serum gave a positive thymol test, while the middle layer of normal serum and of extracted hepatitis serum gave negative thymol tests. The flocculation test was positive with the middle layer in all sera. The serum component giving a positive flocculation reaction appears to be present in both the middle layer and the pellet while that responsible for a positive thymol test is only in the middle layer, where the presence of lipid is also a requisite.

Discussion. Notable differences in the mechanisms of the cephalin flocculation and the thymol test are shown in the observations recorded above. The gamma globulin fraction, upon which the cephalin flocculation depends, is not an essential factor in the thymol test. Also the electrophoretically derived albumin fraction has no effect on the intensity of the thymol test. On the other hand the albumin fraction is an important factor in the flocculation reaction, since normal albumin inhibits flocculation while that derived from hepatitis serum fails to inhibit flocculation.

The presence of lipids is necessary for a positive thymol test, but has little effect on the cephalin flocculation reaction. There is no evidence that an abnormal lipid is present in hepatitis serum since the negative thymol test obtained after ether extraction can be made positive once more by a variety of lipid preparations and even by whole normal serum. Lipids alone give no reaction with the thymol reagent.

It must be assumed that an abnormal constituent in the serum in addition to lipids is required for a positive thymol test in cases of liver diseases. This assumption is based upon the observation that the addition of normal serum or of various lipid preparations restores a positive reaction only to extracted serum which was thymol positive before extraction. The disturbance lies apparently in the serum globulin fraction, but, as has been shown, does not involve primarily the gamma globulins.

Conclusions. The thymol turbidity test tends to parallel the cephalin flocculation test in most liver disorders and other disease, but notable discrepancies are frequent. Fundamental differences in the mechanisms of the two tests have been pointed out. Notable among these are, (1) the effect of salt on the 2 reactions, (2) the importance of lipids, (3) the role of electrophoretically derived albumin fractions and gamma globulins, and (4) the comparative reactions of serum fractions obtained by ultracentrifugation. It is recommended that both tests be employed for clinical purposes since different disturbances in the serum complex are detected by each.

We are indebted to Dr. Dan H. Moore of the Electrophoresis Laboratory, College of Physicians and Surgeons, Columbia University, for preparing the protein fractions used in these studies, and are most grateful to him for his help and interest during the progress of the work.

We acknowledge the valued assistance of Mr. Thomas Kantor for some of the studies on whole and extracted serum cited above.

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Blood Sugar Level and Autonomic Balance.*

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Previous investigations of Gellhorn and collaborators[1] have shown that the excitability of the sympathetic vasomotor center is increased with falling blood sugar level as indicated by the increased blood pressure response of man and animals to anoxia and to hyper-

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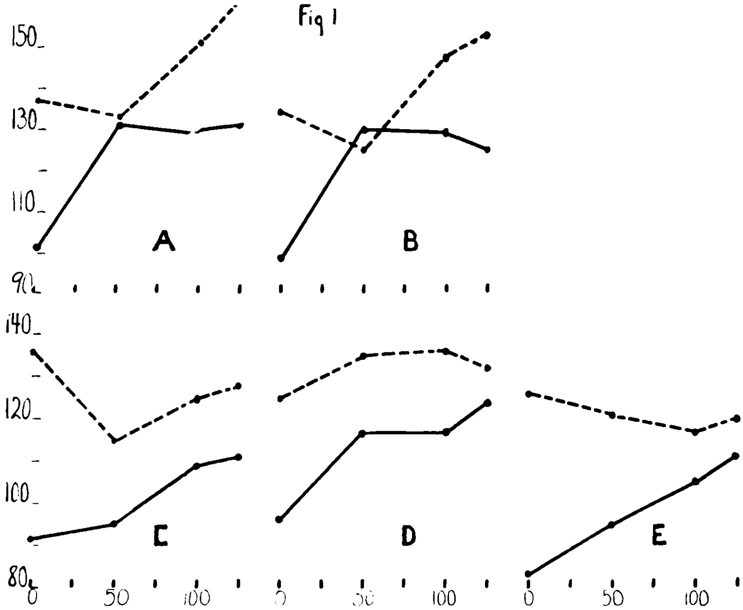
1 Gellhorn, E., Ingraham, R. C., and Moldavsky, L., *J. Neurophysiol.*, 1938, **1**, 301; Ingraham, R. C., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 315; Kraines, S. H., and Gellhorn, E.,



Effect of 5 periods of 25 min. exposure to 280 mm Hg. on the blood sugar of normal rats. Ordinate—mg %; abscissa—time in min.
A, B—Rats of 21 days age.
C, D, E—Rats of 45 days age.
———————— rats fasted for 22 hr.
— — — — — — rats fasted for 4 hr.
Groups A, B, C, D, E consisted of 6, 5, 3, 5, and 6 rats respectively.

capnia. The effect was observed only when hypoglycemic states were compared with conditions in which the blood sugar was normal or above normal. The question as to whether lesser changes in blood sugar level not involving hypoglycemic states may alter the excitability of the sympathetico-adrenal (s.a.) system has not yet been investigated. Nor has its effect on the reactivity of the centers of the vago-insulin (v.i.) system been considered although Zunz and LaBarre[2] showed that cerebral hyperglycemia leads to increased secretion of insulin via the v.i. system. In the following experiments in which these centers were excited by exposure of the experimental animals (rats) to low barometric pressure an attempt was made to answer these questions.

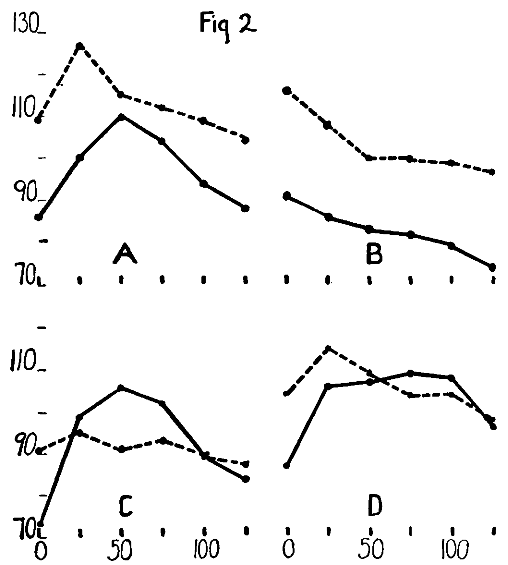
Since, as was shown previously, the autonomic balance is altered by age (Safford and Gellhorn[3]) it was thought of interest to study the influence of various blood sugar levels on this phenomenon in young and adult animals. Therefore, rats varying in age between 21 days and about one year were fasted for 4 and 22 hours and the influence of anoxia on the blood sugar level was determined. In view of the findings of Feldman, Cortell, and Gellhorn[4] this effect is the result of the action of anoxia on both s.a. and v.i. systems. In order to eliminate the effect on the s.a. system and to study the relation of the blood sugar level to the reactivity of the v.i. system alone experiments were performed on adrenodemedullated rats. Finally, a series of experiments was carried out on hypophysectomized animals. As a test for the activity of the s.a. and v.i. systems, the exposure of the rats to 5 periods (25 minutes each) of anoxia in the low pres-

Am. J. Psychiat., 1939, **95**, 1069; Gellhorn, E., Kiely, W. F., and Hamilton, S. L., *Am. J. Physiol.*, 1940, **130**, 256.

2 Zunz, E., and LaBarre, J., *C. R. Soc. de Biol.*, 1927, **96**, 1400.

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

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



Effect of anoxia on adult rats. Procedure as in Fig. 1.

A—Normal rats.

B—Adreno-demedullated rats.

C and D—Hypophysectomized rats.

Rats in groups A and B were fasted 22 and 4 hours, but those of groups C and D 12 and 4 hours respectively.

Groups A, B, C, D consisted of 9, 8, 5, and 5 rats respectively.

sure chamber at 280 mm Hg[5] was chosen as in the preceding study.[3] The rats of the same age which were tested at 2 different fasting levels were litter mates.

Results. Fig. 1 (A and B), illustrating the results on 2 groups of young rats (21 days) shows, as previously reported, a marked s.a. response (hyperglycemia) as the result of exposure to low barometric pressure on animals fasted for 22 hours. If, however, the test was performed on their litter mates after a fasting time of 4 hours only, the results were quite different. Instead of showing a maximum in the hyperglycemic curve after exposure to low barometric pressure for 50 minutes as seen in the 22-hour fasted rats the blood sugar curve falls slightly at this time in the briefly fasted animals. In the later periods, however, the blood sugar rises also in these rats above the original level although, in general, to a lesser degree than was observed after a fasting period of 22 hours. These results are confirmed in the 3 groups of experiments presented in Fig. 1, C to E. The s.a. effect is invariably greater in the 22-hour fasting rats than is observed under otherwise similar conditions after the 4-hour fast. It is interesting to note that in 2 of the 3 groups of 45-day-old rats recorded in Fig. 1, C to E, a hyperglycemic effect is absent after the 4-hour fast although in the corresponding test after a 22-hour fast a distinct hyperglycemia occurred in all animals.

Similar experiments performed on adult rats (Fig. 2, A) shows likewise a greater and more persistent hyperglycemic action of the anoxia test on animals subjected to a 22-hour fast than is observed under similar conditions after a fast of 4 hours.

A final series on the effect of anoxia on rats at different fasting levels was performed on hypophysectomized rats in which the respective fasting times were 12 and 4 hours in order to obtain blood sugar levels comparable to those of the normal and adreno-demedullated groups. Since the responsiveness to anoxia as far as blood sugar is concerned gradually disappears in the first weeks after hypophysectomy[6] the experiments were performed only a few days after the operation. Fig. 2, C and D, shows first that the s.a. response resulting from anoxia is preserved in the hypophysectomized rats and second, that this response is markedly decreased at the higher blood sugar level.

Attention is called to the fact that in the second group (Fig. 2, D) both tests were completed within 5 days after the operation. After the 4-hour fast anoxia caused only a slight and brief rise in blood sugar whereas its effect after the 12-hour fast was much greater and more prolonged. Moreover, the effect of the 2 tests may be compared at the same interval after hypophysectomy since the 12-hour and 4-hour tests were performed on the fifth day in the first and second group respectively (Fig. 2, C and D). Here again a marked difference in the reactivity of the s.a. system is found which is determined by the initial blood sugar level.

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

6 Unpublished observations.

The experiments presented thus far show clearly that in normal and hypophysectomized rats the blood sugar response to anoxia is modified by the prevailing blood sugar level. The higher the latter the less is the s.a. response. This phenomenon can be due either to an increased s.a. response or to a decreased v.i. response with falling blood sugar level or to a combination of both factors. Experiments on adreno-demedullated rats shown in Fig. 2, B, do not give any indication that the v.i. response is dependent on the level of the blood sugar. This experiment was repeated on another group of adreno-demedullated rats with similar results. It is therefore necessary to conclude from the experiments presented in this paper that in unanesthetized rats relatively slight changes in the blood sugar level resulting from different lengths of the fasting time significantly alter the reactivity of the autonomic blood sugar regulating centers. The balance existing between the v.i. and s.a. systems is altered in favor of the s.a. response with falling blood sugar level since no evidence could be obtained for a relation between the responsiveness of centers of the v.i. system to anoxia and the blood sugar level. The experiments show again the great stability of the centers of the v.i. system. Alterations in central autonomic balance induced by such diversified conditions as thyroxinization,[7] different age level,[3] and variations in the blood sugar concentration as the result of fasting seem to be due to the actions of these factors on the centers of the s.a. system only.

Summary. 1. The hyperglycemic effect of lowered barometric pressure (anoxia) on normal rats of different age groups is less in animals subjected to a 4-hour fast than in those fasted for 22 hours. 2. Similar results are obtained for hypophysectomized rats fasted for 4 and 12 hours respectively. 3. In contradistinction to the findings reported in 1 and 2, different durations of fasting have no effect on the vago-insulin system as shown by observations on adreno-demedullated animals.

It is concluded that the reactivity of the sympathetico-adrenal system increases with rising blood sugar level.

7 Gellhorn, E., and Feldman, J., *Endocrinology*, 1941, **29**, 467.

15150

Dietary Requirements for Fertility and Lactation. XXXII. Rice and its By-products as Sources of Pyridoxine and Pantothenic Acid for Reproduction and Lactation.*

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While considerable information is now available on the distribution of various components of the vitamin B complex in natural foods and on their influence on growth, very little is known concerning their biological value as sources of the B vitamins for reproduction and lactation.

In the milling of wheat there is 86% loss of thiamine[1] and 83% loss of nicotinic acid but the losses in pantothenic acid and pyridoxine are only about 50%.[2] In the milling of rice there is 80% loss of thiamine[3] and 66% loss of nicotinic acid[4] and only about 50% losses

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1 Sherwood, R. C., Nordgreen, R., and Andrews, J. S., *Cereal Chemistry*, 1941, **18**, 811.

2 Teply, L. J., Strong, F. M., and Elvehjem, C. A., *J. Nutr.*, 1942, **24**, 167.

3 Kik, M. C., *Cereal Chemistry*, 1943, **20**, 103.

4 Kik, M. C., and Van Landingham, F. B., *Cereal Chemistry*, 1944, **21**, 154.