the Ringer-Heparin solution did not occur in any instance either in the pipette or on the counting chamber.

Other useful points of technique noted since the first report include: (1) autoclaving of the stock Ringer's solution; (2) reduction of the $NaHCO_3$ in the standard Ringer's solution from 0.10 gm. to 0.05 gm. per 1000 cc.; (3) refrigeration of pipettes of diluted blood for delayed counting; (4) reduction of the amount of heparin used for blood counted within 3 hours after taking; (5) the necessity of a thorough mixture through vigorous shaking of the pipettes by hand or machine. It should be pointed out that Hayem's and other fixative solutions should not be used in pipettes employed for Ringer-Heparin solution since hemolysis may occur even after several thorough washings. However, if only Ringer-Heparin solution is used in pipettes, no difficulties with hemolysis will be encountered. Facility in counting is best secured with a fairly strong artificial light, using the low power lens, and keeping the substage shutter closed. The haemocytometer should be so placed that the entire fine ruling may be seen as one field. After standing for at least 15 minutes, to and fro movement of the fine adjustment of the microscope reveals the blood platelets as minute black refractile particles, discrete and distinct. No difficulty in their enumeration has been encountered.

Continued use of the method reaffirms its practicability, speed, and accuracy in the counting of both red blood cells and blood platelets.

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A Delimitation of the Central Nervous Mechanism Involved in Reflex Hyperglycemia.

CHANDLER M. BROOKS. (Introduced by E. N. Harvey.)

From the Department of Biology, Princeton University.

Claude Bernard's sugar puncture experiments furnished some evidence of the existence of a bulbar control of carbohydrate mobilization, but efforts to locate a specific center responsible for the piqûre diabetes have been rather inconclusive. Furthermore, temporary hyperglycemia and glycosuria often follow *any* injury to the brain. On the other hand, numerous investigators have shown that specific centers within the diencephalon may play some part in

the regulation of carbohydrate metabolism. Cannon and Rapport[1] produced additional evidence of a localized central control of the blood sugar level by locating a center in the medulla which is responsible for the reflex activity of the adrenal glands. Griffith[2] studied the various factors involved in a reflex hyperglycemia and found that the liver and the adrenal glands are chiefly responsible for the rise. Bard[3] suggested that a comparison of the reflex rises in blood sugar, obtained from intact animals and from animals after various parts of the central nervous system had been removed, might furnish additional information concerning the location of the centers controlling carbohydrate mobilization.

Before an attempt could be made to detect abnormalities in the reflex hyperglycemic reactions, control experiments had to be performed in which the central nervous system had been left intact. Twenty-six such experiments were performed: 10 of the animals were anesthetized by chloralose (0.1 gm. per kilo) administered by mouth; 10 by intravenously injected sodium barbital (0.28 gm. per kilo) and 6 by intraperitoneally injected sodium barbital (0.3 gm. per kilo). Comparable results were obtained in all cases and therefore sodium barbital, injected intravenously, was generally employed in subsequent experiments. Three blood samples were taken at half-hour intervals before stimulation; after a 3-minute period of interrupted (10 sec. on, 5 sec. off, 10 sec. on, —) tetanic stimulation of the right brachial nerve, blood samples were taken at intervals of one minute, 10 minutes, 30 minutes, one hour, and sometimes 2 hours. In all instances the strength of the stimulus was the same. Folin's[4] method of blood sugar assay was employed, 0.5 cc. of blood being drawn for each sample. Well nourished cats which had not been fed for 24 hours were used.

Reflex rises in blood sugar were studied in 10 thalamic animals, 26 decerebrate animals (10 under chloralose, 10 under sodium barbital injected intravenously, and 6 under sodium barbital injected intraperitoneally), and 10 animals in which the brain stem was divided by a section passing from a point a few millimeters posterior to the *corpora quadrigemina,* dorsally, to the posterior border of the pons ventrally. Ten of the decerebrate preparations had first been used as controls, thus making possible a comparison of the reflex rises obtained from the same animals before and after decerebra-

[1] Cannon, W. B., and Rapport, D., *Am. J. Physiol.*, 1921, **58**, 338.

[2] Griffith, F. R., *Am. J. Physiol.*, 1923, **66**, 618.

[3] Bard, P., *Arch. Neurol. and Psychiat.*, 1929, **22**, 230.

[4] Folin, O., *J. Biol. Chem.*, 1929, **82**, 83.

tion. Records of arterial pressure and heart rate were taken throughout each experiment in order to ascertain whether or not the animal was in good condition. After the transection had been performed an hour or more was allowed to elapse before the stimulation. This permitted the blood sugar to return to an approximately normal level. The reflex rises in blood sugar obtained in these transected animals were practically equal to those evoked in cats with the central nervous system intact (See Table).

TABLE I.

Reflex Rises in Blood Sugar Level Obtained After Transection of the Brain Stem at Various Levels.

Type of Preparation	No. of Animals	Aver. Blood Sugar Level Before stimulation.	Aver. rise in mg. of Sugar per 100 cc. of blood.	Aver. Rise in %.	Deviation from the Rise Obtained in Control Experiments.
Controls	26	130	24	19	
Thalamic	10	147	26	18	—1%
Decerebrate	26	138	29	21	+3%
Section anterior to *Brachium Pontis*	10	164	26	16	—3%
Section posterior to *Brachium Pontis*	5	140	—1	—0.7	No rise
Decapitate	15	154	—15	—9	No rise

Decapitate animals, on the other hand, did not respond to afferent stimulation with an increase in the blood sugar concentration, even though slight rises in arterial pressure and heart rate were obtained. Nor was a reflex hyperglycemia obtained if the medulla oblongata had been transected posterior to the anterior border of the *brachium pontis*. Therefore, it can be concluded that there is a mechanism located in the medulla oblongata, in the region of the vasomotor center, which is essential to a normal rise in blood sugar concentration when an afferent nerve is stimulated. This does not indicate that higher centers may not influence carbohydrate mobilization, but it does indicate that they are not essential to a normal reflex hyperglycemia. The results obtained are summarized in the accompanying table.