TABLE II.
Correlation Between Cold Hemagglutinin Titer and Serum Globulin Content of 33 Kala Azar Cases.

Serum globul'n content, g%	Cold hemagglutinin titers							
	Neg.	1:4	1:8	1:16	1:32	1:64	1:128	1:256
2.1-3				1	1			
3.1-4	5	2	1	1	2			1
4.1-5	1	2	5					
5.1-6	3		3				1	
6.1-7	1	1						
7.1-8			1				1	

among 68 Chinese patients with kala azar. The highest titer obtained was 1:256. The titer bears no relationship to the severity, stage, and treatment of the disease. Our results further suggest that there is no correlation between cold hemagglutinin titer and serum globulin content.

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Influence of Repeated Anoxia, Electroshock and Insulin Hypoglycemia on Reactivity of Sympathetico-Adrenal System.*

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Numerous investigations[1] have been published by the senior author indicating that procedures such as electrically or chemically induced convulsions, insulin coma and anoxia which are used in the "shock therapy" of mental disease involve an excitation of the sympathetico-adrenal and vago-insulin systems in which the effect on the former system predominates.[2] Further experiments[3] showed that stimulation of the sympathetic division of the hypothalamus has a profound influence on cortical activity. This suggested the possibility that procedures leading to sympathetic discharge and increased reactivity of sympathetic autonomic centers may alter cortical activity not only during the experimental procedures but also for some time afterwards. Consequently, experiments were performed in which the behavioral changes following repeated insulin coma and similar procedures were studied by means of the conditioned reflex method. These studies showed that repeated insulin comas or repeated convulsions led to the restitution of previously inhibited conditioned reactions.[4] This result was interpreted to mean that increased discharges originating in the hypothalamus persisted for some time after the animals had been subjected to coma and convulsions and that these discharges altered the reactivity of those cortical centers which were involved in conditioned reflexes.

If this interpretation is correct, it should be possible to relate this effect to the persistence of increased autonomic reactivity.

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1 Gellhorn, E., *Arch. Neurol. and Psychiat.*, 1938, **40**, 125.

2 For a summary of our earlier work, *cf.* Gellhorn, E., *Autonomic Regulations*, New York, 1943.

3 Murphy, J. P., and Gellhorn, E., *J. Neurophysiol.*, 1945, **8**, 341, 431.

4 Gellhorn, E., and Minatoya, H. J., *J. Neurophysiol.*, 1943, **6**, 161; Kessler, M., and Gellhorn, E., *Am. J. Psychiat.*, 1943, **99**, 687; Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 155; *Arch. Neurol. and Psychiat.*, 1946, **56**, 216; PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 375; *Arch. Neurol. and Psychiat.*, 1948, **40**, 125.

TABLE I.
Effect of Anoxia* on Blood Sugar (mg %) of Rats Before and After Repeated Exposure to Low Barometric Pressure.†

No.	Conditions	0′	25′	50′	75′	100′	125′
9	Before repeated exposure	84	93	84	75	73	72
9	After 10 exposures to 350 and 295 mm Hg	80	89	94	86	81	84
9	After 9 additional exposures to 295 mm Hg	75	86	91	102	107	104
9	After a rest of 2 weeks	80	89	84	80	81	84

* Five 25-min. periods at a pressure of 280 mm Hg.
† Exposure to 350 and 295 mm Hg for 4 hours on 5 days of the week.

TABLE II.
Effect of Anoxia* on Blood Sugar (mg %) of Rats Before and After Repeated Electroshocks.

No.	Conditions	0′	25′	50′	75′	100′	125′
10	Before electroshock	84	89	87	79	80	74
10	After 10 daily electroshocks	100	112	106	103	102	102
10	After 14 additional electroshocks in 30 days	81	103	106	104	100	98
10	After a 30-day rest period	88	97	90	88	87	90

* Five 25-min. periods at a pressure of 280 mm Hg.

The present work was undertaken in order to determine, at least indirectly, the state of the sympathetico-adrenal system in animals repeatedly subjected to anoxia, electro-shock and insulin hypoglycemia.

Method. The reactivity of the sympathetico-adrenal system to anoxia was determined before and after subjecting the rats (Sprague-Dawley) to 3 different procedures resembling the shock therapy of mental disease. This anoxia test consisted of an initial determination of blood sugar, and subsequent determinations at 25′ intervals after the rats had been exposed to a barometric pressure of 280 mm Hg for 5 consecutive periods of 25′ each. For further details see the tables and our earlier studies.[5]

Results. I. *The effect of repeated exposures to low barometric pressure on the reactivity of the sympathetico-adrenal system.* Table I summarizes the experiments on the influence of repeated anoxia and shows the following effects:

1. The fasting blood sugar decreases slightly as a result of repeated exposures to anoxia confirming the work of Sundstroem and Michaels.[6]

2. The rise in blood sugar induced by the anoxia test increases as a result of repeated prolonged exposures to low barometric pressure.

3. This effect disappears after a rest period of 2 weeks.

It was pointed out earlier[7] that the effect of anoxia on the blood sugar is a result of the action of low oxygen tension on the centers of the sympathetico-adrenal and vago-insulin systems. In order to avoid misleading conclusions based on averages involving a relatively small number of animals, and to show alterations in the balance of the 2 systems during the course of the experiments the records of each individual animal is presented in Fig. 1 in a form which permits one to see relative predominance in the reactivity of the sympathetico-adrenal system. For this purpose the individual blood sugar curves were graphed and the weight of the graph paper corresponding to the hypoglycemic area was deducted from that representing the hyperglycemic area, the null point being the initial blood sugar. The difference (in mg) is indicated on the ordinate while the abscissa indicates the various stages of the experiment

[5] Safford, H., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1947, **60**, 98, 247.

[6] Sundstroem, E. S., and Michaels, G., The Adrenal Cortex in Adaptation to Altitude, Climate, and Cancer, *Memoirs University of California*, 1942, **12**.

[7] Feldman, J., Cortell, R., and Gellhorn, E., *Am. J. Physiol.*, 1940, **131**, 281.

TABLE III.
Effect of Anoxia* on Blood Sugar (mg %) of Rats Before and After Repeated Insulin Comas.

A—Control							B—After exposure to 11 insulin comas in 28 days						
Rat No.	0′	25′	50′	75′	100′	125′	Rat No.	0′	25′	50′	75′	100′	125′
336	90	102	110	99	96	92	336	110	120	108	106	102	102
337	94	110	115	106	99	90	337	99	116	106	94	87	88
339	92	99	112	110	96	87	339	99	96	82	84	82	82
340	94	110	112	96	94	82	340	100	111	94	94	82	84
341	94	122	106	102	99	96	341	110	118	115	111	102	96
343	84	92	94	82	80	80	343	99	94	115	94	90	94
344	94	134	118	110	106	99	344	106	110	106	97	96	92
345	92	112	90	90	82	82	345	102	87	87	83	72	72
Avg	91	110	107	99	94	87	Avg	103	107	101	95	89	89

* Five 25-minute periods at a pressure of 280 mm Hg.

TABLE IV.
Effect of Adrenalin on Blood Sugar (mg %) of Rats in Different Experimental Conditions.

No.	Condition	0′	30′	60′	90′	Adrenalin
8	Control	92 (100)†	119 (130)	111 (122)	97 (106)	1 cc/kg
8	After 10 electroshocks in 10 days	95 (100)	121 (127)	110 (116)	100 (105)	1:50,000 i.p.
8	Control	83 (100)	137 (165)	122 (146)	107 (129)	1.0 cc/kg
8	After 12 insulin* comas in 15 days	107 (100)	117 (109)	114 (106)	116 (108)	1:25,000 i.p.
8	After a rest of 30 days	90 (100)	132 (147)	124 (138)	112 (124)	
10	22 hr fast	89 (100)	119 (133)	108 (122)	96 (107)	1 cc/kg
10	4 " "	115 (100)	153 (133)	138 (120)	119 (104)	1:50,000 i.p.

* 0.3 unit insulin/kg i.p.
† The number in ( ) is the percentage of the control.

(control test before and after exposure of the rat to a varying number of prolonged periods of anoxia). If the values are below the zero line the reactivity to the anoxia test reveals a predominating vago-insulin response whereas numbers above the base line indicate predominance of the sympathetico-adrenal system. Fig. 1 shows that repeated periods of exposure to low barometric pressure greatly alters the autonomic balance in favor of the sympathetico-adrenal centers. This holds for all animals no matter whether they show under control conditions a predominance of the vago-insulin or of the sympathetico-adrenal system. These effects were reversible in all but one rat.

In a second series of experiments the rats were subjected to repeated electroshocks causing tonic-clonic convulsions by applying a 60 cycle current of 30 ma for 2 seconds. Table II shows that after 10 electroshocks the fasting blood sugar is markedly elevated, but the hyperglycemic effect of the anoxia test is not greatly altered. However, after 14 more electroshocks the resting blood sugar returned to normal values and the average hyperglycemic response in the anoxia test was markedly increased. Moreover, the blood sugar remained above the control value even in the 125 minute sample, whereas in the control series† the slightly hyperglycemic response had reverted into a hypoglycemic reaction. Finally, a control test taken 30 days after cessation of the electroshock treatment showed that the hyperglycemic response had returned approximately to the control level. Fig. 2 shows the individual records which permit their evaluation in terms of sympathetic or parasympathetic balance. The curves indicate that in 8 out of 10 rats the relative responsiveness of the sympathetico-adrenal system increases slightly after 10 convulsions. After 14 more convulsions all animals show a marked sympathetico-adrenal

†*I.e.*, before application of repeated electro shocks.

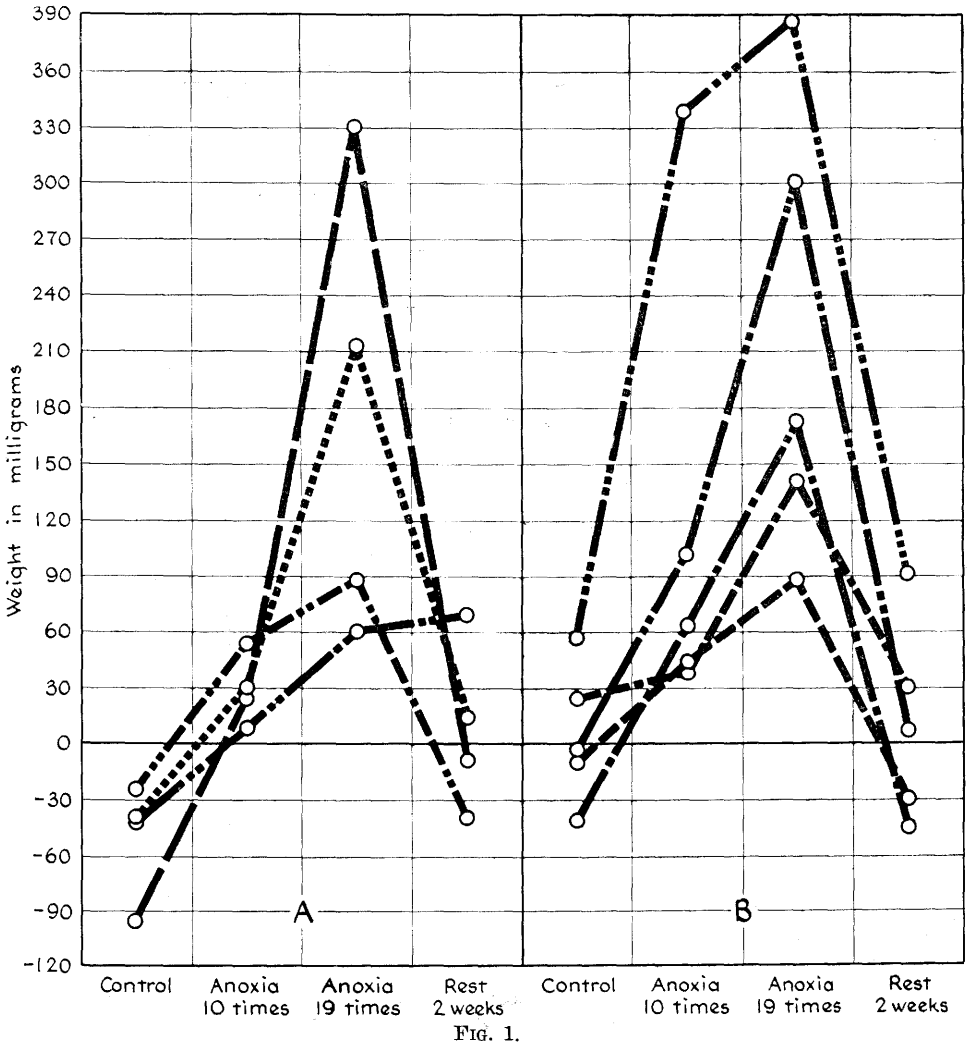

FIG. 1.

The effect of repeated anoxia on the autonomic balance. The blood sugar curve to a standard anoxia test was graphed for each animal and the weight of the area of hypoglycemia was deducted from that of the area of hyperglycemia. Positive values indicate sympathetico-adrenal, negative figures vago-insulin predominance. The figures are divided into A and B in order to avoid superimposition of the curves.

predominance. An interval of 30 days sufficed to restore the original balance of the autonomic centers. In one animal which had shown a marked vago-insulin predominance in the control test taken prior to the administration of the series of convulsions the rest period of 30 days had not only abolished the sympathetico-adrenal predominance caused by the convulsions but actually aggravated the vago-insulin predominance found under control conditions.

The third series of experiments concerns the effect of repeated insulin comas on the autonomic balance as tested by the blood sugar curve resulting from the anoxia test. Table III shows the results on 8 rats before and after exposure to 11 insulin comas. The initial blood sugar values rise after repeated comas, but the hyperglycemic reaction in response to the anoxia test is abolished. The individual blood sugar curves of Table III and particularly their evaluation in terms of autonomic balance by the methods previously employed in Fig. 1 and 2 showed that in all

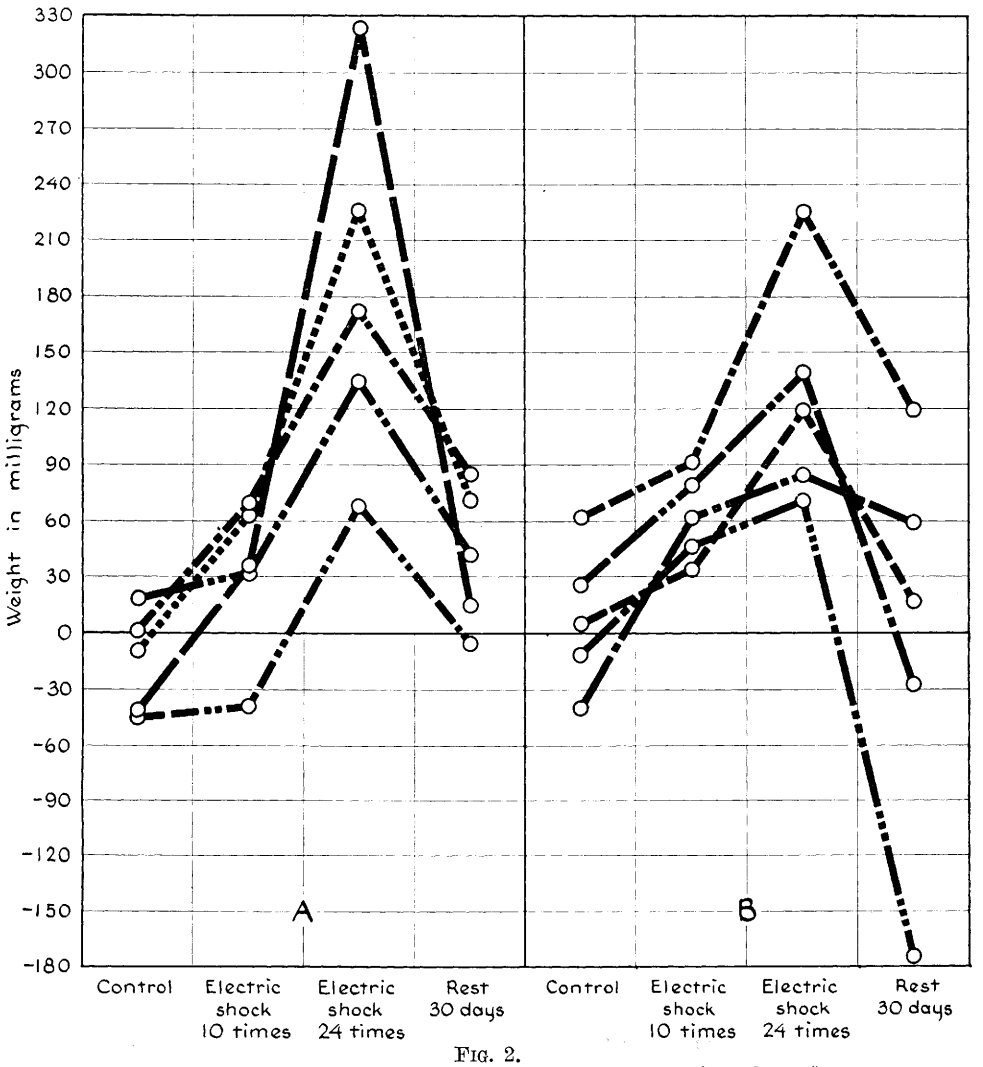

FIG. 2.
The influence of repeated electroshocks on autonomic balance.*

instances the sympathetico-adrenal predominance existing under control conditions was abolished and that in about 50% of the cases the vago-insulin system became dominant.

This striking and unexpected finding could have several causes. One would think primarily of an increased reactivity of the centers of the vago-insulin system, a decreased reactivity of the sympathetico-adrenal system or of a failure of the liver to react to adrenalin with increased glycogenolysis. An increased reactivity of the vago-insulin system was unlikely since our previous investigations had suggested that this system is very stable under various experimental conditions.[8] A decreased reactivity of the centers of the sympathetico-adrenal system was likewise improbable since in the acute experiment hypoglycemia elicits a sympathetico-adrenal discharge. (Cannon, McIver and Bliss).[9] Moreover, studies from this laboratory[10] gave evidence of an increased excitability of the medullary sympathetic center in hypogly-

8 Gellhorn, E., and Feldman, J., *Endocrinology*, 1941, **29**, 467; *Am. J. Physiol.*, 1941, **134**, 603.

9 Cannon, W. B., McIver, M. A., and Bliss, S. W., *Am. J. Physiol.*, 1924, **69**, 46.

* For details of legend *cf.* Fig. 1.

cemia. However, it was found by Gellhorn and Packer[11] that prolonged anoxia abolished the glycogenolytic action of adrenalin and earlier studies from this and other laboratories showed the close relationship between anoxia and hypoglycemia.[12] Therefore, the question was investigated whether the glycogenolytic action of adrenalin was altered in rats as a result of repeated insulin comas. The results (Table IV) show that the injection of 0.04 mg/kg adrenalin i.p. raises the blood sugar 65% in rats under control conditions. After they had been subjected to 12 insulin comas this dose of adrenalin failed to raise the blood sugar significantly. However, the reaction was restored to a great extent after a rest period of 30 days.

Since, as previously noted, repeated insulin comas raise the initial blood sugar level, the effect of this factor was likewise studied by testing the action of adrenalin on blood sugar on rats which had been fasting for 4 and 22 hours respectively. The results show clearly that a considerable variation in the initial blood sugar fails to alter the glycogenolytic action of adrenalin.

Finally, a similar series was performed on rats subjected to repeated electroshock. Table IV shows that repeated convulsions do not alter the hyperglycemic effect of adrenalin; consequently the increased hyperglycemic response to the anoxia test after repeated electroshocks is not due to an altered reactivity of the liver to adrenalin, and therefore appears to be due to an increased secretion of this hormone.

It is planned to repeat these experiments under conditions which permit a more direct determination of the reactivity of the autonomic centers by the assay of the blood for adrenalin.

Summary and Conclusions. The action of a standard anoxia test involving 5 periods of 25 minutes each at 280 mm Hg was used as a method to evaluate the responsiveness of the centers of the sympathetico-adrenal and vago-insulin systems on the basis of blood sugar determinations. It was found that repeated convulsions induced electrically, increased greatly the hyperglycemic response of rats to the standard test. This is interpreted as a sign of increased reactivity of the sympathetico-adrenal system and is accompanied by a marked dominance of this system as compared to the vago-insulin system. Similar results were obtained after frequent exposures to prolonged anoxia. However, repeated insulin comas failed to bring about this shift and showed actually a diminished or no hyperglycemic response to the standard anoxia test. It was shown that in these rats the administration of adrenalin failed to induce glycogenolysis, which explains the reduction in the hyperglycemic response. Whether the reactivity of the centers of the sympathetico-adrenal systems is increased, as it seems to be by prolonged anoxia and electroshock, will be determined in another investigation by more direct methods. The effects described in this paper were reversible after an interval of 2 to 5 weeks. The work on anoxia and electroshock lends support to the idea that procedures similar to those used in the "shock therapy" of mental disease increase the reactivity of the centers of the sympathetico-adrenal system.

[10] Gellhorn, E., Ingraham, R. C., and Moldavsky, *J. Neurophysiol.*, 1938, **1**, 301; Ingraham, R. C., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 315; Gellhorn, E., Kieley, W. F., and Hamilton, S. L., *Am. J. Physiol.*, 1940, **130**, 256.

[11] Gellhorn, E., and Packer, A., *Am. J. Physiol.*, 1940, **129**, 610.

[12] *Cf.* Gellhorn, E., *Autonomic Regulations*, New York, Interscience Publishers, 1943.