lowing the infusion of the sodium acetate.

Discussion. Sodium acetate affords a readily procurable and inexpensive source of fixed base, as rapidly and as completely available as sodium bicarbonate. Solutions are easily prepared, easily sterilized, and indefinitely stable. The pH of 5 different lots of sodium acetate made up in 1/6 molar concentration was found to vary from 7.6 to 8.0. We believe that the use of sodium acetate solutions is indicated in rapid parenteral alkalinization and should be useful in the therapy of most types of metabolic acidosis. However, we wish to emphasize that because the metabolism of acetate has not been specifically defined as to its possible effect on ketone body production in diabetic acidosis, we have not used it to date in this condition.

These studies have not revealed previously undescribed properties of sodium acetate. Indeed the salt has been recommended in standard textbooks[3,4] as a source of fixed base, but its clinical use has been limited. The present observations demonstrate the rapid catabolism of acetate and emphasize certain practical advantages which it possesses for clinical use.

Summary. Studies in dogs and in man demonstrate that sodium acetate is an easily prepared and a rapidly available non-toxic source of fixed base suitable for parenteral administration when alkalinization is indicated.

3 Goodman, L., and Gilman, A., The Pharmacological Basis of Therapeutics, The Macmillan Company, New York, 1941.

4 Sollmann, T., A Manual of Pharmacology, W. B. Saunders Company, Philadelphia, 1948.

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17110. A Method for Assay of Serum Proteolytic Activity.*

G. R. DOWNIE† AND E. E. CLIFFTON.‡ (Introduced by Samuel C. Harvey.)

From the Department of Surgery (Oncology), Yale University School of Medicine.

That blood contains an inactive precursor (plasminogen) which can be activated to form a proteolytic enzyme (plasmin) has been established.[1-7] Study of variations in this enzyme have largely been on the basis of its fibrinolytic properties. Using a method of preparation outlined by Milstone,[6] we have been able to obtain from serum a plasminogen-containing fraction with consistent and reproducible activation in humans and dogs.

Reagents and Materials. 1. *Streptokinase*—the filtrate of a 24-hour culture of Group A, Type 11, Beta hemolytic streptococci, grown in beef heart infusion broth with 0.05% added dextrose.

2. *Protamine Sulphate*§—a solution of protamine sulphate in veronal buffer, 2 mg per ml.

3. *Veronal Buffer*—pH 7.35[8]

4. *Acetic acid—distilled water mixture*—19 volumes of distilled water plus 0.32 volumes of 1% acetic acid.

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† Fellow of Department of Surgery (Oncology), Yale University School of Medicine.

‡ Senior Fellow, American Cancer Society, as recommended by the Committee on Growth of the National Research Council.

1 Christensen, L. R., and MacLeod, C. M., *J. Gen. Physiol.*, 1945, **28**, 559.

2 Christensen, L. R., *J. Gen. Physiol.*, 1946, **30**, 149.

3 Ratnoff, O. D., *J. Exp. Med.*, 1948, **88**, 401.

4 Delezenne, C., and Pozerski, E., *Compt. rend. Soc. Biol.*, 1903, **55**, 690.

5 Kaplan, M. H., Tagnon, J. H., Davidson, C. S., and Taylor, F. H. L., *J. Clin. Invest.*, 1942, **21**, 533.

6 Milstone, H. J., *J. Immunol.*, 1941, **42**, 109.

7 Ferguson, J. H., Travis, B. L., and Gerheim, O. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 285.

§ Eli Lilly and Company, Indianapolis, Ind.

8 Clark, D. G. C., Cliffton, E. E., and Newton, B. L., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 276.

TABLE I.
Sample Reading of Results in %T and Tyrosine Produced After 2, 6, and 24 Hours Incubation with Streptokinase (S), Protamine (P), and Spontaneously (O) Activated Plasminogen. Controlled by Reagent Blanks with 0.5 ml Veronal Buffer Substituted for Plasminogen.

		% T			Tyrosine—mg		
Patient	Activator	2 hr	6 hr	24 hr	2 hr	6 hr	24 hr
B.U.	S	79.5	45	6	.148	.256	.905
	P	91.8	100	59.6	.026	0	.166
	O	93	93.8	84.7	.023	.019	.053
H.D.	S	85	47	7.5	.050	.242	.835
	P	100	90	72	0	.033	.105
	O	96.4	88.5	81	.01	.039	.066
J.S.	S	90.8	86.5	61.5	.03	.045	.155
	P	94	90	58	.018	.033	.175
	O	95	93.5	65	.015	.02	.138
F.G.	S	58.2	24	4.5	.174	.458	1.0
	P	82	69	46.5	.063	.118	.245
	O	80.2	74	58.5	.07	.095	.173
H.C.	S	89	59	18.3	.036	.17	.545
	P	91.2	87.5	69.5	.028	.043	.116
	O	91.3	89.5	86	.027	.035	.048
Reagent blanks	S			100			0
	P			97			.008
	O			100			0

5. *Trichloracetic Acid* (USP), 16%

6. *NaOH* (Reagent grade), 1N

7. *Casein*—a 5% solution, adjusted to a final pH of 7.35-7.45.

8. *Folin-Ciocolteau phenol reagent*—diluted 1:3 with distilled water.

9. *Serum*—obtained from nonhemolyzed blood drawn under sterile conditions.

Method. Plasminogen is prepared by adding 1.5 ml serum to 28.5 ml acetic acid-distilled water mixture, inverting once to mix, and centrifuging at 2000 RPM for 15 minutes. The supernatant is discarded and the precipitate dissolved in 1.5 ml veronal buffer; 0.5 ml of this solution, containing plasminogen, is then added to each of three test tubes. To the first, 0.4 ml streptokinase and 0.1 ml buffer are added; to the second 0.5 ml protamine sulphate solution; and to the third, 0.5 ml buffer. 5 ml of casein solution are added to each tube, mixed by inversion, and a 2.5 ml aliquot taken immediately.

5 ml trichloracetic acid are added to each aliquot, and the resulting coagulum is separated from the sides of the tubes by thorough shaking. Each sample is filtered through Whatman No. 3 filter paper, and a 2.5 ml aliquot of the filtrate is added to 5 ml of 1N NaOH in a 50 ml Erlenmeyer flask. 1.5 ml of diluted phenol reagent is added to each flask in turn as rapidly as possible, and the contents of the flasks are transferred to spectrophotometric cuvettes. Exactly ten minutes after phenol reagent is added to the first flask, the %

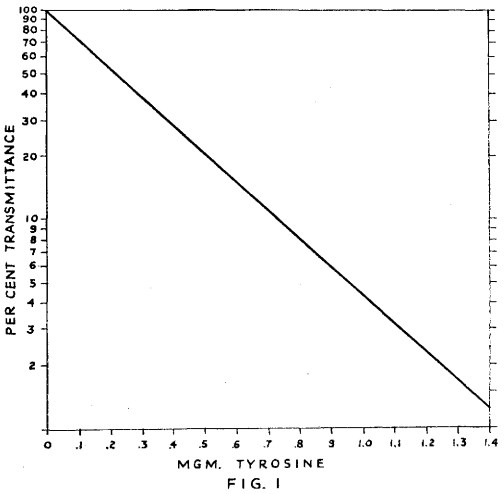

FIG. 1

Calibration curve. Standard tyrosine solution in casein.

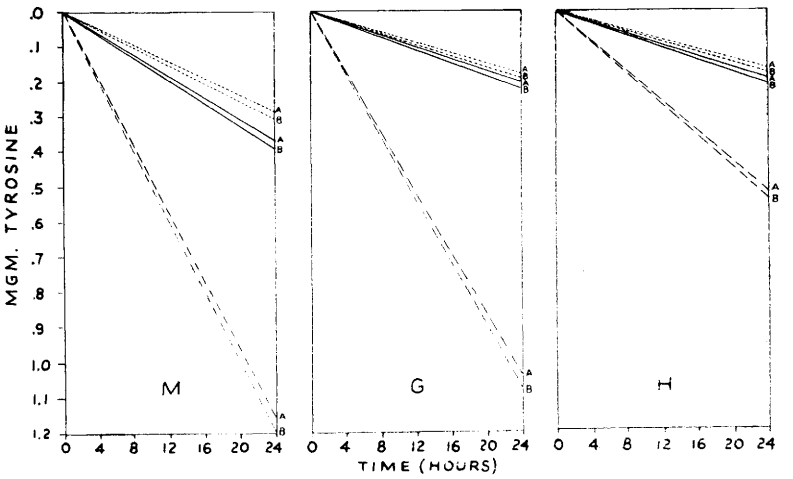

FIG. 2

Graphs of results with duplicate samples of blood of 3 pts. M, G, and H. A and B represent aliquots of each serum taken simultaneously and treated as individual sera.

- - - - - - - - Spontaneous activity.
———— Protamine activated.
– – – – – – Streptokinase activated.

transmittance of each sample is determined in turn in the Coleman Spectrophotometer, set at 675 mu. The original tubes are incubated for 24 hours at 37°C.

The second 2.5 ml aliquot is taken after 24 hours incubation at 37°C, and color is developed as described above. When the samples are read, the first aliquot acts as the "blank" for the second. A stock solution of a blue dye, T-1824, is prepared so that its optical density is greater than the optical densities of the first aliquots of the unknowns. In reading the first aliquots in the spectrophotometer, the color-producing agents already present in the digest mixtures are negated by setting the galvanometer index at 100% T, or 0 concentration. With this as a reference point, the %T of the dye is then taken for each sample. When the corresponding 24-hour sample is read, the dye, as the "blank", is set at the T value obtained the day before, and the % T of the unknown is then read directly.

Calculation. Standard amounts of tyrosine were added to 5 ml of casein solution, and 2.5 ml aliquots were taken and treated as above. These were read against a similar aliquot of casein without added tyrosine. A calibration curve was prepared, so the % T of unknown samples can be expressed directly as mg of tyrosine produced (Fig. 1, Table 1).

Results and Discussion. Aliquots of sera tested after 2, 6 and 24 hours' incubation showed steadily increasing amounts of tyrosine produced, and for experimental purposes the 24 hour period was chosen (Table I). Reproducibility was established by taking aliquots of serum simultaneously and treating them as individual samples (Fig. 2).

Demonstration of the activation of plasminogen by streptokinase and the slow spontan-

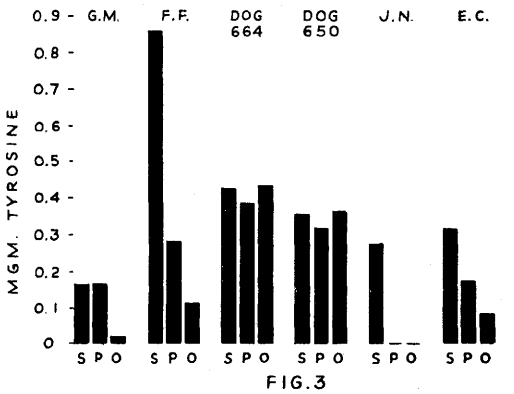

FIG. 3

Columnar graphs showing activation by streptokinase (S), protamine (P), and spontaneous activation (O). 4 representative patients and 2 dogs. Note similarity of activation by agents in dogs as compared to humans.

eous activation confirms previous studies of this relationship. The activation by protamine is definite, though in most instances not as great as is that by streptokinase (Fig. 3). This is at variance with the previous impression that the fibrinolytic activity of protamine was due to inactivation of antifibrinolysin.[10] In the present study protamine sulphate (up to 1.2 mg/tube) failed to diminish antiproteolytic activity tested by the method previously reported.[8]

Dog sera differ from human in that their spontaneous activation is of the same order as the protamine and streptokinase activation (Fig. 3).

Summary. A method for preparation of plasminogen from small quantities of blood has been described. Activation by streptokinase, protamine sulphate, and spontaneous activity have been observed. Studies of other activators and of species differences are in progress.

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10 Scroggie, A. E., Jaques, L. B., Rocha e Silva, M., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 326.

17111. Automatic Measurement of Alveolar CO_2 in Small Animals.

KENNETH A. BLINN AND WERNER K. NOELL.
(Introduced by Albert W. Hetherington.)
From the Department of Neuropsychiatry, USAF School of Aviation Medicine, Randolph Field, Texas

The following paper will describe the application of an infra-red analyzer to alveolar carbon dioxide studies in small animals such as the rabbit, where rapid respiration and small respiratory volumes pose severe problems. As reported elsewhere,[1] this analyzer has been used successfully in measuring the alveolar CO_2 tension in humans during hyperventilation as it is performed in routine electroencephalography. The analyzer was developed for other purposes by Luft[2] and applied to CO_2 measurement by Schmeiser.[3] It measures the infrared absorption of one gas in a mixture by using a fixed quantity of the same kind of gas as a thermomechanical metering device. This method allows an analysis which is rapid enough to fully record a change in CO_2 tension in one-seventh of a second. Thus, in human studies, alveolar air sampling devices are unnecessary, and a portion of the expired air is drawn continuously through the analyzer. The latency of measurement then depends solely upon the speed of transport of this air through the test chamber. The only additional equipment required is a long expiration tube to prevent dilution of the continuous sample by outside air. The measured CO_2 value after a certain expired volume can be safely regarded as the conventional "alveolar CO_2 tension."

Fig. I illustrates the CO_2 analyzer in simplified form. A hot-wire infra-red source (S) projects a beam of infra-red through a transparent (mica) test cell (T) containing the air for CO_2 analysis. The remaining infra-red is absorbed in a following chamber (A), one wall of which is a thin metal membrane. The temperature increase due to the absorption displaces the membrane by increasing the pressure, thus measuring the amount of infra-red reaching the chamber. If the chamber is filled with CO_2, it becomes sensitive to only those wave lengths which the CO_2 in the test cell absorbs. Thus, it is insensitive to any other gases appearing in the test cell. To balance the system, an identical arrangement (S', C, A') is attached to the other side of the membrane, and the two sides "buck" each other. Displacements of the membrane are measured by utilizing it as one plate of a condenser and

1 Blinn, K. A., and Noell, W. K., *EEG Clin. Neurophysiol.*, 1949, in press.

2 Luft, K., *Z. f. Tech. Phys.*, 1943, **24**, 97.

3 Schmeiser, K., unpublished work.