The absolutely and relatively refractory phases of these fibers have been determined by plotting the curve of return of irritability after a maximal first stimulus. The potentials were recorded after conduction to allow separation out from the earlier waves (Gasser and Erlanger).[5] Condenser charges were employed usually up to 160 volts and from .01 to .1 microfarad capacity. These condensers were connected through 2 contacts of the circuit breaker and charged in series with the bridge containing the nerve, the whole being balanced for capacity and resistance. To prevent the first condenser shunting the second, the circuit breaker was made to open the first circuit before the second was closed, the condenser charging through the nerve circuit during this interval. Both repeated and single pairs of stimuli were employed and it was found that stimuli applied more frequently than at the rate of 1 per second resulted in a prolongation of the absolutely refractory phase. The nerve was supplied with oxygen at room temperature. Preliminary experiments on the effect of anoxemia on these fibers and the knowledge that in the ordinary myelinated fibers, failure of response occurs when the normal absolutely refractory period of less than one sigma has increased only 100 to 300%, both indicate that the long absolutely refractory period found for these fibers is not the result of depression by anoxemia. They have an absolutely refractory period of 4 to 6 sigma when the action potential is still maximal, as indicated by the results of single stimuli.

These measurements of the properties of the unmyelinated fibers are being used to differentiate between types of fibers found in the same nerve.

4298

The Direct Estimation of True Blood Sugar.

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Blood sugar values obtained by Somogyi's[1] fermentation technique very probably represent the *true sugar* of blood. This procedure, while the standard for comparison, is somewhat laborious,

[5] Gasser, H. S., and Erlanger, Joseph, *Am. J. Physiol.*, 1925, lxxiii, 613.

[1] Somogyi, M., *J. Biol. Chem.*, 1927, lxxv, 33.

requiring 2 determinations for one result. It has been found that blood filtrates prepared by precipitation with acid mercuric sulphate followed by neutralization with barium carbonate and removal of the mercury with zinc give true sugar values as judged by the fermentation procedure. Barium carbonate possesses an advantage as a neutralizing agent in that it not only removes the sulphate from solution but automatically adjusts the pH to a uniformly definite value at which reducing non-sugars are efficiently precipitated by the mercury.

Method: 5 cc. of blood (1 vol.) are laked in 50 cc. of water (10 vol.) and 5 cc. (1 vol.) of acid mercuric sulphate (30% $HgSO_4$ in 10% H_2SO_4) added, with shaking, from a burette. The flask is stoppered and thoroughly shaken to break up the gel. 9-10 gm. of dry precipitated barium carbonate are added, the flask is rotated for a few seconds until most of the CO_2 has escaped, and then stoppered and shaken. Carbon dioxide is released until no further pressure develops in the flask and the mixture reacts neutral to litmus paper. If neutralization does not take place promptly, add 1-2 gm. additional barium carbonate. The mixture is poured upon a rapid filter. The filtrate (about 22 cc.) is treated with one drop of saturated sodium sulphate (to remove traces of barium) a pinch of zinc dust, and the flask shaken for a second or two. Zinc is filtered off and the sugar determined by the Shaffer-Hartmann method using an unpublished modification of the reagent by Dr. Somogyi. It has been found better than his published modification and is of the composition:

	gm. per liter
Sodium carbonate, anhydrous	20
Sodium bicarbonate	25
Rochelle salt	25
Copper sulphate, cryst.	7
Potassium oxalate	5
Potassium iodide	10
Potassium iodate	0.8

The time required for the preparation of filtrates is about the same as by the method of Folin-Wu. The mixture filters quickly and rapid filters may be used. Upon standing, a slight precipitate of zinc carbonate separates from the filtrates which, however, in no way interferes with the sugar determination. The filtrates contain a minimum of electrolytes because all the precipitating agent is removed by the barium carbonate and zinc. Nitrogen determinations show the filtrates of normal blood to contain no detectable N. The

technique is useful in preparing filtrates of tissue extracts, etc. Further work is in progress and a more detailed report will be published elsewhere.

4299

A Method for the Preparation of Blood Filtrates for Analysis.

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The use of zinc salts for the precipitation of blood proteins furnishes a method of preparing filtrates in which fermentable sugar (glucose) is the only substance to reduce alkaline copper solutions. The reducing substances other than sugar—responsible for the "residual reduction" of fermented blood filtrates—are precipitated along with the proteins so that sugar determinations in zinc filtrates give true blood sugar values.

In a simple method of deproteinization we employ the following 2 reagents:

Reagent I. 12.5 g. of zinc sulphate ($ZnSo_4 7H_2O$) are dissolved in water, 125 cc. of 0.25 normal sulfuric acid are added, and made up with water to one liter.

Reagent II is a 0.75 normal solution of sodium hydroxide.

The 2 reagents have to be in such a definite relation that when Reagent I is titrated against Reagent II in the presence of phenol phtalein, 25 cc. of I should require 3.35 to 3.40 of II to produce a permanent pink color.

Procedure: In order to prepare a 1:10 filtrate, lake one volume of blood in 8 volumes of Reagent I, add one volume of Reagent II, stopper tightly, shake well, filter after a few minutes through a dry filter paper. Centrifuge before filtration if a maximum yield of filtrate is wanted.

For the preparation of filtrates of concentrations other than 1:10, we use a somewhat different technique and pair of reagents. Reagent *A* is a 19.5% zinc sulphate solution, Reagent *B* a normal sodium hydroxide. One volume of blood requires one half a volume of each of these reagents for deproteinization. If, for instance, a 1:5 filtrate is desired, lake one volume of blood in 3 volumes of water, introduce one half a volume of Reagent *A*, mix, then add one half a volume of Reagent *B*, stopper tightly, shake well, filter