The author wishes to express his sincere appreciation to Dr R. E. Hungate for an introduction to the fascinating cattle protozoa and for many stimulating discussions during the course of the experiments. The investigation was supported in part by funds for biological and medical research by the State of Washington, initiative measure number 171.

REFERENCES

- Carroll, E. J. & Hungate, R. E. (1954). *J. appl. Microbiol.* **2**, 205.
 Elsdon, S. R. (1946). *Biochem. J.* **40**, 252.
 Friedmann, T. E., Cotonio, M. & Shaffer, P. A. (1927). *J. biol. Chem.* **73**, 335.
 Gutierrez, J. (1953). *J. Bact.* **66**, 123.
 Heald, P. J. & Oxford, A. E. (1953). *Biochem. J.* **53**, 506.
 Heald, P. J., Oxford, A. E. & Sugden, B. (1952). *Nature, Lond.*, **169**, 1055.
 Hungate, R. E. (1940). *Bot. Gaz.* **102**, 382.
 Hungate, R. E. (1942). *Biol. Bull., Woods Hole*, **83**, 303.
 Hungate, R. E. (1943). *Ann. ent. Soc. Amer.* **36**, 730.
 Hungate, R. E. (1950). *Bact. Rev.* **14**, 1.
 Johns, A. T. (1951). *J. gen. Microbiol.* **5**, 317, 326, 337.
 Sugden, B. & Oxford, A. E. (1952). *J. gen. Microbiol.* **7**, 145.

A Colorimetric Method for the Estimation of Serum Magnesium

By A. J. SMITH

Department of Chemical Pathology, King's College (Durham University) and the Royal Victoria Infirmary, Newcastle upon Tyne

(Received 29 December 1954)

In clinical work, where general electrolyte depletion occurs, there may be disturbances of serum magnesium which require correction. These deficiencies have been shown by Flink, Stutzman, Anderson, Konig & Frazer (1954) to occur sometimes in chronic alcoholics and in patients on continuous intravenous therapy. These authors reported neurological symptoms similar to those described in animals by Kruse, Orent & McCollum (1932) and by Greenberg & Tufts (1938). Evidence of change in the magnesium levels of the cerebrospinal fluid in human subjects was found by McCance & Watchorn (1932) in cases of chronic meningeal inflammation, but no symptoms were ascribed to this. Smith (1949) showed that a raised serum magnesium concentration in the dog caused a flaccid paralysis. The importance of magnesium is also evident in intermediary metabolism, since magnesium is needed as an enzyme activator in oxidative decarboxylation and is involved in adenosine triphosphate energy-release mechanisms (Baldwin, 1947). Interest in magnesium levels in clinical work is increasing and there is a need for a method of serum magnesium estimation suitable for a routine clinical laboratory.

The earliest method for the estimation of magnesium was gravimetrically as magnesium ammonium phosphate (McCrudden, 1909), and in the following period many micro-methods applicable to serum were based on the estimation of phosphate in the precipitates by various procedures. Decolorization of ferric thiocyanate was used by Marriott & Howland (1917), and by Kramer & Tisdall (1921), who combined calcium and magnesium estimations by consecutive precipitation of calcium as oxalate, and magnesium as magnesium ammonium phos-

phate. Denis (1920) used a nephelometric method with strychnine molybdate. Upon the introduction of the colorimetric estimation of phosphate by Bell & Doisy (1920), this was applied to the magnesium ammonium phosphate precipitate by Denis (1922), Hammett & Adams (1922), and with modifications by Briggs (1922*a, b*, 1924). The colorimetric method of Fiske & Subbarow (1925) was subsequently used until Simonsen, Westover & Wertman (1947) introduced molybdivanadate for the estimation of phosphate.

A different principle was used by Greenberg & Mackey (1932), in which calcium was removed and magnesium precipitated by 8-hydroxyquinoline, which was estimated volumetrically; but this method has not been generally used owing to difficulty in handling the light precipitate.

Hirschfelder & Serles (1934) introduced the method based on an estimation of magnesium as a specific coloured lake using Titan Yellow in alkaline solution. The lake had, however, to be suspended by a colloidal dispersing agent; soluble starch was used but tends to give opalescent solutions. Garner (1946) used gum ghatti as the dispersing agent to give clear solutions. A further modification was introduced by Kunkel, Pearson & Schweigert (1947) using hydroxylamine hydrochloride with Titan Yellow. The most recent examination of Titan Yellow methods was made by Orange & Rhein (1951), who selected polyvinyl choride as a colloidal dispersing agent.

Buckley, Gibson & Bartolotti (1951) introduced titration with ethylenediaminetetraacetate to estimate magnesium using Eriochrome Black T as an indicator after the determination of calcium

using murexide as an indicator. Eriochrome Black T was also used by Sobel & Hanok (1951) in the determination of total magnesium and calcium by ethylenediaminetetraacetate.

The estimation of a soluble magnesium-dye complex was introduced by Bacon (1951) to determine magnesium and calcium using the dye Solochrome Cyanin R.S. Finally, Harvey, Komarmy & Wyatt (1953) used the estimation of the magnesium-Eriochrome Black T complex to determine magnesium in water after the removal of calcium.

The estimation of the magnesium-Eriochrome Black T soluble complex is used in this paper to determine serum magnesium.

MATERIALS

Absolute methanol. A.R. methanol was used.

Eriochrome Black T. This was obtained from Light and Co. Ltd. Except for a small residue it dissolved in methanol.

Buffer. Buffer as used by Harvey *et al.* (1953) is made by dissolving 6.75 g. of ammonium chloride A.R. in distilled water, adding 57 ml. conc. ammonia A.R., and making up to 1 l. with distilled water, giving a pH of 10.1.

Ammonia. 8% (v/v) of conc. ammonia A.R. was used.

Trichloroacetic acid. A.R. 10% (w/v) solution was used.

Saturated ammonium oxalate. This was the A.R. reagent.

Standard stock solution of magnesium acetate. This was A.R., containing 1 mg./ml. magnesium preserved with chloroform.

Primary standard of magnesium acetate. This was prepared from stock standard to contain 40 $\mu\text{g./ml.}$ magnesium.

Working standard of magnesium acetate. This was prepared from primary standard to contain 4 $\mu\text{g./ml.}$ magnesium.

The glassware used must be specially clean.

EXPERIMENTAL

Results with pure solutions

Choice of wavelength. The dye forms a dark red solution in absolute methanol, a blue one when this is made alkaline and a red complex with magnesium when alkaline. The absorption spectra of both dye and complex in alkaline solution show broad bands with maxima well separated but with each band overlapping the maximum of the other. Spectrophotometric estimation depends on the choice of a wavelength at which changes in the proportions of dye and complex in a mixture gives the greatest changes in the optical density. In Fig. 1 the absorption curves are shown for the dye (0.01% at pH 10.1) alone and with added magnesium 0.8 $\mu\text{g./ml.}$ In both cases the buffer forms 25% of the solution measured. At this magnesium concentration the major proportion of the dye is in the form of the magnesium complex. The greatest increase in absorption on adding magnesium occurs at 520 $\text{m}\mu$, as was also found by Harvey *et al.* (1953). The calibration for the desired range of magnesium concentrations thus depends on the resultant of an increased density at 520 $\text{m}\mu$. due to the formation of the complex and a decrease in the density due to the excess dye.

The measurement of absorption was made on a Unicam spectrophotometer SP.600 using a 0.2 cm. light path.

Choice of dye concentration. Harvey *et al.* (1953) used a dye strength of 0.004% in the solution measured for the magnesium range of 0–1.4 $\mu\text{g./ml.}$ This has been found to be insufficient for the higher concentrations of this range. Fig. 2. shows the relation between magnesium concentration and optical density at 520 $\text{m}\mu$. with dye concentrations

Fig. 1. Absorption spectra of Eriochrome Black T 0.01% at pH 10.1 for dye alone, ○—○; and with the addition of 0.8 $\mu\text{g./ml.}$ magnesium, ●—●.

Fig. 2. Measurement of optical densities of magnesium solutions containing from 0 to 1.0 $\mu\text{g./ml.}$ at pH 10.1 with varying dye strengths: 0.01%, ●—●; 0.008%, ○—○; and 0.004%, ×—×.

of 0.004, 0.008 and 0.01% at pH 10.1. The concentration of 0.01% dye has been selected as the minimum giving a linear relationship between magnesium concentration and optical density over the desired range. Although for low magnesium concentrations this involves estimating a small increment on a high density owing to the excess dye, the sensitivity is adequate. Measurements were made as before on a 0.2 cm. light path.

The age of the dye solution influences the densities measured because of a gradual change in dye structure. For

2-3 days this does not affect the linear relationship of density to magnesium concentration, but after 5 days the values for higher magnesium concentrations cease to be on a straight line. The extinctions for a given concentration of magnesium are not exactly reproducible owing to the small residue of methanol-insoluble material present in the dye. This, however, has no effect on the reproducibility with one batch of dye solution. The solutions are well mixed before and after addition of the dye solution and read within 5 min. The fading of the colour is very slow but variable.

Effect of trichloroacetic acid. When the standards are treated by the same procedure as is used for serum, the trichloroacetic acid causes a decrease in the slope of the calibration line obtained with pure magnesium solutions.

For the pure standards a volume of the working standard solution (2 ml. for 0.5 μ g. Mg/ml.) is made up to 13 ml. with distilled water, 5 ml. buffer are added and then 2 ml. dye. For the standard treated with trichloroacetic acid, double the volume of working standard is used (4 ml. for 0.4 μ g. Mg/ml.), 0.4 ml. saturated ammonium oxalate and 10 ml. trichloroacetic acid 10% (w/v) are added and the volume is made up to 20 ml. with distilled water. This is filtered through a no. 42 Whatman 9 cm. paper and 10 ml. of the filtrate are taken. Three ml. ammonia 8% (v/v), 5 ml. buffer pH 10.1 and 2 ml. dye 0.01% are added. The results for these standards are given in Fig. 3.

Fig. 3. Optical densities read at pH 10.1 with dye concentration of 0.01% for magnesium concentrations from 0 to 1.0 μ g./ml. as pure solutions, ●—●; and when treated in the same procedure as used for serum, ○—○.

Application to serum

Procedure. Calcium must be removed by precipitation as oxalate and may be determined by the method of Kramer & Tisdall (1921) on the same sample as the magnesium. The supernatant fluid after removal of the calcium is deproteinized with trichloroacetic acid and the spectrophotometric method applied to the protein-free filtrate. A calibration is made in parallel with each batch of analyses using a control on reagents and a set of standards treated with the reagents to correspond with the serum filtrate.

To 2 ml. serum are added 2 ml. distilled water and 1 ml. saturated ammonium oxalate. These are mixed, allowed to stand 30 min. then centrifuged

for 5 min. at 3000 rev./min. The supernatant is removed and 2 ml. are added to 8 ml. distilled water. Then 10 ml. 10% trichloroacetic acid are added with mixing. After 10 min. this is filtered (9 cm. no. 42 Whatman paper) and 10 ml. of filtrate are taken. To the filtrate are added 3 ml. 8% (v/v) ammonia and 5 ml. buffer. This is mixed, 2 ml. dye solution (0.10% in absolute methanol) are added and mixed again. In parallel is taken a series containing 0, 4, 8, and 16 μ g. Mg, to which are added 0.4 ml. saturated ammonium oxalate, 10 ml. 10% (w/v) trichloroacetic acid and the volume is made up to 20 ml. with distilled water. This is filtered, 10 ml. filtrate are taken and treated in the same way as the serum filtrate. The densities are read at 520 m μ . within 5 min. from addition of the dye, the results for control and standards are plotted and unknowns read from the graph.

Table 1. Recovery of magnesium after adding to demineralized serum

	Mg added (μ g./ml.)	Mg estimated (μ g./ml.)	% recovery
Expt. 1			
Sample A	0	0	—
B	0	0	—
C	0.2	0.19	95
D	0.2	0.19	95
Expt. 2			
Sample A	0	0	—
B	0	0	—
C	0.4	0.40	100
D	0.4	0.39	97.5

Control tests. Adequate separation of calcium and magnesium by oxalate was to be expected as the solubilities of the two oxalates are respectively 0.00068 g./100 ml. at 25° (Richards, McCaffrey & Bisbee, 1901) and 0.036 g./100 ml. (Kolthoff & Sandell, 1950). The amount of calcium in the supernatant in the presence of the excess oxalate is 0.000142 mg./100 ml. calculated from the solubility product of calcium oxalate. The absence of magnesium from the precipitate was checked first by dissolving it and testing with Titan Yellow. No magnesium was detected. Secondly, experiments on the recovery of magnesium added to magnesium- and calcium-free serum, demineralized by passing through a column of Biodeminrolit (The Permutit Co. Ltd.), showed not more than 5% loss. The results of these experiments are shown in Table 1.

To examine for loss, if any, in the protein precipitate, precipitation was compared with digestion as a means of removing protein using a sample of pooled serum. The digestion was carried out on 2 ml. of the supernatant from the calcium precipitation, adding 0.4 ml. conc. sulphuric acid A.R., 1 ml. 60% perchloric acid and 1 ml. conc. nitric acid A.R. This mixture was heated on a microburner until clear and then concentrated to 1 ml. The sample was next diluted with 5 ml. distilled water, 4 ml. conc. ammonia A.R. were added, and the volume was made up to 20 ml. with distilled water. From the diluted digest 10 ml. were treated as 10 ml. of filtrate as in the method above. The

results are given in Table 2 and show no significant difference between the estimations using digestion or protein precipitation.

Recoveries were performed in duplicate on different specimens of serum and the results are given in Table 3. The first six were measured using 2 ml. of the supernatant and in the solutions read the sensitivity of measurement is $\pm 0.005 \mu\text{g./ml.}$ over the range 0.1–1.0 $\mu\text{g./ml.}$ magnesium. The second six were measured using 1 ml. of the supernatant to keep within the calibration range and the sensitivity of reading is $\pm 0.01 \mu\text{g./ml.}$ over the same range. Table 3 shows the accuracy of the method to be within $\pm 5\%$ for serum magnesium, which is sufficient for most clinical work. The accuracy could, however, be increased by working within a smaller range to allow reduction of dye

strength and therefore reducing the extinction due to the excess dye.

Normal values for serum magnesium

A short series of values for adults under 30 years was determined on venous blood collected not less than 2 hr. after a meal. The values in mg./100 ml. serum were 2.05, 2.50, 2.40, 2.20, 1.85, 1.90, 1.95, 1.90, 2.10, 2.20, 2.20, 1.75, 1.90, 1.85, 2.35, 2.00, 2.10, 2.35, 2.20, 2.05, 2.35, 2.30, 2.05, 1.90, the range being 1.75 to 2.50, the mean value 2.1, and standard deviation ± 0.20 .

DISCUSSION

Previous methods using the precipitation of magnesium ammonium phosphate have not proved entirely satisfactory, and the methods using Titan Yellow have given variable results probably due to the nature of the complex formed with the colloidal dispersing agent. The method described uses a soluble coloured complex for the estimation of magnesium and the normal range determined falls within the normal values determined by other workers. A comparison of their values is given in Table 4 and where possible the number of normal subjects tested is given together with the mean value.

Table 2. *Comparison of digestion with precipitation for protein removal*

Magnesium determined by measuring extinction E at 520 $m\mu$.

Precipitated		Digested	
$E_{1 \text{ cm.}}$	Mg (mg./100 ml.)	$E_{1 \text{ cm.}}$	Mg (mg./100 ml.)
1.735	1.95	1.735	1.95
1.740	2.00	1.735	1.95
1.740	2.00	1.740	2.00
1.735	1.95	1.740	2.00
1.740	2.00	1.735	1.95
1.735	1.95	1.740	2.00

Table 3. *Recovery of magnesium from serum*

Serum	Added Mg (mg./100 ml. serum)	Mg found (duplicate estimations) (mg./100 ml. serum)		Mg recovery average (mg./100 ml.)	% recovery
<i>a</i>	0.0	2.20	2.20	1.97	98
	2.0	4.20	4.15		
<i>b</i>	0.0	1.90	1.90	2.00	100
	2.0	3.90	3.90		
<i>c</i>	0.0	1.80	1.80	2.05	102
	2.0	3.85	3.85		
<i>d</i>	0.0	2.25	2.25	2.00	100
	2.0	4.25	4.25		
<i>e</i>	0.0	2.20	2.20	1.95	97
	2.0	4.15	4.15		
<i>f</i>	0.0	2.10	2.10	1.90	95
	2.0	4.00	4.00		
<i>g</i>	0.0	2.05	2.05	4.00	100
	4.0	6.10	6.00		
<i>h</i>	0.0	2.45	2.45	3.90	97
	4.0	6.40	6.30		
<i>i</i>	0.0	2.30	2.30	4.00	100
	4.0	6.30	6.30		
<i>j</i>	0.0	1.85	1.85	3.95	99
	4.0	5.80	5.80		
<i>k</i>	0.0	1.80	1.80	4.07	102
	4.0	5.85	5.90		
<i>l</i>	0.0	2.45	2.45	3.80	95
	4.0	6.25	6.25		

Table 4. *Magnesium content of serum*The magnesium was precipitated as MgNH_4PO_4 except where indicated.

Reference	Colour reagents	Normal serum range	No. of normals	Normal mean (mg./100 ml.)
Marriott & Howland (1917)	$\text{Fe}(\text{CNS})_3$	2.2-3.5	—	—
Kramer & Tisdall (1921)	$\text{Fe}(\text{CNS})_3$	1.8-2.3	7	—
Denis (1920)	Strychnine molybdate	1.6-3.5	—	—
Denis (1922)	Bell & Doisy (1920)	2.0-2.2	8	2.1
Briggs (1922 <i>a, b</i>)		2.23-2.5	5	2.3
Watchorn (1925)		2.2-2.6	10	—
Watchorn (1926)		2.24-2.58	200	—
Watchorn & McCance (1931)		2.6-2.88	3	2.33
Watchorn & McCance (1932)		2.42-2.59	8	2.48
Hald (1933)		1.7-2.7	6	2.1
Hald & Eisenman (1937)		1.44-2.5	12	—
Snyder & Katzenelbogen (1942)		1.3-2.3	—	—
Bissell (1945)		1.88-2.65	18	2.25
Copeland & Sunderman (1952)	Fiske & Subbarow (1925)	1.75-2.37	17	—
Soffer, Cohn, Grossman, Jacobs & Sobotka (1941)	Molybdate + SnCl_2	2.1-2.7	14	2.5
Cope & Wolf (1942)	Molybdate + SnCl_2	1.73-2.42	17	2.01
Lavietto & Dine (1942)	Benedict & Theis (1924)	1.77-2.22	—	1.88
Simonsen <i>et al.</i> (1947)	Molybdivanadate	1.7-2.3	—	—
Stutzman & Amatzio (1953)	Molybdivanadate	2.04-2.33	—	—
Flink <i>et al.</i> (1954)	Molybdivanadate	2.05-2.53	—	—
Kunkel <i>et al.</i> (1947)	Titan Yellow + hydroxylamine*	2.02-2.22	6	2.14
Orange & Rhein (1951)	Titan Yellow + NaOH + polyvinyl chloride*	1.90-2.5	—	—
Greenberg & Mackey (1932)	Titration†	2.2	—	—
Sobel & Hanok (1951)	Titration by ethylenediamine-tetraacetate with Eriochrome Black T as indicator*	1.58-2.27	—	—

* No Mg precipitation.

† Mg precipitated as Mg 8-hydroxyquinolate.

SUMMARY

1. A colorimetric method is given for determining magnesium in serum with an accuracy of $\pm 5\%$.

2. The method can be combined with the determination of calcium in a single sample of serum.

3. A short series of serum magnesium levels in young adults is given, together with a series of normal levels determined by other authors.

The author wishes to thank Dr F. K. Herbert for her assistance in the writing of this paper.

REFERENCES

- Bacon, A. (1951). *Metallurgia, Manchr.* **44**, 207.
- Baldwin, E. (1947). *Dynamic Aspects of Biochemistry*, 1st ed. p. 359. London: Cambridge University Press.
- Bell, R. D. & Doisy, E. A. (1920). *J. biol. Chem.* **44**, 55.
- Benedict, S. R. & Theis, R. C. (1924). *J. biol. Chem.* **61**, 63.
- Bissell, G. W. (1945). *Amer. J. med. Sci.* **210**, 195.
- Briggs, A. P. (1922*a*). *J. biol. Chem.* **52**, 349.
- Briggs, A. P. (1922*b*). *J. biol. Chem.* **53**, 13.
- Briggs, A. P. (1924). *J. biol. Chem.* **59**, 225.
- Buckley, E. S., Gibson, J. G. & Bartolotti, T. R. (1951). *J. Lab. clin. Med.* **38**, 751.
- Cope, C. C. & Wolf, B. (1942). *Biochem. J.* **36**, 413.
- Copeland, B. E. & Sunderman, F. W. (1952). *J. biol. Chem.* **197**, 331.
- Denis, W. (1920). *J. biol. Chem.* **41**, 363.
- Denis, W. (1922). *J. biol. Chem.* **52**, 411.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Flink, E. B., Stutzman, F. L., Anderson, A. R., Konig, T. & Frazer, R. (1954). *J. Lab. clin. Med.* **43**, 169.
- Garner, R. J. (1946). *Biochem. J.* **40**, 828.
- Greenberg, D. M. & Mackey, M. A. (1932). *J. biol. Chem.* **96**, 419.
- Greenberg, D. M. & Tufts, E. V. (1938). *Amer. J. Physiol.* **121**, 416.
- Hald, P. M. (1933). *J. biol. Chem.* **103**, 471.
- Hald, P. M. & Eisenman, A. J. (1937). *J. biol. Chem.* **118**, 275.
- Hammett, F. S. & Adams, E. T. (1922). *J. biol. Chem.* **52**, 211.
- Harvey, A. E., Komarmy, J. M. & Wyatt, G. M. (1953). *Analyt. Chem.* **25**, 498.
- Hirschfelder, A. D. & Serles, E. R. (1934). *J. biol. Chem.* **104**, 635.
- Kolthoff, I. M. & Sandell, E. B. (1950). *Textbook of Quantitative Inorganic Analysis*, p. 356 (1st English Ed.) London: Macmillan and Co.
- Kramer, B. & Tisdall, F. F. (1921). *J. biol. Chem.* **47**, 475.
- Kruse, H. D., Orent, E. R. & McCollum, E. V. (1932). *J. biol. Chem.* **96**, 519.
- Kunkel, H. O., Pearson, P. B. & Schweigert, B. S. (1947). *J. Lab. clin. Med.* **32**, 1027.
- Lavietto, P. H. & Dine, R. (1942). *J. clin. Invest.* **21**, 781.
- McCance, R. A. & Watchorn, E. (1932). *Brain*, **55**, 91.
- McCrudden, F. H. (1909). *J. biol. Chem.* **7**, 83.

- Marriott, W. M. & Howland, J. (1917). *J. biol. Chem.* **32**, 233.
- Orange, M. & Rhein, H. C. (1951). *J. biol. Chem.* **189**, 379.
- Richards, T. W., McCaffrey, C. F. & Bisbee, H. (1901). *Proc. Amer. Acad. Arts Sci.* **36**, 375.
- Simonson, D. G., Westover, L. M. & Wertman, M. (1947). *J. biol. Chem.* **169**, 39.
- Smith, S. G. (1949). *Arch. Biochem.* **20**, 473.
- Snyder, R. & Katzenelbogen, S. (1942). *J. biol. Chem.* **143**, 223.

- Sobel, A. E. & Hanok, A. (1951). *Proc. Soc. exp. Biol., N.Y.*, **77**, 737.
- Soffer, L. J., Cohn, C., Grossman, E. B., Jacobs, M. & Sobotka, H. (1941). *J. clin. Invest.* **20**, 429.
- Stutzman, F. L. & Amatzio, D. S. (1953). *J. Lab. clin. Med.* **41**, 215.
- Watchorn, E. (1925). *Quart. J. Med.* **18**, 288.
- Watchorn, E. (1926). *Brit. J. exp. Path.* **7**, 120.
- Watchorn, E. & McCance, R. A. (1931). *Quart. J. Med.* **24**, 371.
- Watchorn, E. & McCance, R. A. (1932). *Biochem. J.* **26**, 54.

A Simple Technique for Counting Milligram Samples of Protein Labelled with ^{14}C or ^{35}S

BY J. GARROW AND E. A. PIPER

*Medical Research Council, Tropical Metabolic Research Unit, West Indies and
National Institute for Medical Research, Mill Hill, London, N.W. 7*

(Received 17 November 1954)

Radioactive sulphur and carbon have been extensively used in the investigation of protein metabolism. In the study of protein-malnourished children in Jamaica, one of us (J.G.) wished to use ^{35}S as a tracer element and for this purpose a counting apparatus was needed which would fulfil two requirements: (1) It must be capable of measuring ^{35}S activity of the order of 10^{-11}C in very small samples of tissue since, for example, a needle biopsy of liver yields about 5 mg. of tissue of which about 1 mg. is protein. (2) It should be as simple as possible to operate and maintain.

By a combination of a gas-flow counter, a thin sample film technique and a compact scaler—E.H.T.—timer unit, both requirements have been reasonably well satisfied. No part of the technique is essentially original; the merits of a gas-flow counter are well discussed by Tait & Haggis (1949), a thin film technique has been described by Fager (1947), and the scaler unit is based on an A.E.R.E., Harwell, design and was made at the National Institute for Medical Research. A similar scaler, type 1221b, with separate E.H.T. unit is now manufactured by Ericsson Telephones Ltd., Beeston, Nottingham.

The counting gas is helium, which is bubbled through absolute ethanol over sodium sulphate, and then again through ethanol at 0° . The gas passes slowly through the counter at a pressure of about 4 cm. on an alcohol manometer, and then out through a mercury trap. The pulses from the anode of the counter are passed to a probe unit (A.E.R.E., Harwell, type 1014a) which feeds the scaler.

The shape of the counter is shown in Fig. 1. It is made of brass, nickel-plated, and is cylindrical with

a concentric anode wire, a construction which provides a uniform field and a small plateau slope. The cathode diameter is chosen to provide a convenient working voltage, and the anode wire length is just sufficient to give the maximum counting rate from the sample. The anode wire is made of tungsten and is supported at each end, so

Fig. 1. Longitudinal section through gas flow counter.
Scale 1:4.

that a very thin wire can be used and a low starting voltage and long plateau are obtained. The sample is introduced at the side and is arranged within the sensitive volume of the counter. Although sample changing is easier with the end-window Geiger-Müller counter, that type of counter has a lower counting efficiency. In the present counter, large areas of insulating material and polythene planchets have been avoided, as they may give rise to static effects which distort the field.