The values for water threshold R will range, therefore, from *c.* 0·995 for a urine flow of 0·5 ml./min. to *c.* 0·8 for a urine flow of 20 ml./min.

In order to obtain a more useful scale of values, we suggest the use of the expression $1/C$ and the multiplication of the values by 10^3. These values then express the volume of urine excreted from each 1,000 ml. of glomerular filtrate and can be obtained without knowing the volume of urine formed per minute. In this way, the concentrating power of the kidney towards water can be obtained from samples of urine without any precautions for complete collection of the sample. The fact that no injections or accurate urine sampling are required make this a useful tool in the clinical examination of renal disease, and when the values for 'water excretory threshold' are taken in conjunction with values of the index E [6] for the various urinary solutes, one obtains a reliable assessment of the power of the kidney to deal with the normal urinary constituents and the extent of any glomerular or tubular damage.

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Absence of Hyaluronidase from Reptilian Testes

The occurrence in mammalian testes and aqueous extracts of spermatozoa of a substance causing increased spread of fluids injected intradermally was described by Hoffman and Duran-Reynals[1], and McClean[2]. These observations have since been confirmed by numerous workers[3], and the 'spreading factor' has been shown to be identical with the enzyme hyaluronidase first described by Meyer, Dubos and Smythe[4]. The work of McClean and Rowlands[5], Fekete and Duran-Reynals[6], and others has indicated the probable role of seminal hyaluronidase in the fertilization process, namely, solution of the gel cementing the follicle cells around freshly ovulated ova, thereby denuding the latter and so enabling spermatozoa to penetrate them. In human semen, a close correlation has been shown to exist between sperm density and hyaluronidase content, azoospermic semen being devoid of the enzyme[7,8,9]. It has also been shown that a similar relationship holds in the case of the bull, rabbit and boar, though not in the dog or fowl[9]; in the dog, the hyaluronidase content of semen was found to be small and seemed to vary independently of the sperm density, while in the fowl it was absent altogether from six out of ten samples tested and appeared to be present in the remainder in trivial amounts only.

The interest of the findings in fowl semen lay in the fact that in birds the ova are never surrounded by follicle-cell cumuli; in other words, according to the hypothesis outlined above, the presence of hyaluronidase would not be necessary to enable fertilization to occur. Since reptilian ova, like those of birds, are also devoid of cumuli when ovulated, it seemed well worth while to determine whether or not reptiles produce seminal hyaluronidase. Assay of semen in this case was out of the question, and it was consequently decided to extract reptilian testes according to a method known to give high yields of the enzyme from rabbit testes[9]. Briefly, this consisted in grinding the testes (which had been kept on ice for not longer than 48 hours until ready for use) with sand, extracting with water at 0° C. and filtering the extract. The extracts were tested for hyaluronidase activity by the viscosimetric method described by Swyer and Emmens[10].

The nature of the testicular material used is shown in the table; in all cases the animals, so far as could be ascertained, were sexually mature. In no case was any hyaluronidase detectable in the extracts.

	Species	Moist weight of testes (gm.)	Total volume of extract (ml.)
1	Anaconda (*Eunectes murinus*)	2·86	25
2	,, ,, ,,	4·34	25
3	,, ,, ,,	3·64	25
4	Flap-necked chameleon (*Chamæleon dilepis*)	0·193	10
5	*Python regius*	0·56	10
6	River Jack viper (*Bitis gabonica*)	1·35	10
7	,, ,, ,,	0·465	10
8	Margouillat lizard (*Agama agama*)	0·25	10
9	Green mamba (*Dendraspis viridis*)	0·21	10

The absence of hyaluronidase from reptilian testes is, of course, in accord with the hypothesis mentioned above, and may therefore be regarded as additional support for it.

My sincere thanks are due to the Zoological Society of London for providing the reptilian material used.

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Adsorption Analysis of Oxidized Insulin

A special modification of adsorption chromatography on charcoal has been shown by Tiselius[1] to be a useful and selective method of analysis for mixtures containing closely related substances. The method was originally designed for the investigation of breakdown products of proteins, though it has not yet been extensively applied to relatively high-molecular polypeptides, since these are often such complex mixtures as to render any type of analysis extremely difficult.

The oxidation of insulin with performic acid yields a product in which it was believed that the molecule was split into its separate polypeptide chains[2]. This

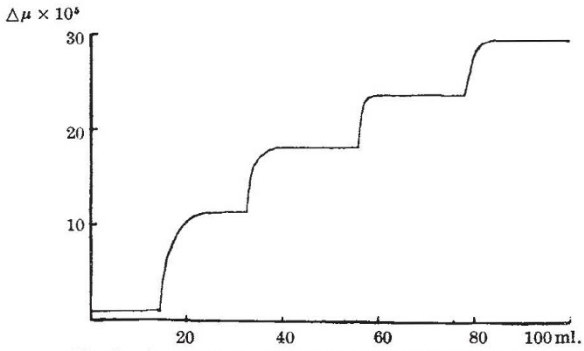

Fig. 1. ADSORPTION ANALYSIS OF OXIDIZED INSULIN

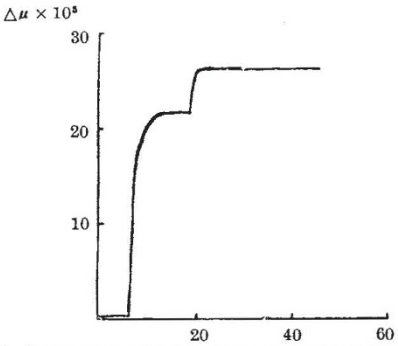

Fig. 2. ADSORPTION ANALYSIS OF FRACTION *A* OF OXIDIZED INSULIN

product should be a relatively simple mixture of polypeptides of average molecular weight 3,000, and in order to determine its complexity it was subjected to adsorption analysis. Fig. 1 shows a typical result of a frontal analysis of a 0·2 per cent solution of the oxidized insulin by the interferometric method[1], using a filter of volume 875 π c.mm. It can be seen that the mixture consists of at least four components. Considerable differences in the relative concentrations and retention volumes on the various steps were observed in different experiments, though the general appearance was the same. The front fraction was found to contain predominantly but not entirely glycyl terminal residues[2], and little of the basic amino-acids. Fig. 2 shows a frontal analysis of the crude fraction *A* [2] on a filter of volume 250 π c.mm. Both steps had only glycyl terminal residues and no arginine or lysine, suggesting that any phenylalanyl peptides present had not been eluted from the charcoal, due to their low concentration. The retention volumes of these substances is very much affected by their concentration; thus when fraction *B* was studied three steps were formed, the first large step containing predominantly phenylalanyl terminal residues.

At this stage of the work it should not be assumed that the four steps of the frontal analysis represent the four separate peptide chains of the insulin submolecule; but it is hoped that further work will help to characterize the various components.

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Estimation of Potassium in Composts and Sewage Sludges

IT is known that the presence of silicious matter in organic substances interferes with the potash estimations. In most carbonaceous substances the silica content is small but, even so, it is often desirable to 'dehydrate' the silica, prior to the potash extraction, by the use of perchloric acid. In the cases of dried sewage sludge and composts, such as those prepared from town refuse or from sewage sludge and straw, the silicious matter forms a major portion of ash which may comprise more than 80 per cent of the dry matter. In such cases the interference of silicious matter is so serious that only 10 per cent or less of the total potash content may be extractable with hot water, after ignition, or about 20 per cent if warm dilute hydrochloric acid is used. The accompanying table shows the actual amounts of potash extracted from one gram sample of town refuse.

	Method of extraction	Mgm. K_2O
1	Extraction with hot water after ignition	0·40
2	,, ,, warm dilute HCl after ignition	1·10
3	,, ,, hot water before ignition	1·25
4	,, ,, 1% citric acid before ignition	2·20
5	,, ,, cold dilute HCl ,, ,,	2·70
6	,, ,, conc. HNO_3 + $HClO_4$	3·00
7	Lawrence-Smith method before ignition	4·28
8	,, ,, ,, after ,,	5·00

The only satisfactory method found suitable for the estimation of potash in organic substances containing large quantities of silicious matter consisted of ignition, followed by the fusion of ash by the Lawrence-Smith method and the separation of potash by any of the conventional methods.

Method 8 was the only one which gave a complete recovery of the added potassium chloride, while method 1 yielded only 20·4 per cent and method 7 92·7 per cent of the added potassium chloride.

This work was done under the auspices of the Agricultural Research Council at the University of Reading.

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Limitations of the Colony Count Test for Assessing the Total Number of Viable Bacteria in Raw and Heated Milk

DURING the course of an investigation on the rate of growth of *Str. lactis* in raw and heated milk, it was found that the rate of growth as estimated by the colony count test at 37° C. was very much greater in heated milk (milk heated to 70° C. for one hour), but that the reduction of methylene blue and resazurin was the same, in spite of this apparent greater metabolic activity of the organisms.

NUMBER OF VIABLE ORGANISMS PRESENT AFTER 4 HR. INCUBATION AT 37° C. AS ESTIMATED BY THE COLONY COUNT TEST

	Colony count per ml.	Methylene blue reduction time
Raw milk	5,800,000	½ hour
Heated milk	80,800,000	2½ hour

In an attempt to throw some light on these anomalous results, smears were made for direct microscopical examination, and it was found that the organisms in the raw milk grew in long chains,