day brains. But the results were negative throughout.

Finally, an attempt was made to discover whether embryo mouse brain culture virus could be cultivated directly in cultures prepared from the brains of 5 day old mice, or whether it was necessary to adapt the virus gradually by passing it through an intervening series of cultures prepared from the brains of 1 and 3 day old mice. Accordingly, virus from embryo mouse brain cultures and from brain cultures from 5 day mice were each transferred directly to sister cultures prepared from the brains of 5 day old mice that were litter mates. The mouse titrations made at the end of the experiment gave identical results. It would appear, therefore, that the intermediate culture passages were quite unnecessary as a means of adapting the embryo brain virus to brain cultures from 5 day mice.

Summary and Conclusions. (1) The rabies virus has been propagated for as long as 57 passages (transfers) in cultures of chopped mouse embryo brain suspended in rabbit serum and buffered salt solutions. Under conditions developed in shorter, more recent experiments, the virus multiplied to the extent that mice were killed regularly by the culture material in dilutions as high as 10^{-6} (1:1,000,000); and it was possible to produce typical rabies in mice with 1/33,000,000 cc of the culture virus. When ox serum ultrafiltrate was substituted for rabbit serum, the virus titer was somewhat less. (2) Comparative experiments showed the importance of grinding the culture material for mouse titrations. But no significant increase in virus output was ever obtained by subjecting the cultures to continuous gassing with mixtures of O_2, CO_2 and N_2. (3) A duplicate series of cultures incubated for 9 passages at 35° C and at 37.5° C, respectively, showed no consistent difference in the amount of virus produced. (4) Attempts to cultivate the rabies virus in chick embryo brain cultures were unsuccessful even when the virus had been adapted to chick tissue by serial intracerebral passage in embryonated eggs. (5) Rabies virus from a mouse brain frozen at —60° C for 60 days has been cultivated for 24 successive passages in cultures of brain tissue from embryo mice (5 passages), 1 day old mice (3 passages), 3 day old mice (2 passages), 5 day old mice (10 passages) and 14 day old mice (4 passages), respectively. Culture virus developed in brain material from 5 day old mice, failed to multiply in cultures of brain tissue from 48 day old mice.

15102

Age and Autonomic Balance.*

HELEN SAFFORD AND ERNST GELLHORN.

From the Laboratory of Neurophysiology, Department of Physiology, University of Minnesota, Minneapolis.

It has been shown by Feldman, Cortell, and Gellhorn[1] that anoxia causes an excitation of autonomic centers leading to a discharge of the vago-insulin (v.i.) and sympathetico-adrenal (s.a.) systems. If the responsiveness of these centers to anoxia or similar procedures is taken as a standard the question may be investigated as to whether external or internal factors alter the reactivity of these structures, and thereby influence the central autonomic balance. The effect of temperature[2] and fever[3] and of changes in the endo-

* Aided by a grant from the Josiah Macy, Jr., Foundation.

1 Feldman, J., Cortell, R., and Gellhorn, E., *Am. J. Physiol.*, 1940, **131**, 281.

2 Gellhorn, E., and Feldman, J., *Am. J. Physiol.*, 1941, **133**, 670.

3 Feldman, J., and Gellhorn, E., *Endocrinology*, 1941, **29**, 141.

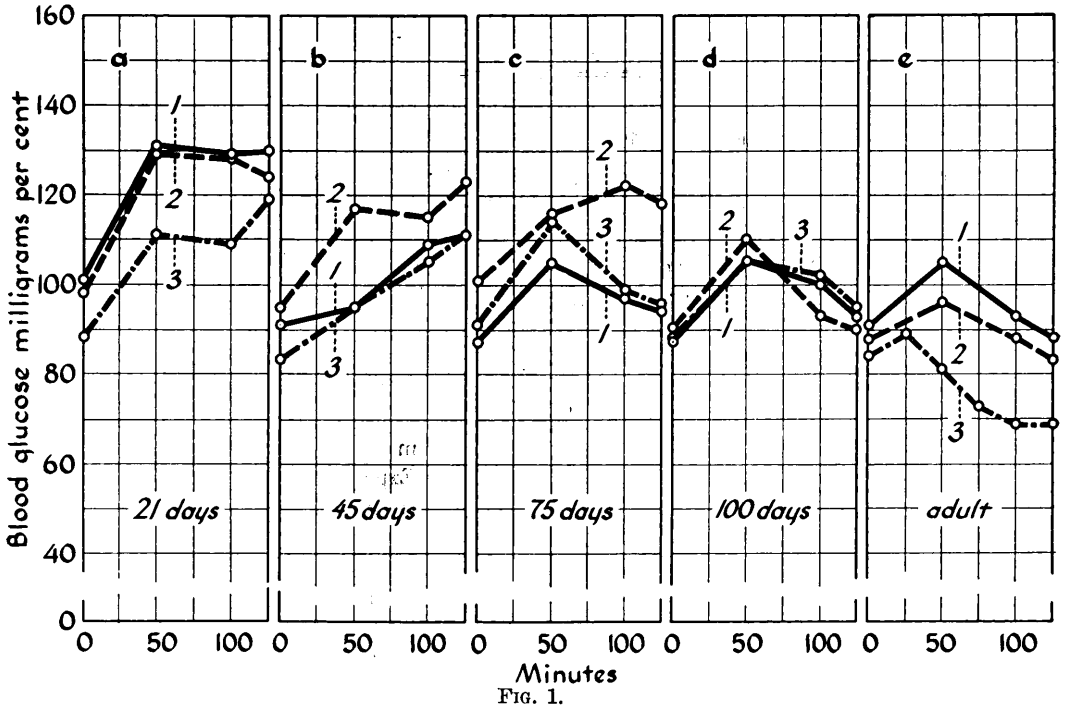

FIG. 1.

Influence of 5 periods of 25 min. each at 280 mm Hg on the blood sugar of rats at various age levels. Blood samples were taken after 50, 100, and 125 min. of anoxia. At each age level 3 groups of rats were used.

crine (thyroid)[4] and in the ionic balance,[5] has been reported previously. In view of the fact that experimental evidence seems to indicate a deviation in the reactivity of these centers in psychotic patients from that found in normal individuals (Gellhorn, Feldman, and Allen)[6] a better knowledge of internal physiological factors which have a central autonomic action appears desirable. The present investigation deals with the effect of age.

Methods. The experiments were performed on normal and adreno-demedullated rats at ages varying from 21 days to 1 to 2 years. After a fast of 22 hours the rats were subjected to a lowered barometric pressure of 280 mm Hg. for five periods of 25 minutes each. Blood sugar samples were taken before and after 50, 100, and 125 minutes exposure and analyzed using the Folin ferricyanide method as modified by Jeghers and Myers.[7] Three groups of rats, totaling between 14 and 25 animals were tested at each age level. In order to determine the reactivity of the v.i. system separately adreno-demedullated rats were tested at the age of 50 days and after they had become adults.

Results. Fig. 1 gives a graphic record of the results and demonstrates that age causes a progressive shift in the curves of hyperglycemia in rats subjected to the test period of lowered barometric pressure. The adult animals show at the beginning of the period of anoxia only a hyperglycemic phase which diminishes during the test period and gradually progresses into a state of hypoglycemia. Bearing in mind that hypoglycemia is a result of central stimulation of the v.i. system and that hyperglycemia is due to the excitation of the s.a. system,[1] the results indicate that in adult animals the s.a. system predominates at first and that later on the v.i. system comes into prominence. This method of repeated ex-

[4] Gellhorn, E., and Feldman, J., *Endocrinology*, 1941, **29**, 467.

[5] Gellhorn, E., and Feldman, J., *Am. J. Physiol.*, 1941, **134**, 603.

[6] Gellhorn, E., Feldman, J., and Allen, A., *Arch. Neurol. and Psychiat.*, 1942, **47**, 234.

[7] Jeghers, H. J., and Myers, V. C., *J. Lab. and Clin. Med.*, 1930, **15**, 982.

TABLE I.
Effect of Anoxia (5 Periods of 25′ each at 280 mm Hg) on the Blood Sugar (mg %) of Adrenodemedullated Rats.

Anoxia				
0′	50′	100′	125′	Remarks
104	82	79	83	Avg of 10 rats, 50 days
113	102	87	86	" " 8 " adults (more than 200 days)

posure to anoxia as first described by Van Middlesworth[8] is apparently a more effective activator of the v.i. system than the exposure of rats to 7% O_2 for 2 hours which was previously used in this laboratory. With the latter method the blood sugar was generally elevated at the end of the experimental period whereas with the present method a considerable number of rats showed a fall in blood sugar below the control level.

If these results are compared with those obtained on rats of 21 days of age it is obvious that the hyperglycemic phase is greatly accentuated in degree and duration in the young animals. In no instance was a fall in blood sugar below the control level observed, and in general, it was found that the rise in blood sugar observed after 50 minutes was maintained or even increased at the end of the experiment.

The experiments performed on rats at the at the age of 45 days were similar to those obtained at an earlier age. However, at the next stage (75 days) there is a definite change in the reactivity of the autonomic centers as disclosed by the blood sugar reaction in anoxia. In only one of the three groups is the reaction similar to that observed in younger animals. The other two groups (1 and 3) show only a temporary hyperglycemia which gradually diminishes in the later periods of anoxia. In rats at the age of 100 days this reaction approaches that seen in adult animals (older than six months). The latter show, however, as previously mentioned, a further reduction in duration and extent of the hyperglycemic phase and the appearance of hypoglycemia.

Since previous investigations showed that in experiments involving anoxia hyperglycemia depends on the s.a. and hypoglycemia on the v.i. system the progressive changes in the blood sugar curve discussed in this paper seem to indicate a shift in the reactivity of these centers. The results could be due either to a diminution in the reactivity of the s.a. system, an increase in the excitability of the v.i. system or a combination of these two factors. Experiments on adreno-demedullated rats which in anoxia react through the v.i. system only showed that the degree of hypoglycemia seen under these conditions differed not significantly between mature and immature rats (Table I). The maximal fall in blood sugar is almost the same in both groups although the v.i. effect starts earlier in the young animals. The difference between the two groups is, however, too slight to account for the effect of age seen in normal animals. The interpretation seems therefore warranted that in rats with progressing age the reactivity of the centers of the s.a. system diminishes whereas that of the v.i. system remains constant.

An attempt was made also to determine whether at very old age (rats older than two years) a further alteration in autonomic reactivity takes place. However rats of this age readily succumbed to the anoxia employed, apparently due to an insufficient adjustment of the vascular and respiratory systems.

Conclusion. When rats of varying age groups (21, 45, 75, 100, and more than 200 days) are exposed to five successive periods of anoxia (each lasting 25 minutes at 280 mm Hg.) the blood sugar curve shows a definite dependence on the age of the animal. Hyperglycemia is greatest and sustained in very young animals (21 and 45 days). In rats of 75 and 100 days the hyperglycemic phase is greatest initially and diminishes gradually as the experiment progresses. Finally, in adult animals the initial hyperglycemic phase is definitely lessened. At the end of the test

[8] Van Middlesworth, L., Kline, R. F., and Britton, S. W., *Am. J. Physiol.*, 1944, **140**, 474.

period the blood sugar is either about normal or definitely hypoglycemic as an indication that the vago-insulin system has come into prominence. This shift in the balance is, however, not due to an alteration in the activity of the vago-insulin system, but is a result of a diminished excitability of the sympathetico-adrenal system as age increases since the hypoglycemic effect of anoxia on the vago-insulin system as shown by experiments on adreno-demedullated rats is not influenced by the age of the animals.

15103 P

Effect of Glucose Injections on Fasting Ketonemia After Low Protein Diets.

HERBERT C. TIDWELL AND C. R. TREADWELL. (Introduced by Donald Slaughter.)
From the Department of Biochemistry, Southwestern Medical College, Dallas.

The rapid onset of a marked fasting ketosis following low protein diets has been attributed by MacKay *et al.*[1,2] to a lack of "stored" protein available for the formation of antiketogenic material during fasting. Their conclusions were based on the higher nitrogen excretion observed during fasting after the high protein intakes. The difference in nitrogen excretion between groups on 5 and 25% protein diets averaged approximately 6 mg per square decimeter of body surface per day during the first 48 hours. In a comparable experiment we observed a difference in excretion of 8 mg of nitrogen. It seemed unlikely that 30 mg of glucose per square decimeter of body surface, the amount available from the metabolism of protein containing 8 mg of nitrogen, would have an appreciable effect on the metabolism of the rat. However, Deuel[3] has reported a marked lowering of ketonuria after giving 100 mg of glucose per square decimeter of body surface daily.

The present study was designed to determine the effect of glucose available from the additional protein metabolized after higher protein intakes upon fasting ketonemia in the rat. Two groups of 12 male rats, weighing approximately 224 g, were fed for 3 weeks a 5% protein, high fat diet similar to that used previously[4] and then fasted for 48 hours. Twice daily during the fasting period one group received intraperitoneally a 5% glucose solution containing 15 mg of glucose per square decimeter of body surface. The other group received similar volumes of normal saline.

The blood ketone levels at the end of the 48-hour fast were 25.6 $\pm$ 1.3* mg % of acetone in the glucose injected animals and 28.5 $\pm$ 1.0 mg % for the controls. There was a slight lowering of the ketonemia by even this small amount of glucose but not to the level (18.8 $\pm$ 1.7 mg %) obtained on animals receiving a 25% protein diet. These results do not support the suggestion that the greater protein catabolism in the animals with the larger protein stores can account for sufficient antiketogenic material to prevent the marked fasting ketosis following low protein diets.

The available carbohydrate from the excess protein metabolized by the animals on the high protein diets does not appear to be the major factor responsible for the markedly decreased fasting ketosis in such animals and the effect must at least in part be otherwise explained. Other data we are accumulating suggests that an increased rate of metabolism in the animals on the low protein diets plays a more important role than the lack of protein stores.

[1] MacKay, E. M., Carne, H. O., Wick, A. N., and Visscher, F. E., *J. Biol. Chem.*, 1941, **141**, 889.

[2] MacKay, E. M., Visscher, F. E., and Wick, A. N., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 514.

[3] Deuel, H. J., Jr., Hallman, L. F., and Murray, S., *J. Biol. Chem.*, 1938, **124**, 385.

[4] Treadwell, C. R., King, W. C., Bebb, K. C., and Tidwell, H. C., *J. Biol. Chem.*, 1942, **143**, 203.

* Standard error of the mean included.