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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REFERENCES

- Consden, R., Gordon, A. H., and Martin, A. J. P. (1944). *Biochem. J.*, **38**, 224.
 Fowden, L. (1951). *Ibid.*, **48**, 327.
 Husfeldt, E. (1931). *Z. physiol. Chem.*, **194**, 137.
 Longcope, W. T., and Donhauser, J. L. (1908). *J. exp. Med.*, **10**, 618.
 Minor, A. H., and Burnett, L. (1948). *Blood*, **3**, 799.
 Müller, F. (1888). Unpublished data cited by Opie, E. L. (1922). *Physiol. Rev.*, **2**, 552.
 Nour-Eldin, F., and Wilkinson, J. F. (1955). *Brit. J. Haemat.*, **1**, 358.
 Oelkers, H. A. (1931). *Arch. exp. Path. Pharmacol.*, **161**, 344.
 Partridge, S. M. (1948). *Biochem. J.*, **42**, 238.
 Stern, K., Birmingham, M. K., Cullen, A., and Richer, R. (1951). *J. clin. Invest.*, **30**, 84.

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.

FIG. 1.—Photograph of pleural fluid clot from Sula's medium after 30 days' incubation showing colonies of tubercle bacilli. ($\times 4$.)

The medium is sterilized in a steamer, and when cool 10% sterile ascitic fluid added.

In the method described here this medium was made in double strength (with the exception of the ascitic fluid), and distributed in 100 ml. amounts in 10 oz. bottles with previously marked levels indicating 100 and 200 ml. Pleural fluid was withdrawn and at once placed as follows :

- (1) 100 ml. into 100 ml. of double strength Sula's medium.
- (2) 20 ml. into a universal container with 0.3 ml. of 20% citrate.

In the laboratory penicillin was added to (1) to a final concentration of 10 units per ml., and the bottle incubated at 37° C. The second specimen (2) was centrifuged and the deposit inoculated on Löwenstein-Jensen medium and into a guinea-pig in the usual way.

In Sula's medium growth has been obtained in as short a time as 10 days and after as long as two months (average 20 days). Growth usually appears as a granular

deposit in the foot of the bottle, or, if a clot has formed, as colonies in the clot. Confirmatory Ziehl-Neelsen-stained films are made.

Results

Specimens examined	121	
Positive	86	(71%)
Distributed as follows :		
Fluid culture	16	(13.2%)
Guinea-pig inoculation	16	(13.2%)
Löwenstein-Jensen cultures	16	(13.2%)
Fluid culture	26	(21.4%)
Guinea-pig inoculation	26	(21.4%)
Fluid culture	86	(71%)

Where culture alone was positive confirmation of virulence was obtained by further guinea-pig inoculation.

It will be seen from the above results that the fluid culture method is greatly superior to Löwenstein-Jensen culture and guinea-pig inoculation; also in no case was it negative when either of the two latter methods was positive. The final clinical diagnoses of the cases from which the 35 negative specimens were taken were as follows :

Tuberculosis	9
Bronchial carcinoma	7
Lobar pneumonia with effusion	9
Empyema of gall bladder with pleural effusion	1
Pulmonary embolus with pleural effusion	1
Coronary thrombosis with pleural effusion	1
Uræmia, chronic pyelonephritis	1
Not yet ascertained	6

This method can also be used in the examination of other body fluids and has been used with success in the culture of tubercle bacilli from cerebrospinal fluids. (In this case 5–10 ml. of fluid is added to 10 ml. of double strength medium in a universal container.)

Summary

An improved method for the cultivation of tubercle bacilli from pleural fluid using Sula's medium is described. Results of the examination of 121 specimens of fluid are given. Comparison is made between this method, guinea-pig inoculation, and culture on Löwenstein-Jensen media. This method, by allowing handling of larger quantities of fluid, gives greatly superior results, and has proved practicable for routine use.

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REFERENCES

- Close, H. G. (1946). *Lancet*, **1**, 193.
 Eberle, A. J. F. (1949). *Tubercle (Lond.)*, **30**, 146.
 Sula, L. (1947). *Schweiz. Z. allg. path. Bakt.*, **10**, 125.