mined by our electrical method present some interesting problems; possibly these cases are due to over-hydration, and further studies correlating our results with the clinical state of the patient might be informative.

Time is of great importance in a busy biochemistry department, where there is an increasing demand for electrolyte estimations, particularly in post-operative cases. A constant temperature of $37^{\circ}\text{C.} \pm 0.5$ is essential during the estimation, as the results are affected by temperature variations. This method is so simple that, given suitable apparatus, the whole estimation can be completed within a few minutes. Only those bloods with a total electrolyte figure outside the normal range require further analysis.

Summary

An apparatus for the determination of the electro-conductivity of plasma is described. The investigation of 118 samples of plasma confirmed that the normal range for plasma electrolyte values is 290–310 mEq./l. Thirty-seven cases which showed an abnormal distribution of electrolytes fell either above or below the normal range.

It is suggested that this method could be used as a screening test to eliminate a large percentage of cases with a normal electrolyte balance, thus relieving the biochemical department of a considerable portion of routine normal investigations.

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While the results in this paper apparently show that cases with an abnormal electrolyte distribution usually have a total electrolyte value outside the normal range, further work is required before a normal result by conductimetric methods can be definitely accepted as indicating a normal sodium and potassium distribution in cases where any abnormality might otherwise be anticipated.—ED.

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The Estimation of Peptidase Activity in the White Blood Cells by Paper Chromatography

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Following our observations on the amino-acid content of white blood cells in human leukaemias (Nour-Eldin and Wilkinson, 1955), it appeared of interest to examine the content of enzymes related to amino-acid metabolism in these cells. Quantitative paper chromatography (Fowden, 1951) was proposed as a method for estimating the amino-acid released by hydrolysis of the substrate when testing peptidase activity.

The occurrence of proteolytic enzymes in the cells of purulent exudates was first demonstrated by Müller (1888). Longcope and Donhauser (1908) reported that the blood of patients with marked leucocytosis seemed to contain enzymes capable of digesting coagulated blood serum. That the white blood cells contained peptidases was demonstrated by Husfeldt (1931) and by Oelkers (1931). Stern, Birmingham, Cullen, and Richer (1951) showed that the peptidase activity of packed white blood cells was nearly five and 500 times that of red blood cells and plasma, respectively, and was higher in senile people than in young individuals.

In the present paper the peptidase activity in the white blood cells obtained from normal individuals and cases of leukaemia and Hodgkin's disease is determined, using paper chromatography for the estimation of the amino-acids arising from the hydrolysis of the substrates.

Experimental

Leucocytes.—The white blood cells were separated by the method of Minor and Burnett (1948).

Substrates.—The substrates used were glycylglycine (G.G.), glycylglycylglycine (G.G.G.), and leucylglycylglycine (L.G.G.).

Extraction of Peptidases.—The white blood cells were extracted by such a quantity of 30% glycerol that each 0.1 ml. of the final extract was derived from two million cells. The extraction was carried out for four hours at room temperature.

Reaction Mixture.—This was prepared in well-stoppered 8 mm. tubes. It comprised 1 ml. 0.1 M substrate, 0.2 ml. 0.01 M cobalt sulphate, 0.2 ml. glycerol extract of white blood cells, 0.3 ml. distilled water, and 0.3 ml.

0.07 M phosphate buffer pH 7.8. This quantity of buffer was found to bring the pH of the mixture to 7.2, and its buffering effect, plus that of the substrate, maintained the pH within less than 0.1 unit. The mixture was shaken and incubated at 40° C. for two hours in case of L.G.G. and G.G.G. and four hours in case of G.G. After this period 2 ml. of 0.5% hydrochloric acid was added to the mixture to inhibit the reaction. A control was run at the same time using an equal volume of 30% glycerol instead of the white blood cell extract. For paper chromatography 0.04 ml. of the final mixture was used.

Paper Chromatography.—The method used for quantitative estimation of the amino-acids was essentially that described by Fowden (1951). Table I shows the R_F

TABLE I
 R_F VALUE OF PEPTIDES AND AMINO-ACIDS

	Phenol/ Ammonia	Butanol-Acetic Acid
Glycylglycylglycine (G.G.G.) ..	0.73-0.78	0.18-0.19
Glycylglycine (G.G.) ..	0.59-0.66	0.16-0.18
Leucylglycylglycine (L.G.G.) ..	0.87-0.89	0.48-0.50
Glycine ..	0.40-0.42	0.20-0.21
Leucine ..	0.83-0.85	0.60-0.62

values observed for the peptides and the compounds resulting from their hydrolysis. It is apparent that glycine could be separated from G.G. and G.G.G. by a one-dimensional chromatogram run in phenol (Consden, Gordon, and Martin, 1944), while leucine could be separated from L.G.G. and G.G. by running the mixture in butanol acetic acid (Partridge, 1948). In the case of the substrate G.G.G., it was realized that by estimating glycine the actual measurement was the combined effect of the tripeptidase, plus the dipeptidase activity on the dipeptide (G.G.) in the first reaction. A direct approach appeared preferable, namely, to measure the amount of the tripeptide remaining after the completion of the reaction and subtracting it from the original quantity to give the actual amount of substrate broken down by the tripeptidase. However, irregular results were obtained on heating for 25 minutes. Although more consistent results were obtained after 35 minutes' heating, there was a loss of 20 to 30% apparently due to tailing. Consequently, this attempt had to be abandoned and glycine only was measured, ignoring the side reaction, which was presumed to be negligible, as the dipeptide would be in a low concentration. In the case of L.G.G. this question did not arise, as the glycine formed would be separated from leucine on the chromatogram. The control was run at the same time as the test mixture, and the area of paper in the appropriate position for glycine was cut out and used as a blank.

Calculations.—All calculations were based on the assumption that complete splitting of one peptide linkage is 100%. The results were expressed as percentage hydrolysis per million white blood cells per ml. in one hour in 0.05 M substrate solution.

TABLE II
PEPTIDASE ACTIVITY IN THE WHITE BLOOD CELLS

	Day	W.B.C. (10 ⁹ / c.mm.)	% Hydrolysis per Hour— per 1 m. W.B.C. per. ml.			Remarks
			(G.G.G.)	(L.G.G.)	(G.G.)	
<i>Normal</i>						
F.N.		9.1	7.0	6.4	3.0	
F.N.		7.6	6.4	6.6	3.5	
F.N.			4.6	5.4	3.8	
J.K.			6.6	7.0	3.4	
S.C.			7.1	5.4	3.3	
D.T.			5.4	6.0	3.0	
K.H.		8.4	5.9	5.6	2.7	
K.H.		11.1	6.0	5.1	2.0	
Average			6.1	6.0	3.1	
S.D.			0.7	0.7	0.6	
<i>Patient</i>						
E.R.	7	6.9	6.4	6.0	3.0	6. mg. nitro- gen mustard on 8th, 13th, and 16th days
Hodgkin's	8	7.9	7.0	6.1	3.4	
disease	9	10.2	5.8	5.0	4.0	
	14	7.2	4.9	6.0	4.5	
	18	6.9	5.4	5.0	3.5	
Average			5.9	5.6	4.1	
S.D.			0.8	0.6	0.8	
G.G.	4	75.2	9.0	8.2	4.5	5 mg. tri- ethylene melamine on 6th day; 1 mg. on 8th, 9th, and 10th days
Chronic	5	70.4	8.0	6.0	3.6	
lymphatic	6	72.4	6.2	7.0	4.2	
leukaemia	8	47.2	8.2	8.0	4.2	
	11	20.7	7.3	7.3	2.5	
	12	10.2	8.4	4.8	4.0	
Average			7.8	6.9	3.8	
S.D.			0.9	1.3	0.7	
N.L.	1	30.0	12.7	12.5	5.7	A.C.T.H., 25 mg. on 7th and 150 mg. on 8th day
Acute	4	29.5	14.2	14.0	5.0	
leukaemia	6	59.2	12.5	10.3	6.2	
	7	68.7	11.1	12.1	7.0	
	9	92.4	14.1	13.4	6.0	
	10	59.4	12.7	10.9	5.0	
Average			12.9	12.2	5.8	
S.D.			1.2	1.2	1.1	
M.G.	1	733.0	10.0	10.0	5.4	5 mg. tri- ethylene melamine on 6th and 7th days
Chronic	2	695.0	10.3	10.0	4.5	
myeloid	3	605.5	11.0	10.7	5.0	
leukaemia	5	565.0	12.1	11.2	4.0	
	19	200.5	9.9	12.1	3.9	
	20	131.0	10.1	8.5	5.4	
	21	110.5	8.7	8.1	3.4	
	22	86.0	11.1	10.7	5.5	
	26	87.0	10.9	11.2	3.8	
M.M.	1	225.0	11.1	10.8	5.1	25 mg. "my- leran" daily starting on 10th day for six days
Chronic	3	253.0	9.2	9.0	5.4	
myeloid	5	207.0	8.9	9.1	4.2	
leukaemia	6	221.0	9.1	9.0	6.1	
	7	208.0	10.7	10.0	3.9	
	10	204.0	12.3	11.1	4.5	
	13	221.0	9.7	9.1	3.7	
	14	244.0	7.9	8.3	4.7	
	18	198.0	11.4	10.0	5.1	
	19	165.0	12.1	10.1	4.5	
	20	131.0	10.0	12.0	5.9	
	21	101.0	13.0	10.9	3.6	
	25	29.5	11.0	8.6	3.9	
N.T.	1	110.0	9.3	9.0	3.6	25 mg. "my- leran" daily for 6 days starting on 4th day
Chronic	2	120.0	9.7	8.7	3.7	
myeloid	8	96.0	8.9	9.3	5.1	
leukaemia	11	100.0	11.3	10.0	4.5	
	15	84.0	7.9	8.5	5.4	
	18	33.0	12.5	10.5	3.4	
Average			10.4	9.9	4.9	
S.D.			1.4	1.2	0.9	

Results

The di- and tri-peptidase activity in white blood cells showed little variation from day to day (Table II). This variation bore no relation to the changes in the number of white blood cells per c.mm. observed in cases of leukaemia. However, the activity of the enzymes differed in the diverse types of white blood cells examined. White blood cells from cases of acute and chronic myeloid leukaemias gave higher peptidase activity than those obtained from normal subjects and cases of Hodgkin's disease and chronic lymphatic leukaemia.

Discussion

The amino-acids glycine and leucine split from glycylglycine and glycylglycylglycine and leucylglycylglycine by the action of peptidases in the white blood cells were quantitatively estimated after being separated from the other compounds in the incubation mixture by one-dimensional paper chromatography. This method could be utilized to determine the activity of other peptidases if the resulting amino-acid could be separated from the peptides in the mixture by paper chromatography.

The activity of peptidases, acting on the above-mentioned peptides, in the white blood cells from cases of acute leukaemia and chronic myeloid leukaemia was found to be higher than that in white blood cells in cases of chronic lymphatic leukaemia, Hodgkin's disease, and normal individuals. However, the intracellular enzyme activity seemed to remain practically unchanged throughout, irrespective of the number of white blood cells or of their amino-acid content, which was found by Nour-Eldin and Wilkinson (1955) to show daily variations. This suggests that changes in the amino-acid content and distribution in the white blood cells do not derive from inhibition of enzymes of this sort.

Summary

Peptidases in white blood cells were more active in cells from acute and chronic myeloid leukaemia than in normal and chronic lymphatic leukaemia cells. The activity of the peptidases was measured by utilizing quantitative paper chromatography for determining the amino-acids resulting from hydrolysis of the peptides.

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A Modification of Sula's Method for the Cultivation of Tubercle Bacilli from Pleural Fluid

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While primary pleural effusion in young adults is accepted in the majority of cases as being of tuberculous aetiology, routine examination of the fluid by the usual methods of culture and animal inoculation gives a surprisingly low percentage of positive results. A method of examination in common use is to take approximately 20 ml. of fluid into a universal container with 0.3 ml. of 20% sodium citrate and to inoculate the centrifuged deposit into a guinea-pig and on to slopes of Löwenstein-Jensen medium. As it is becoming increasingly important to isolate the tubercle bacilli in order to carry out sensitivity tests to the various antibiotics and chemotherapeutic agents, an improved method was sought.

Close (1946) and Eberle (1949) described methods which gave a high proportion of positive results, but these techniques are time consuming, involve considerable handling of the specimen, and therefore are not suitable for routine use. Sula (1947) described a method whereby up to 300 ml. of exudate was taken into sterile flasks and allowed to clot; the fluid was then withdrawn and replaced with an equal quantity of medium. After incubation at 37° C. for varying periods (10 days to two months) colonies of tubercle bacilli could be seen growing in the clot in positive cases. In the series he reported, 23 of 47 cultures showed growth of *M. tuberculosis*. This method has been used and proved to be successful (Fig. 1), but it was found that a clot did not appear in every specimen of fluid examined, and also that contamination sometimes arose as a result of the handling. This paper describes a routine with Sula's medium which we have used in the cultivation of fluids from 121 cases of pleural effusion in parallel with culture on Löwenstein-Jensen medium and guinea-pig inoculation of the centrifuged deposit of 20 ml. of citrated fluid.

Medium

The medium used is that described by Sula (1947).

Na ₂ HPO ₄	..	..	..	2.5 g.
KH ₂ PO ₄	..	..	..	1.5 g.
Sod. cit. (neutr.)	..	..	..	1.5 g.
MgSO ₄	..	..	..	0.5 g.
Asparagin	..	..	..	2.0 g.
Alanin	..	..	..	0.15 g.
Glycerin	..	..	..	25 ml.
Ferriammonium cit.	..	..	..	0.05 g.
Malachite green	..	..	..	1 ml. (0.2% aqueous sol.)
Aq. dest.	..	..	..	1,000 ml.