across the output terminals and adjusting the cathode resistor of the 6F6 tube until the desired current is obtained. If a square wave of 180° duration is assumed the meter reading is approximately ½ the peak current. The time of stimulation was controlled by placing the relay contacts of an electronic photo timer across the output terminals in such a manner as to act as a short except during stimulation.

Results. The first part of Table I shows that the incidence of convulsions decreased as the average weight increases from 52 to 282 g corresponding to an age ranging from 4 to 50 weeks. A current of 24 ma induced convulsions in all rats up to an average weight of 120 g but led only to 6% convulsions when rats of a mean weight of 282 g are used. Moreover the table shows that the severity of the convulsions declined with increasing age. The convulsive stimulus produced clonic-tonic convulsions in 100% of the younger and 0% of the older group listed in Section I. The convulsions were exclusively of the clonic type in the latter group. These results were confirmed in a second series of experiments in which older rats were subjected to a stimulus of 27 ma. Here again a decline in convulsive respon-

† Unanesthetized. The type of electroshock used was found to be painless in man.

‡ Constructed by Mr. John Killen.

TABLE II.
The Influence of Eserine on the Effect of Electroshock.

No. of rats	Current	Number of convulsant animals: Without eserine	A %	With eserine*	B %	$\frac{B-A}{A} \times 100$	% of *maximally* convulsant rats: Without eserine	With eserine
87	20 ma†	19	22				5	
87	20 ma			27	31	+ 42%		4
63	23 ma	17	27				8	
74	23 ma			38	51	+123%		25
48	25 ma	5	10				4	
47	25 ma			9	19	+ 80%		12

* 1 mg/kg subcutaneously.
† Alternating current, 60 sec., applied for 0.4 seconds.

siveness resulted from increased weight and age.

A final group shows the convulsive reactivity of adult rats of similar weight but of different age. All rats received their food (dog chow) ad libitum, but the rats of 1½ years of age and older had the tendency to lose weight. They could therefore easily be matched in weight by a younger group. In this experiment (Table I, pt. 3) adult rats averaging 25 and 68 weeks of age respectively were shocked with 29 ma. The results show clearly that age is a decisive factor since incidence and severity of convulsions decrease with increasing age although weight differences are absent.

Discussion. Although the threshold for convulsions of individual rats was not determined the fact that a current of 24 ma causes severe tonic-clonic convulsions in 100% of the young rats and brief clonic convulsions in only 6% of rats of an average age of 50 weeks indicates that the convulsive threshold for electrical currents increases with increasing age. This phenomenon is not confined to the first months of life until maturity is reached but applies to rats of more than 6 months of age. However the data are inadequate to answer the question as to whether the decline in convulsive reactivity progresses to old age.

Since the blood-brain barrier is not involved in these experiments the convulsive reactivity of the brain itself seems to vary with increasing age. As was shown in a previous paper[7] the convulsive response to a large number of convulsant drugs is increased through pretreatment with mecholyl or eserine under conditions of topical application to the cortex. This may suggest that the effect dealt with in this paper is related to the acetylcholine metabolism of the brain. The increasing concentration of brain cholinesterase (Nachmansohn,[8] Welsh, and Hyde[9]) and the diminishing sensitivity of rats to DFP with increasing age (Freedman and Himwich[10]) are compatible with this interpretation. In order to clarify further the relation of acetylcholine metabolism to convulsive activity in general, experiments were performed on the effect of eserine on the reactivity of rats to electrically induced convulsions. Eserine administered intravenously is known to alter the pulse rate as blood pressure falls, and such circulatory changes are known to modify greatly the convulsive activity (Gellhorn *et alii*[11]). The subcutaneous injection of eserine, however, sensitizes the animal to electrically induced convulsions (Table II) but does not alter the pulse rate (Table III). This suggests

[7] Hyde, J., Beckett, S., and Gellhorn, E., in press.

[8] Nachmansohn, D., *Bull. Soc. Chem. Biol.*, 1939, **21**, 761.

[9] Welsh, J. H., and Hyde, J. E., *J. Neurophysiol.*, 1944, **7**, 41.

[10] Freedman, A. M., and Himwich, H. E., *Symposium on Military Physiology, Digest series No. 4*, 1947.

[11] Gellhorn, E., Yesinick, L., and Kessler, M., *Am. J. Physiol.*, 1942, **137**, 396.

TABLE III.
Effect of Eserine on Pulse Rate of Rats.*

No.	Wt in g	Pulse rate/min. Before	Pulse rate/min. 30' after es.
1	250	324	321
2	210	345	336
3	230	372	388
4	280	480	479

* Eserine sulfate (1 mg/kg) was injected subcutaneously and the effect on the pulse was determined by recording the EKG with an Offner oscillograph.

that eserine directly sensitizes the brain substance to electroshock, quite apart from changes in reactivity resulting from circulatory effects.

There seems to be no good evidence to relate the age dependent susceptibility of rats to convulsions to the water content of the brain (Yannet and Darrow[12]). Donaldson[13] notes that the water content of the brain of the albino rat although declining from the 1st to the 50th day remains constant from there on to the age of 300 days and that no variation occurs in Norway rats varying in weight between 195 and 460 g. However our experiments show clearly that within these ranges of age and weight convulsive reactivity changes greatly.

Finally attention is called to the fact that the excitability of the sympathetic centers declines with increasing age (Safford and Gellhorn[14]). The relation of this fact to the topic of the present paper remains to be determined.

Summary. Experiments on rats varying in age between 4 weeks and more than 1½ years show that the incidence and severity of convulsions induced by application of an electrical current to the head declines with increasing age. This decline is not confined to the period preceding sexual maturity. Comparison of adult rats of different age but similar weight shows that the susceptibility to convulsions is related to age and not solely to weight. Furthermore it is shown that eserine administered subcutaneously increases the susceptibility to electrically induced convulsions without altering the circulation.

12 Yannet, H., and Darrow, D. C., *J. Biol. Chem.*, 1938, **123**, 295.

13 Donaldson, H. H., *The rat, Memoirs of the Wistar Inst. of Anat. and Biol., No. 6, Philadelphia*, 1924.

14 Safford, H., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 98.

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A Comparison of Cardiac Outputs, Determined by Direct Fick and Pressure Pulse Methods.

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A method of determining cardiac output that is more rapid than the standard procedures, and with less dependence on skilled technical help has long been needed. With the publication by Hamilton and Remington[1] of a method for determining stroke volume from a pressure pulse this need has been met if their results are reproducible by others. In addition, their method has the added advantage, for the pharmacologist, of being able to follow the actions of a drug on the output from stroke to stroke.

Using the dye method as their basis of comparison, they found a correlation of $r = +0.994$ with an average variation between the two measurements of $\pm 8.2\%$

1 Hamilton, W. F., and Remington, J. W., *Am. J. Physiol.*, 1947, **148**, 14.