found in the secretions of man, horse, rabbit, cat, guinea pig and rat were very small.

2. With male goats, cattle, sheep and dogs, appreciable quantities were found, mainly in the lipid fraction of the hair, i.e. the ether extract from which the free acids had been removed. The acids (including some C_2 and C_3 acids), were therefore present in this fraction in combined form. The aggregates of volatile fatty acids C_2–C_{10} in the lipid fractions amounted for the four species of animals mentioned respectively to about 3, 3, 1·5 and 5 %.

3. In the lipid fractions relating to the three species of ruminants all seven volatile acids were present. However in cattle, C_8 and C_{10} acids were preponderant, whilst in sheep C_4 and C_5 acids predominated. In male goats the seven volatile acids were more evenly distributed, but C_8 and C_{10} acids predominated. The lipid fractions from eight dogs contained virtually C_5 acid only.

5. It is questionable whether the offensive smell of male goats is really due to C_6 and C_8 as is generally assumed.

REFERENCES

Dukes, H. H. (1947). *The Physiology of Domestic Animals*, p. 427.

French, T. H., Hunter, G. D., Martin, A. J. P. & Popják, G. (1951). *Biochem. J.* **48**, vi.

Lovern, J. A. (1934). *Biochem. J.* **28**, 394.

Nijkamp, H. J. (1951). *Analytica Chimica Acta*, **5**, 325.

Ramsey, L. L. & Patterson, W. J. (1948). *J. Ass. off. agric. Chem., Wash.*, **31**, 139.

Zwaardemaker, H. (1895). *Die Physiologie des Geruchs.*

The Occurrence of Growth Factors for *Lactobacillus leichmannii*, *Streptococcus faecalis* and *Leuconostoc citrovorum* in the Tissues of Pernicious Anaemia Patients and Controls

By R. H. GIRDWOOD
Department of Medicine, University of Edinburgh

(*Received* 27 *December* 1951)

The chief therapeutic substances that have been shown to be of value in the treatment of pernicious anaemia in man are: vitamin B_{12} (cyanocobalamin) which is a growth factor for *Lactobacillus leichmannii*; pteroylglutamic acid (probably folic acid) which is a growth factor for *Streptococcus faecalis*; 5-formyl-5:6:7:8-tetrahydropteroylglutamic acid (the citrovorum factor or probably folinic acid) which is a growth factor for *Strep. faecalis* and for *Leuconostoc citrovorum*. The metabolic interrelationships of these factors are uncertain and have already been discussed (Girdwood, 1952*a*, *b*).

The present investigation commenced with a study by microbiological assay methods of the content of vitamin B_{12} and of growth factors for *Strep. faecalis* in the tissues of a patient who died of untreated pernicious anaemia. Later, published work suggested that the citrovorum factor is of importance in relation to haemopoiesis (Nichol & Welch, 1950), and accordingly we commenced studies of the amounts of citrovorum factor in human tissues. Swendseid, Bethell & Ackermann (1951) have introduced a differential microbiological assay procedure for measuring the extent to which the apparent folic acid content of tissues is, in fact, due to citrovorum factor. By a similar approach, and using autopsy material, we have found that in certain human tissues the whole of the material with folic acid activity is citrovorum factor. This is in keeping with the findings of Swendseid *et al.* (1951) who carried out their investigations on fresh mouse livers.

EXPERIMENTAL

Various tissues and organs were obtained at autopsy from the following patients.

Case I. A man aged 65, was admitted to hospital with severe untreated pernicious anaemia diagnosed on the clinical and haematological findings which included sternal puncture. The haemoglobin level was 5·7 g./100 ml., R.B.C. 1·95 millions/cu.mm. Before treatment commenced the patient died suddenly of a coronary thrombosis.

Case II. A woman aged 79, was admitted to hospital with untreated pernicious anaemia (Hb 5·3 g./100 ml., R.B.C. 1·35 millions/cu.mm.). She was given 2 ml. of liver extract on admission (containing about 6 μg. of vitamin B_{12}/ml.) and the following day 100 μg. of vitamin B_{12} together with a transfusion of a pint of blood. She died on the third day in hospital through having aspirated her vomitus into the trachea and bronchi. At the time of death the marrow was not completely converted to the normoblastic state.

Case III. A woman aged 57 was admitted to hospital with hypertensive cardiac failure. There was collapse of the left lung and at autopsy a bronchial carcinoma was found to be present. No visible tumour deposits were present in the tissues that were used for assay. The patient was not anaemic.

Case IV. A man aged 75 was admitted to hospital with a cerebral thrombosis and died the following day. There was no anaemia.

In addition, a sample of liver weighing approximately 2 g. was obtained at operation from a patient (case V) who was undergoing a partial gastrectomy operation for peptic ulcer.

METHODS

All pH estimations were made with glass electrodes.

Buffer solutions

Stock solutions used for the preparation of buffer solutions were (i) 27·228 g. KH_2PO_4 dissolved in distilled water and made up to 1 l. (ii) 35·612 g. $Na_2HPO_4.2H_2O$ dissolved in distilled water and made up to 1 l. 0·2 M-Phosphate buffer solution at pH 7·5 was prepared by mixing 84·2 ml. of solution (i) with 15·8 ml. of solution (ii). 0·2 M-Phosphate buffer solution at pH 6·8 was prepared by mixing equal parts of solutions (i) and (ii).

Preparation of tissues

Tissue extractions were performed by two methods in each instance.

Method A. Approximately 1 g. of tissue was homogenized in 10 ml. of 0·2 M-phosphate buffer solution at pH 7·5. There were added a further 10 ml. of the same buffer solution and the sample was incubated at 37° for 24 hr. The material was then steamed for 10 min. at 100° and filtered. The volume was made up to 50 ml., the final pH being adjusted to 6·8 with 0·1 N-HCl.

Method B. To approximately 1 g. of tissue there were added 20 mg. of pancreatin which itself was found to have negligible growth-promoting activity for the test organisms. Then 10 ml. of 0·2 M-phosphate buffer at pH 6·8 were added and the further steps carried out as in method *A*, the buffer used throughout being of pH 6·8. The final pH was 6·8.

Vitamin assay procedures

Vitamin B_{12}. The content of this was measured using a modification of the method of Hoffmann, Stokstad, Hutchings, Dornbush & Jukes (1949), with *Lb. leichmannii* as test organism. Thioglycollic acid was not included in the medium, the samples instead being added aseptically to the autoclaved medium. It has recently been shown (Pierce, Page, Stokstad & Jukes, 1949) that related forms other than vitamin B_{12} itself occur in nature, and that these forms differ in that they lack a cyano group. For this reason, in the later stages of the investigation, the samples were treated with NaCN to convert alternative forms to cyanocobalamin. This was done after homogenization and incubation.

It is known that certain substances other than vitamin B_{12}, notably various deoxyribosides, will support the growth of *Lb. leichmannii* under the conditions of the assay. Correction for this effect was made by destroying the vitamin B_{12} with alkali and re-assaying for growth activity.

Pteroylglutamic acid and citrovorum factor. The growth of *Strep. faecalis* in a medium deficient in pteroylglutamic acid is stimulated either by pteroylglutamic acid or by citrovorum factor, whereas under the conditions of the assay the growth of *L. citrovorum* depends only upon citrovorum factor and not upon pteroylglutamic acid.

In the earlier stages of the investigation, estimations of combined pteroylglutamic acid and citrovorum factor were made using *Strep. faecalis* and the medium of Teply & Elvehjem (1945). Measurement of growth was by estimation of turbidity. Later, when assays of citrovorum factor were performed these were done by a differential assay method as described by Swendseid *et al.* (1951). Thus, assays of citrovorum factor alone were made with *L. citrovorum* as test organism. For this a modification of the method of Sauberlich & Baumann (1948) was employed, acid-hydrolysed casein being used and thymidine added to the medium since the latter substance reduces the requirement of *L. citrovorum* for citrovorum factor (Broquist, Stokstad & Jukes, 1950). The Ca salt of citrovorum factor, supplied by the kindness of Dr H. Broquist of Lederle Laboratories, Inc., New York, was used to give the standard curves, and concentrations were measured in terms of citrovorum-factor activity.

At the same time assays were performed using *Strep. faecalis* as test organisms, with two series of standard curves produced by pteroylglutamic acid and citrovorum factor, respectively. It was thus possible to measure 'total apparent pteroylglutamic acid activity' (FAA) and, at the same time 'apparent citrovorum activity' (ACF) with *Strep. faecalis.* Since citrovorum factor has been used as a standard in both the *L. citrovorum* and *Strep. faecalis* assays, if the activity of a given specimen in stimulating *Strep. faecalis* was greater than its activity in stimulating *L. citrovorum*, pteroylglutamic acid must have been present. If the results were the same in each instance, only citrovorum factor was present. Since the true content of citrovorum factor is known from the *L. citrovorum* assay, and there are both pteroylglutamic acid and citrovorum factor standards in the *Strep. faecalis* assay, it is possible to calculate to what extent citrovorum factor is falsely showing itself as total apparent pteroylglutamic acid activity and hence to calculate true pteroylglutamic acid content.

These assays cannot differentiate between free and conjugated forms of the various haematopoietic factors.

Under the conditions of the assay reported here, the stimulation of growth of *Strep. faecalis* produced by 1·0 mg. of citrovorum factor (calculated as the free acid) was equivalent to that produced by 0·58–0·78 mg. of pteroylglutamic acid. There were variations with individual assays but the results were consistently within this range. This is in keeping with the findings of Broquist (1951), but differs from the experience of Swendseid *et al.* (1951) who used folinic acid provided by Dr William Shive as standard, and found that 1·0 mg. of folinic acid had the growth-promoting activity of 1·5 mg. of pteroylglutamic acid.

RESULTS

Significance of assay procedures

Nichol & Welch (1950) have shown that pteroylglutamic acid may be converted to citrovorum factor in tissues under certain conditions, and hence we have followed the practice of Swendseid *et al.* (1951) of adding pteroylglutamic acid to samples of

Table 1. *Recovery of pteroylglutamic acid and citrovorum factor added to the buffer in which human tissues were homogenized*

Organ	Method of extraction	Supplement added to homogenate	Citrovorum factor activity (*L. citrovorum* assay) (μg./g.)	Pteroylglutamic acid activity (*Strep. faecalis* assay) (μg./g.)
Liver	*B*	None	0·67	0·61
	B	10 μg. PGA/g.	0·68	9·8
	B	4·38 μg. CF/g.	4·3	4·1
	A	None	0·45	0·47
	A	10 μg. PGA/g.	0·44	9·0
	A	4·38 μg. CF/g.	4·1	3·9
Liver	*B*	None	1·0	0·79
	B	0·922 μg. CF/g.	1·8	1·4
Kidney	*B*	None	0·22	0·10
	B	0·8 μg. PGA/g.	0·25	0·89
	B	0·922 μg. CF/g.	0·97	0·73

Table 2. *Results of repeated assays on different specimens of same organs*

Case	Organ	Method of estimation	Vitamin B_{12} content (*Lb. leichmannii* assay) (μg./100 g.)	Growth factors for *Strep. faecalis* (as PGA) (μg./100 g.)	Citrovorum factor content (*L. citrovorum* assay) (μg./100 g.)
III	Liver	*B*	22, 29, 25	160, 170, 160	150, 160, 180
		A	23, 26, 24	170, 150	150, 150
IV	Liver	*B*	58, 53, 50	390, 410	530, 520

certain of the tissues prior to incubation to see whether such conversion occurred under the conditions of our assay. When pteroylglutamic acid had been added to the fluid in which the tissues were homogenized, there was no increase in the ability of samples to stimulate the growth of *L. citrovorum* although the added pteroylglutamic acid could be recovered to the extent of 80–98 %, as shown by the *Strep. faecalis* assay (Table 1). When citrovorum factor was added before homogenization, recoveries were equally satisfactory, and hence there was no evidence of conversion of pteroylglutamic acid to citrovorum factor or citrovorum factor to pteroylglutamic acid.

In all instances, assays were performed at least in duplicate, usually in quadruplicate, and the accuracy of the method was checked by performing repeated assays on several specimens of the same tissue, separately weighed and homogenized (Table 2).

Content of vitamin B_{12} *and of growth factors for* Streptococcus faecalis *in a patient with untreated pernicious anaemia*

The results of this investigation are given in Table 3. It will be seen that no vitamin B_{12} was detected in any organs or tissues. In the assay method employed each tube contained 10 ml. of fluid of which 5 ml. comprised double strength medium. When 0·2 μmg. of vitamin B_{12} was added to a series of such assay tubes containing 0·5, 1, 2, 3 and 4 ml. respectively of extracts of each of the tissues of this patient, prepared as in method *B*, but brought to a final dilution of 100 ml., growth occurred equally in all tubes except in the case of the extract of skeletal muscle where it was progressively less with increasing content of tissue extract. Since

Table 3. *Vitamin* B_{12} *and total apparent pteroylglutamic acid content of the tissues and organs of a patient with untreated pernicious anaemia case I*

(The vitamin B_{12} content (*Lb. leichmannii* assay) without cyanide was nil in all cases.)

Organ or tissue	Method of extraction	Total apparent folic acid activity (*Strep. faecalis* assay) (μg./100 g.)
Liver	*B*	83
	A	90
Kidney	*B*	24
	A	5·5
Spleen	*B*	14
	A	8·9
Lung	*B*	Negligible
	A	Negligible
Brain	*B*	5·4
	A	—
Stomach	*B*	Negligible
	A	Negligible
Intestine	*B*	14
	A	3·9
Tongue	*B*	12
	A	3·4
Muscle	*B*	8·2
	A	4·1

the amount of growth in all other instances was equal to that produced by 0·2 μmg. of vitamin B_{12} in water, it appears that in most of the tissues no inhibitors were present to prevent the growth-stimulating effect of vitamin B_{12} for *Lb. leichmannii*.

what was found in cases III and IV, an appreciable part of the apparent pteroylglutamic acid content was not due to citrovorum factor. The patient received approximately 112 μg. of vitamin B_{12} by injection before death. The liver weighed 1120 g.

Table 4. *Content of haemopoietic factors in the tissues of non-anaemic human patients*

Case no.	Organ or tissue	Method of extraction	Vitamin B_{12} content (*Lb. leichmannii* assay) Without cyanide (μg./100 g.)	Vitamin B_{12} content (*Lb. leichmannii* assay) After cyanide treatment (μg./100 g.)	Total apparent folic acid activity (FAA) *Strep. faecalis* assay (μg./100 g.)	Apparent citrovorum-factor activity (ACF) *Strep. faecalis* assay (μg./100 g.)	True citrovorum-factor activity (CF) *L. citrovorum* assay (μg./100 g.)	True folic acid activity (PGA) (μg./100 g.)
III	Liver	*B*	25	28	130	170	160	Negligible
		A	24	28	138	160	150	Negligible
	Kidney	*B*	16	15	79	84	90	Nil
		A	7·1	7·1	16	16	14	Negligible
	Spleen	*B*	3·9	4·7	39	47	47	Nil
		A	4·6	5·0	6·0	7·8	9·9	Nil
	Lung	*B*	5·1	5·2	65	76	42	30
		A	4·0	4·2	33	36	9·4	26
	Brain	*B*	9·1	10	38	48	14	27
		A	7·0	8·7	12	18	1·7	11
	Muscle	*B*	8·3	8·4	39	42	24	21
		A	7·8	6·4	7·5	13	3·9	4·5
	Skin	*B*	1·3	1·5	24	25	7·8	18
		A	1·4	1·2	15	17	Negligible	15
IV	Liver	*B*	54	67	400	520	520	Nil
		A	55	74	400	510	540	Nil
	Kidney	*B*	25	32	38	38	40	Nil
		A	33	37	34	41	42	Nil
	Spleen	*B*	36	39	71	78	29	49
		A	12	21	16	19	5·8	11
	Brain	*B*	22	22	17	20	3·7	14
		A	5·9	6·1	Negligible	Negligible	Negligible	Negligible
	Stomach	*B*	22	30	38	40	23	21
		A	6·2	7·8	21	20	8·1	15
	Intestine	*B*	28	27	23	26	3·1	20
		A	15	16	20	20	5·3	16
	Muscle	*B*	11	16	18	20	17	5·4
		A	9·9	12	16	13	9·1	9·7
V	Liver	*B*	50	—	430	550	500	41
		A	46	—	310	390	300	81

Content of vitamin B_{12}, pteroylglutamic acid and citrovorum factor in the organs and tissues of control patients

The results of this investigation are given in Table 4. It will be seen that in the liver and kidney almost all the apparent pteroylglutamic acid was, in fact, citrovorum factor, but that in most instances the other organs and tissues contained both of these forms.

Content of haemopoietic factors in the organs and tissues of a pernicious anaemia patient at the commencement of treatment

The results of this investigation are given in Table 5. It will be seen that here, in contrast to

and the kidneys each weighed 120 g. If the highest figure obtained for B_{12} content (method *B*, treated with sodium cyanide) is taken to be the correct one, then the liver contained 260 μg. of vitamin B_{12} and in the kidneys there was a further 17·8 μg.

DISCUSSION

These results give an indication of the occurrence of vitamin B_{12}, pteroylglutamic acid and citrovorum factor in human tissues and indicate that in the liver and kidneys the apparent pteroylglutamic acid is largely citrovorum factor. It is possible, but unlikely, that this was due to post-mortem conversion of pteroylglutamic acid to citrovorum factor because (1) when pteroylglutamic acid was

incubated with liver or kidney, which did not appear themselves to contain appreciable amounts of this substance, conversion to citrovorum factor did not occur; (2) similar results were found by Swendseid *et al.* using fresh mouse livers; (3) in the liver obtained at surgical operation, growth of *Strep. faecalis* was very largely due to citrovorum factor; and (4) in the liver of the pernicious anaemia patient (case II) there was no increase of the percentage of growth activity for *Strep. faecalis*, due to citrovorum factor, after the organ had been kept frozen for 4 months. Unfortunately, it was not possible to carry out assays for citrovorum factor at the time when the tissues of case I were investigated, since at that time citrovorum factor for use as a standard was not available.

No vitamin B_{12} was found in the tissues and organs of case I, and except with the extract of muscle there was no evidence for the presence of any inhibitors that would prevent added vitamin B_{12} from supporting the growth of the test organism. The findings for the vitamin B_{12} content of the organs in case II are more difficult to interpret. It is known that when vitamin B_{12} is given by injection in doses of 100 μg. or more, there is a considerable and rapid excretion of the vitamin in the urine, and yet the combined vitamin B_{12} contents of the liver and kidneys appear to be greater than the amount that was injected. There is already some evidence that the tissues and organs of patients with pernicious

Table 5. *Content of haemopoietic factors in the organs and tissues of a pernicious anaemia patient at commencement of treatment. Case II*

Organ	Method of extraction	Vitamin B_{12} content (*Lb. lechmannii* assay) Without cyanide (μg./100 g.)	Vitamin B_{12} content (*Lb. lechmannii* assay) After cyanide treatment (μg./100 g.)	Total apparent folic acid activity (FAA) *Strep. faecalis* assay (μg./100 g.)	Apparent citrovorum-factor activity (ACF) *Strep. faecalis* assay (μg./100 g.)	True citrovorum-factor activity (CF) *L. citrovorum* assay (μg./100 g.)	True folic acid activity (PGA) (μg./100 g.)
Liver	*B*	14	23	120	140	79	62
	A	3·2	4·1	65	66	26	45
Kidney	*B*	5·0	7·3	46	43	46	Nil
	A	2·6	2·9	11	15	12	2·0
Skin	*B*	0·27	—	11	16	1·5	9·7
	A	0·26	Negligible	Negligible	Negligible	Negligible	Negligible

It appears that the liver of case II, a pernicious anaemia patient at the commencement of treatment, differs from that of cases III and IV in that pteroylglutamic acid itself was present to an appreciable extent either in a free or conjugate form. However, further work on the livers of untreated patients with megaloblastic anaemia and on many more normal livers is necessary before any claim can be made that one of the metabolic errors in pernicious anaemia is a failure in the conversion of pteroylglutamic acid to citrovorum factor. The fact that the therapeutic dosage of pteroylglutamic acid and of citrovorum factor is similar (Davidson & Girdwood, 1951) suggests that this cannot be the primary metabolic fault.

It will be noted that in common with other workers we found that with certain tissues the content of haemopoietic factors was greater when pancreatin extraction was used, and there is no reason to believe that the higher figure in each instance is not the correct one. In most instances the addition of sodium cyanide after homogenization caused little increase in the content of vitamin B_{12} suggesting that little vitamin B_{12b} was present, though Cuthbertson (1951) states that cyanide is more effective if added prior to homogenization.

anaemia in relapse are not always completely devoid of vitamin B_{12} (Girdwood, 1951; Drouett, Wolff & Rauber, 1951), but if this is the true explanation it would appear reasonable to assume that the vitamin B_{12} present in the tissues of such patients is in a 'bound' form that is not available for haemopoiesis. An alternative interpretation would be that some other substance is present that stimulates the growth of *Lb. leichmannii* under the conditions of the assay despite the use of a technique that includes alkaline hydrolysis.

SUMMARY

1. An account is given of the distribution of vitamin B_{12}, pteroylglutamic acid and citrovorum factor in the tissues and organs at autopsy of two non-anaemic patients. The liver and kidneys contained relatively large amounts of citrovorum factor, but no pteroylglutamic acid.

2. The tissues and organs of a patient with untreated pernicious anaemia contained no vitamin B_{12}. In another pernicious anaemia patient who died at the commencement of treatment, the amount

of vitamin B_{12} recovered appeared to be greater than the amount given. The liver of this patient contained appreciable amounts of both pteroylglutamic acid and citrovorum factor.

The author is most grateful to Mr Robert Brown for assisting him with many of the assays, and to Prof. L. S. P. Davidson for his interest and encouragement. He also desires to thank Dr T. G. Brady for supplying thymidine, Dr H. Broquist (Lederle Laboratories Inc.) for supplying citrovorum factor, Dr Augustus Gibson (Merck and Co. Inc.) and Dr Frank Hartley (British Drug Houses Ltd.) for supplying vitamin B_{12} standards, and Dr W. F. J. Cuthbertson (Glaxo Laboratories Ltd.) and Dr G. E. Shaw (Evans Medical House) for supplying other materials and for giving valuable advice.

REFERENCES

Broquist, H. P. (1951). Personal communication.
Broquist, H. P., Stokstad, E. L. R. & Jukes, T. H. (1950). *J. biol. Chem.* **185**, 399.
Cuthbertson, W. F. J. (1951). Personal communication.
Davidson, L. S. P. & Girdwood, R. H. (1951). *Lancet*, **2**, 1193.
Drouett, P. L., Wolff, K. & Rauber, G. (1951). Quoted in *J. Amer. med. Ass.* **146**, 493.
Girdwood, R. H. (1951). *Brit. J. Nutrit.* **5**, xi.
Girdwood, R. H. (1952*a*). *Blood*, **7**, 77.
Girdwood, R. H. (1952*b*). *Brit. J. Nutrit.* (in the Press).
Hoffmann, C. E., Stokstad, E. L. R., Hutchings, B. L., Dornbush, A. C. & Jukes, T. H. (1949). *J. biol. Chem.* **181**, 635.
Nichol, C. A. & Welch, A. D. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 403.
Pierce, J. V., Page, A. C. jun., Stokstad, E. L. R. & Jukes, T. H. (1949). *J. Amer. chem. Soc.* **71**, 2952.
Sauberlich, H. E. & Baumann, C. A. (1948). *J. biol. Chem.* **176**, 165.
Swendseid, M. E., Bethell, F. H. & Ackermann, W. W. (1951). *J. biol. Chem.* **190**, 791.
Teply, L. J. & Elvehjem, C. A. (1945). *J. biol. Chem.* **157**, 303.

Some Observations on the Hydrolysis and Extraction of Formaldehydogenic Corticosteroids

By R. I. S. BAYLISS
*Department of Medicine, Presbyterian Medical Centre, Columbia University, New York City**

(*Received* 22 *November* 1951)

Of the methods recently reviewed by Dorfman (1950) and Callow (1950) for estimating urinary corticosteroids only a few have been generally adopted. In the bioassay procedure of Venning, Kazmin & Bell (1946) use is made of the ability of certain 11-oxysteroids to promote the deposition of glycogen in the liver of adrenalectomized mice. The chemical procedures are of two main types. The first depends on the reducing properties of steroids with a primary or secondary α-ketol group, or an αβ-unsaturated 3-keto group or both. Talbot, Saltzman, Wixom & Wolfe (1945) used this property to reduce cupric ions, and Heard & Sobel (1946) phosphomolybdic acid. Since urine may contain a wide variety of reducing agents, the specificity of the assay depends upon the efficiency of the extraction and the purification of the extract. The second chemical method depends upon the liberation of formaldehyde when steroids with a free terminal vicinal glycol or hydroxyketone group are oxidized with periodic acid (Lowenstein, Corcoran & Page, 1946; Daughaday, Jaffe & Williams, 1948; Corcoran, Page & Dustan, 1950). The liberated formaldehyde reacts with chromotropic acid to give a lavender colour.

More recently, Zaffaroni, Burton & Keutman (1950) and Burton, Zaffaroni & Keutman (1951) have evolved a paper partition chromatographic procedure which permits separation of urinary corticoids into individual components. Such fractionation is an advance on the less specific methods, but unfortunately permits only semi-quantitative determinations.

All these methods require the extraction of corticosteroids from urine. Hormones which are excreted in the free form as neutral lipid-soluble substances are readily extracted by organic solvents. Others, however, are excreted in water-soluble conjugated form as sulphates and glucuronides (Klyne, 1946; Klyne & Marrian, 1945; Lieberman & Dobriner, 1948) and these must be hydrolysed before extraction. In the method of Talbot *et al.* (1945) the urine is extracted without adjusting the pH, and the steroids obtained are

* Present address: Department of Medicine, Postgraduate Medical School, London, W. 12.