

Central expiratory potentials, of the tonic type, were commonly of a much higher frequency than those previously recorded in respiratory muscles (Gesell), suggesting the existence of a step-down mechanism. There is also some evidence that certain potential frequencies may be a multiple of a lower rate of discharge.

Continuously occurring discrete potentials of a uniform frequency have been encountered. These potentials have been changed by inspiratory and expiratory mechanical asphyxia to either the inspiratory or expiratory type.

Electrodes inserted into silent regions may from time to time register respiratory potentials as resting cells come into activity. Frequently these silent regions yielded respiratory potentials during mechanical asphyxias. Potentials emanating from a previously silent region, if from a true respiratory center, indicate that the completeness of participation of the center varies with the magnitude of breathing.

The exact cellular or fiber source of central potentials is being sought by placement of minute lesions at the site of the lead-off electrodes immediately following photographic registration of potentials. The fact that both expiratory and inspiratory potentials can be recorded from one placement of the electrodes, or that one type may give way to the other with a minute displacement of the electrodes, indicates a close anatomical association between the so-called inspiratory and expiratory centers.

7862 C

A New Method of Determining Plasma Fibrin.

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The usual method of estimating the fibrinogen content of the blood is by recalcifying the oxalated plasma in order to convert the fibrinogen into fibrin.¹ The quantity of fibrin precipitated is then determined by the Kjeldahl, the gravimetric, the colorimetric, or the refractometric methods.

¹ Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Williams and Wilkins, Baltimore, 1932. This method of precipitating the fibrin was developed by Cullen and Van Slyke, *J. Biol. Chem.*, 1920, **41**, 587.

The method of determining plasma fibrin presented here is based on the coagulant activity of certain types of snake venom. Martin² was the first to demonstrate the striking activity of the venoms of certain Australian snakes in causing coagulation of blood both *in vivo* and *in vitro*. Lamb³ found that minute doses of such venoms could also coagulate *in vitro* blood which had lost the capacity for spontaneous coagulation on account of the addition of citrates, oxalates or fluorides. Among the venoms noted for their coagulating action are those of the following snakes: *Notechis scutatis* (tiger snake), *Pseudechis porphyriacus* (black snake), *Echis carinata* (phoorsa) and *Viperi russelli* (daboia). The former 2 species are found in Australia and the latter 2 in India. In addition, certain species of the Lachesis group of vipers are known to exhibit this property. In the present investigation, tiger snake venom, which apparently has the most powerful coagulating action,* was used. In all probability, the venoms of the other species mentioned can also be used.

The actual technique of estimating the concentration of fibrinogen (as fibrin) in the plasma with the aid of tiger snake venom is as follows: One cc. of oxalated plasma is diluted 25 to 30 times with normal saline solution. (The quantity of powdered potassium oxalate used for collecting the blood need not be accurately measured.) To the diluted plasma is added 0.3 cc. of a 1 to 5,000 dilution of dried tiger snake venom in normal saline. Coagulation usually occurs within 2 or 3 minutes. The clotted plasma is transferred to dry filter paper on a funnel. When practically all the fluid has drained through, distilled water is poured on the filter, whereupon the fibrin clot will usually float free in one piece. The distilled water is allowed to remain in contact with the clot for 10 minutes, after which time the water is drained off. This process of washing the clot is repeated 4 or 5 times, when the clot should be colorless. The entire clot can then be picked up by twisting it on a glass rod. The clot is transferred to a piece of dry filter paper, and all the fluid remaining is gently expressed. It can then be thoroughly dried on a watch glass kept in an incubator at 37°C. over night, or in a hot air oven at 110°C. for one hour. The combined weight of 5 (or more) dried clots which have been obtained in this way can be de-

² Martin, C. J., *Proc. Roy. Soc. of New South Wales*, 1896, **30**, 150 (cited after Kellaway, *Med. J. of Australia*, 1929).

³ Lamb, G., 1903 (cited after Kellaway).

*According to Mellanby (*J. Physiol.*, 1909, **38**, 441), the amount of tiger snake venom which coagulates 2 cc. of bird plasma in 4 minutes is .00003 mg.

terminated fairly accurately in an ordinary quantitative balance. The amount of fibrin in 100 cc. of plasma is then obtained by multiplying by the proper factor (20, if the clots from 5 cc. of plasma are weighed). If a micro or assay balance is available, the clot from one cc. of plasma is ample for the determination.

The question could arise whether the substance precipitated by the venom is really fibrin, and if it is fibrin, whether the precipitation is complete. The simplest way to answer this question is by comparing the results obtained by this method with those obtained when the fibrin is precipitated by recalcifying the plasma. Accordingly, parallel micro-Kjeldahl determinations were made on fibrin clots from the same specimen of plasma by the 2 methods of precipitation. For example, in Table I are given the results for 2 cases which were

TABLE I.
Comparison of Results Obtained when Fibrin is Precipitated by Venom and by Calcium

Diagnosis		Estimation by Micro-Kjeldahl Method				Mg. Fibrin in 100 cc. Plasma Estimated by Weighing Directly Fibrin
		Mg. Fibrin N in 100 cc. Plasma		Mg. Fibrin in 100 cc. Plasma ($N \times 6.25$)		
		Precipitated by Venom	Precipitated by Calcium	Precipitated by Venom	Precipitated by Calcium	
Pneumonia	(1)	144.8	143.9			
	(2)	155.5	144.9			
	(3)		153.6			932.
	Mean	150.2	147.5	938.4	921.4	
Acute Gastro-enteritis	(1)	93.5	90.7			
	(2)	96.6	91.3			
	(3)	94.1	92.7			586.
	Mean	94.7	91.6	592.4	572.3	

thoroughly studied. It will be seen that the nitrogen values obtained by the 2 methods agree very closely. Furthermore, the fibrin values derived by multiplying the fibrin N by the factor 6.25 are almost identical with that found by directly weighing the dried clot obtained as described above. Of course the clot obtained by recalcifying the plasma cannot be weighed directly, since it contains calcium oxalate.

In addition, the results obtained by Wu's colorimetric method⁴ were the same, whether the fibrin was precipitated by venom or by calcium. However, the colorimetric method itself is not entirely reliable, but this aspect will be taken up in a later paper.

⁴ Wu, H., *J. Biol. Chem.*, 1922, **51**, 33.

Perhaps the principal advantage of this method of determining blood fibrin is its simplicity. The major difficulty is in obtaining the venom. However, since only a minute amount is required for each test, a small supply will last for a very long time. Even the quantities used in the present study (0.06 mg. of the dried venom per test) were far greater than necessary.

We wish to express our indebtedness to Dr. C. H. Kellaway, Director of the Walter and Eliza Hall Institute of Melbourne, Australia, who supplied us with the venom used in this investigation.

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Acquired Resistance of Liver Cells to the Toxic Action of Uranium Nitrate.*

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A statement has been made previously concerning the type, distribution and severity of the injury to the liver by the use through subcutaneous injection of a solution of uranium nitrate.^{1, 2} In addition, observations were recorded² concerning the types of repair processes developing in the liver as a result of the reaction of the liver to this hepatotoxic agent, and the resistance or lack of resistance which such processes manifested when the liver was subjected to the toxic action of chloroform, administered by inhalation.

The present series of experiments are concerned with the injury induced to hepatic epithelium by the subcutaneous use of uranium nitrate in the amount of either 2 or 4 mg. per kilogram, the type of repair process which is inaugurated by such injuries and the resistance which certain cells in such areas of repair may acquire to secondary intoxications by uranium. During the course of the experiments biopsy material has been obtained from the liver for cytological study and at such periods tests of hepatic function have been made by the use of phenoltetrachlorophthalein according to the technique devised by Rosenthal.³

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¹ MacNider, Wm. deB., *Proc. Soc. Exp. Biol. and Med.*, 1919, **16**, 82.

² MacNider, Wm. deB., *Trans. Assn. Am. Physicians*, 1934, **49**, 14.

³ Rosenthal, S. M., *Bull. Johns Hopkins Hosp.*, 1922, **33**, 432.