These extracts were much lighter coloured than those from the fulvic extracts but the products obtained had properties identical with those of the fulvic constituents previously discussed.

Table 4. *Fractionation of water-soluble fraction of soil organic matter*

Water-soluble organic matter (g./100 g. organic matter)		Water-soluble nitrogen (mg./100 g. organic matter)	
Total water extract	5.98	Total nitrogen	210
Fraction A	3.15		
Fraction B	0.38		
Fraction C	1.06		
Fraction D	1.14		
Nitrogen adsorbed (complex nitrogen)		37.6 %.	
Organic matter adsorbed (complex substances)		47.3 %.	

As might be expected, a much higher proportion of the simpler substances (fraction A) was observed here. These results show that the fulvic constituents are present in the soil as such. Fraction C especially is easily extracted by water.

SUMMARY

1. A considerable advance has been made in the study of the fulvic extract of soil organic matter. A new method has been introduced whereby the fulvic extract is separated by a selective adsorption technique into four distinct fractions.

2. These fractions have been obtained from the fulvic extracts from four widely diverse types of soil.

3. A preliminary examination of the chemical properties of the fractions has been made.

4. A pure polysaccharide containing uronic acid groups has been isolated from the four soils. The presence of a glycosidic fraction in the four soils has also been shown.

5. The fulvic nitrogen has been shown to be in part contained in nitrogenous acidic complexes and in part as simple degradation products.

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A Polarographic Study of Acid-insoluble Fractions from Iodo-proteins

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In the study of iodinated proteins, attempts to correlate the thyroxine iodine and the biological activity of these thyro-active substances have met with varied success. Deanesly & Parkes (1945) express the view that most of the acid-insoluble iodine as obtained by the accepted method (British Pharmacopoeia, 1932) is not true thyroxine iodine and that more evidence is required before the actual relationship between biological activity and thyroxine content can be measured.

More recently, Reineke & Turner (1945) claim to

have obtained close correlation between biological activity and the thyroxine content of iodinated casein. The method adopted was to hydrolyze the iodinated protein in a baryta solution and to extract the hydrolysate with *n*-butanol. The thyroxine content was then calculated from iodine estimations done on the purified *n*-butanol extracts.

The present paper describes some preliminary work on the application of the polarographic wave of thyroxine to certain acid-insoluble fractions obtained from the baryta hydrolysis of iodinated proteins.

METHODS

Samples of iodinated 'Ardein'* (ground nut protein) and iodinated casein were hydrolyzed by baryta. A partially purified acid-insoluble active fraction was obtained by subsequent treatment according to the method of Ludwig & von Mutzenbecher (1939) as indicated in the following diagram.

The Cambridge Polarograph, the technique and the capillary characteristics used were similar to those described in the polarographic determination of thyroxine (Simpson & Traill, 1946). Half-wave potentials were there recorded against a saturated calomel electrode, but for the present analytical method the reference electrode used was the normal quiescent mercury pool anode.

RESULTS

Determination of apparent thyroxine in active fractions

When a small amount of an acid-insoluble active fraction, obtained from an iodo-Ardein, was dissolved in a mixture of 0.5N-Na₂CO₃ and ethanol (3:2), a composite polarographic wave between -1.1 and -1.9 V. was obtained. Addition of tetramethyl-ammonium bromide to the above test solution yielded a more clear-cut reproducible wave similar to that produced by pure thyroxine. When small quantities of pure thyroxine were added to the test solution, the wave height increased by proportionate amounts. In the following, it is assumed that thyroxine is the only substance in the acid-insoluble fraction which produces this characteristic wave structure. A calibration curve was made relating wave height and concentration of added thyroxine in a solution of the active fraction.

(a) *Acid-insoluble fraction from the baryta hydrolysis of iodinated Ardein* (PB 13). Active fraction (PB 13C) (0.52 g.) was dissolved in 52 ml. of a mixture of 0.5N-Na₂CO₃ and ethanol (3:2), i.e. 1 ml. of solution contained 0.01 g. of PB 13C. A solution

was prepared of 0.06 g. of thyroxine sodium B.P. in 20 ml. of the same ethanolic carbonate solution (1 ml. of this solution contained 0.003 g. of thyroxine sodium). Polarographic test solutions were prepared as in Table 1.

It is seen from Table 1 that each 10 ml. portion of test solution contains the same quantity of base

electrolytes. Polarograms were recorded at a galvanometer sensitivity of 1/300, without removal of oxygen, and are reproduced in Fig. 1 a, b.

Table 1. *Polarographic test solutions*

No. of test solution	Solution PB 13C (ml.)	Pure thyroxine sodium solution (ml.)	0.5N-Na ₂ CO ₃ /ethanol solution (3:2) (ml.)	Tetra-methyl-ammonium bromide (g.)
1	2	—	8	0.2
2	2	1	7	0.2
3	2	2	6	0.2
4	2	4	4	0.2
5	2	6	2	0.2
6	2	6.75	1.25	0.2
7	3	—	7	0.2
8	4	—	6	0.2

The relationship between the polarographic wave height and thyroxine sodium content is given in Table 2.

Table 2. *Relation between polarographic wave height and thyroxine content*

No. of test solution	Thyroxine sodium added (g.)	Polarographic wave height	Wave height difference
1	0.000	11.0	0.0
2	0.003	13.0	2.0
3	0.006	15.0	4.0
4	0.012	19.25	8.25
5	0.018	23.0	12.0
6	0.0203	24.5	13.5
7	—	15.75	4.75
8	—	20.25	9.25

* The word 'Ardein' is a registered trademark.

The figures in the last column of Table 2 were obtained by subtracting the wave height for test solution no. 1 from the values for the remaining solutions. The calibration curve relating thyroxine concentration with wave height difference is shown in Fig. 2 (a). From this curve, the quantity of

These are reproduced in Fig. 3 a, b. From the calibration curve, Fig. 2 (b), relating polarographic wave height and concentration of added thyroxine sodium in this case, the thyroxine quantities equivalent to the extra 1 ml. and 2 ml. of PB 25 C solution were found to be 0.0046 and 0.00935 g. respectively.

Fig. 2. (a) Calibration curve relating thyroxine concentration with polarographic wave height for determination on acid-insoluble fraction from iodinated Ardein. (b) Similar curve for iodinated casein fraction.

thyroxine equivalent to the additional 1 ml. and 2 ml. of PB 13 C solution in test solutions nos. 7 and 8 respectively can be determined. Thus (a) 1 ml. of PB 13 C solution is equivalent to 0.00705 g. of thyroxine sodium, and (b) 2 ml. PB 13 C solution are equivalent to 0.0139 g. of thyroxine sodium. The percentage of apparent thyroxine sodium in this active fraction is therefore (a) 70.5; (b) 69.5; average 70.0.

The percentage thyroxine sodium in this acid-insoluble fraction calculated from the iodine content assuming all the iodine present to be thyroxine iodine, was found to be 68.6. The iodine analysis was carried out according to the British Pharmacopoeia (1936 Addendum) method for total iodine determination (Table 3) on thyroxine sodium.

(b) *Acid-insoluble fraction from the baryta hydrolysis of iodinated casein* (PB 25). Active fraction (PB 25 C), 0.2 g. was dissolved in 35 ml. of a mixture of 0.5N- Na_2CO_3 and ethanol (3 : 2) (1 ml. of solution contained 0.00571 g. of PB 25 C). A solution of thyroxine sodium in the same ethanolic carbonate solution containing 0.002 g./ml. was prepared. Polarographic test solutions were prepared in a similar manner to that used above in Table 1, omitting no. 6 test solution.

Polarograms were recorded without removal of oxygen and at a galvanometer sensitivity of 1/150.

The percentage of apparent thyroxine sodium in this active fraction is therefore (a) 80.6; (b) 81.9; average 81.3. The percentage thyroxine sodium content of the active fraction PB 25 C as calculated from the iodine content assuming all the iodine present to be thyroxine iodine was 78.2.

Table 3. *Determinations of thyroxine in acid-insoluble fractions of iodinated protein hydrolysates*

Iodinated protein batch no.	Iodine content of active fraction (%)	Thyroxine sodium content calculated on iodine analysis (%)	Thyroxine sodium, polarographic method (%)
Ardein PB4	48.6	76.4	77.2
Ardein PB5	35.3	55.5	50.9
Ardein PB7	38.8	61.0	62.9
Ardein PB10	37.5	58.9	58.78 56.58 56.15
Casein PB12	49.0	77.0	77.2
Ardein PB13	43.5	68.4	73.10 69.30 70.00
Casein PB25	49.7	78.2	81.30 77.40 79.83

A number of active fractions was treated as in the above examples. Thyroxine values were determined for each by the polarographic and iodine methods and the results are given in Table 3.

Thyroxine content of iodinated protein

The amount of acid-insoluble fraction, which was isolated from 100 g. of iodo-protein by the method of separation outlined above, ranged from 0.7 to 1.2 g. Taking the values for thyroxine contents as given in Table 3 then the amount of thyroxine in the original iodo-protein is about 0.6 %. This value agrees well with the amount of thyroxine (0.4 g.) which has actually been isolated from iodinated casein samples by Reineke & Turner (1943).

Thyroxine determination in thyroid powder

An acid-insoluble fraction was obtained from a quantity of thyroid powder. Preliminary work has shown that it is possible to obtain polarographic thyroxine waves in solutions of the acid-insoluble fraction similar to those used in the iodinated protein work. The apparent thyroxine content of this acid-insoluble fraction as determined polarographically was 8.8 % and the thyroxine content as calculated from the iodine content and assuming all the iodine present to be thyroxine iodine was 9.1 %.

DISCUSSION

It is desired to emphasize that the main object in presenting the above observations is to indicate that clear-cut and measurable polarographic waves can be obtained in solutions of an acid-insoluble fraction from iodinated proteins and thyroid powder. If varying amounts of thyroxine are added to these solutions then a linear relationship exists between thyroxine concentration and wave height. If this

wave is assumed to be due to thyroxine then the thyroxine content of the acid-insoluble fractions agrees with that derived from the iodine estimations, assuming all the iodine present to be thyroxine iodine. The thyroxine content of these iodinated protein samples on this basis is about 0.6 %. This would indicate that the amount of non-thyroxine iodine compounds in these fractions is rather small, since 0.4 g. of thyroxine has been isolated from 100 g. of iodinated casein. Further work could profitably be done on the effect of any non-thyroxine, acid-insoluble iodine compounds on the polarographic wave of thyroxine.

The polarographic method may be of some immediate interest in the case of thyroglobulin, since in this protein thyroxine is the only known compound containing acid-insoluble iodine (Harington & Randall, 1929).

SUMMARY

1. The baryta hydrolysis of an iodinated protein (100 g.) yields an acid-insoluble active fraction (about 1 g.). A thyroxine value for this fraction can be obtained polarographically. This value agrees fairly closely with that calculated from the acid-insoluble iodine value, assuming all the iodine present to be thyroxine iodine, and is about 0.6 % of the original iodinated protein.

2. A similar agreement between the two thyroxine values was obtained with an acid-insoluble fraction obtained from thyroid powder.

3. In the case of fractions from iodinated protein, non-thyroxine iodine compounds may interfere with the polarographic waves obtained and further work on this may be necessary.

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Fig. 1*a*. Fig. 1*b*.

Fig. 1 *a, b*. Polarograms of acid-insoluble fraction from iodinated Ardein, without removal of oxygen from test solution. See data in Table 2.

Fig. 3 *a*. Fig. 3 *b*.

Fig. 3 *a, b*. Polarograms of acid-insoluble fraction from iodinated casein without removal of oxygen from test solution. Calibration curve relating thyroxine sodium added, to polarographic wave height is shown in Fig. 2 (*b*).

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