When the D.N.P.H. reaction is allowed to proceed for 5 minutes at 20° C. the Δ^4-3-ketone group reacts quantitatively with relatively little interference from other ketone groups. Heating for 90 minutes at 59° C. causes, in addition, the complete reaction of C-20 ketones in a dihydroxy-acetone side chain. Since, according to a recent hypothesis [Dorfman, 1954], the major metabolic pathway of steroids involves reduction of the Δ^4-3-ketone group, simultaneous determinations of the reaction at room temperature for five minutes and after heating at 52° C. for 120 minutes, may assist in differentiating biologically active corticosteroids (e.g., compounds E and F) which contain the Δ^4-3-ketone group from their inactive metabolites (Tetrahydro-E and F). This important application of the Gornall-MacDonald reaction is being investigated in normal subjects and in diseased states.

We acknowledge with thanks the statistical analysis of our results by Dr. A. Adelstein.

REFERENCES

DORFMAN, R. I. (1954). *Recent Progress in Hormone Research*, **9**, 5.
GORNALL, A. G. and MACDONALD, M. P. (1953). *J. biol. Chem.*, **201**, 279.
LURIE, D., ALLDIS, D. and BLOOMBERG, B. M. (1954). *S. Afr. J. med. Sci.*, **19**, 121
PORTER, C. C. and SILBER, R. H. (1950). *J. biol. Chem.*, **185**, 201.

PROTEIN IN FISH SCALE, *by C. Solomons* (*Joint Dental Research Unit of the Council for Scientific and Industrial Research and the University of the Witwatersrand, Johannesburg: and the National Chemical Research Laboratory, Pretoria*).

This communication describes two procedures that are being used to investigate the proteins in pilchard scale.

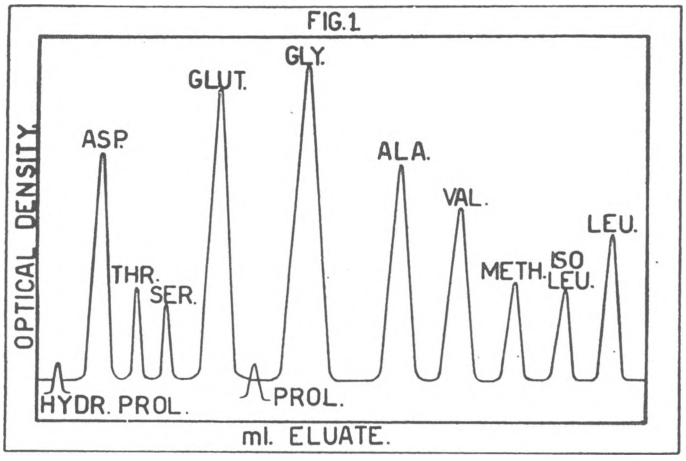
Preparation of material. The scales are washed with water, dried, and extracted with hexane. Demineralization is effected by soaking the scales in trichloracetic acid at 5° C. The protein material is then separated into two fractions by heating the demineralized scales in water. The gelatin fraction dissolves, leaving insoluble ichthylepidin. The major components of the oven dried scales are mineral matter, 56 per cent; gelatin, 23 per cent; and ichthylepidin, 21 per cent.

1. *Estimation of terminal amino groups.* It is possible to determine the sequence of amino acid residues, molecular weight, and degree of homogeneity of proteins by using the quantitative reaction between fluoro-dinitro-benzene and terminal amino groups [Sanger, 1945]. This technique was successfully applied to the study of the insoluble protein ichthylepidin. The results obtained are shown in Table I.

TABLE I

	Approx. Molecular Weight	N—Terminal Amino Acids
Scales	1,500,000	All yielded glycine, alanine, valine, glutamic acid, aspartic acid, serine and threonine.
Demineralized scale ..	1,200,000	
Ichthylepidin	200,000	

2. *Determination of amino acid composition.* Mixtures of amino acids in protein hydrolysates can be separated quantitatively by elution with buffers from a chromato-



graphic column of sulphonated polystyrene resin (Dowex 50). One ml. fractions of the eluate were analysed by colorimetric reaction with ninhydrin (Fig. 1). The gelatin fraction of fish scale was hydrolysed, and several amino acids determined [Moore and Stein, 1951]:—

TABLE II

Some amino acids in gelatin	Per cent
Hydroxyproline	8·4
Aspartic acid	7·7
Threonine	1·6
Serine	1·4
Glutamic acid	10·5
Proline	12·9
Glycine	25·8
Alanine	12·6
Valine	2·2
Methionine	0·62
Isoleucine	1·3
Leucine	2·3

REFERENCES

MOORE, S. and STEIN, W. (1951). *J. biol. Chem.*, **192**, 663.
SANGER, F. (1945). *Biochem. J.*, **39**, 507.

THE EFFECT OF HEAT STRESS ON MINE WORKERS WITH REGARD TO LOSS OF WATER AND ELECTROLYTES, *by Walter M. Politzer* (*Biochemistry Department, South African Institute for Medical Research*), *Michael E. Barry* (*Van Dyk Mines Native Hospital*) *and Arthur King* (*St. Helena Gold Mines Health Dept.*)

See this Journal, **19**, 155-160, 1954.