Comparison between a microdiffusion and an ion exchange method for the determination of plasma ammonium

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SYNOPSIS The results of plasma-ammonium determinations by an ion-exchange method and by a microdiffusion method were compared on the same specimens with each other and with ammonium determinations carried out on the corresponding whole-blood specimens by the microdiffusion method. The plasma-ammonium levels of whole-blood specimens were higher than those of the corresponding plasma specimens, but the difference was not constant. Recoveries of added NH_4 from plasma by the microdiffusion method ranged from 65 to 144%. Recoveries of NH_4 from the same specimens by the ion-exchange method ranged from 93 to 96%. Of the two, the ion-exchange method was the only one of value.

Studies on venous plasma-ammonium levels in cardiac disorders have been reported (Acland and Strong, 1965; 1966) in which plasma ammonium was determined by the ion-exchange technique of Fenton (1962) slightly modified (Fenton and Williams, 1968). Plasma-ammonium levels ranging from 13.5 to 49.5 $\mu\text{g.}$ of ammonium nitrogen per 100 ml. were recorded in a group of normal subjects and patients in whom there was no reason to suspect the presence of hepatic or respiratory disease. Somewhat higher normal ranges for the venous plasma ammonium level, determined after microdiffusion (Seligson and Seligson, 1951; Seligson and Hirahara, 1957), have been reported by Bessman and Evans (1955), by Conn (1960), and by Humoller, Barak, and Holthaus (1964). The difference between the normal ranges of plasma ammonium determined by the two methods has been attributed to the formation of ammonia from other nitrogenous constituents of plasma in the highly alkaline conditions used in the microdiffusion step (Dienst, 1961).

The normal range for the ammonium level of whole blood has been reported to be higher than that of plasma by Humoller *et al.* (1964) who used a microdiffusion technique. They also obtained evidence which suggested that the different ranges for plasma and whole blood resulted from pH differences between cells and plasma.

The object of the present investigation was to find out whether there was a consistent difference (1) between plasma-ammonium levels determined after microdiffusion and after ion exchange, and (2) between whole-blood ammonium and plasma ammonium determined after microdiffusion. If these differences were consistent, the results of ammonium determinations on different starting materials or by different methods could be made comparable by applying the appropriate corrections.

METHODS

BLOOD SAMPLES Blood was collected from an antecubital vein by means of a plastic disposable syringe (not heparinized), introduced directly into plastic 96×15 mm. stoppered tubes (Stayne Laboratories Ltd.), heparinized as described by Fenton and Williams (1968), and cooled to 0°C. in an ice-water bath. The ammonium content of whole blood was determined immediately. Plasma was separated within 30 min. by centrifugation at 0°C. If the plasma samples were not to be subjected immediately to microdiffusion or ion exchange, they were kept frozen at -10°C. for not longer than 24 hours.

PLASMA AMMONIUM DETERMINATION AFTER ION-EXCHANGE A 'column blank' (2 ml. of deionized water taken through the whole analysis) and a 'column recovery' (2 ml. of a standard $(\text{NH}_4)_2\text{SO}_4$ solution containing 100 $\mu\text{g.}$ of $\text{NH}_4^+ - \text{N}$ per 100 ml.) were run with each batch of determinations. A calibration curve was constructed for each batch, using standard solutions composed of 4 ml. of $4\text{M} - \text{NaCl}$ and 4 ml. of an NH_4^+ standard, to which

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alkaline phenol reagent and hypochlorite were added in the usual way. The ammonium standards used corresponded to plasma ammonium levels of 50, 100, and 200 $\mu\text{g. of NH}_4^+ - \text{N}$ per 100 ml. respectively. Distilled water from a Manesty still was deionized before use by passage through a column of Amberlite MB - 1 resin. The method of determination (Fenton and Williams, 1968) was modified from the original description (Fenton, 1962). Instead of the nylon net diaphragm in the ion exchange columns we used a pledget of cottonwool, which had been previously treated for half an hour with 0.35 N-NaOCl then washed thoroughly with water and stored in 4M-NaCl. The pledgets reduced the rate of fluid flow sufficiently and we did not fit hypodermic needles to the columns. The ion-exchange method was not used for NH_4^+ determinations on whole blood.

PLASMA AMMONIUM DETERMINATION BY MICRODIFFUSION
Microdiffusion was carried out in 30 ml. glass Universal containers, as described by Seligson and Hirahara (1957), except that a stainless steel rod was not used as a stirrer. The rubber bungs, in which the glass rods were mounted for microdiffusion, were stored over concentrated H_2SO_4 . The diffused ammonium was dissolved from the glass rod and estimated spectrophotometrically by the method of Brown, Duda, Korkes, and Handler (1957).

RESULTS AND DISCUSSION

Plasma ammonium levels were measured in nine subjects who were either healthy or had no evidence of liver or respiratory disease. Since it appeared that the size of the difference between whole blood ammonium and plasma ammonium was not consistent, whole blood ammonium was not determined in the remaining five patients. Instead, the recovery of ammonium from plasma by both methods was measured by carrying out determinations on a mixture of plasma and a 10 $\mu\text{g./ml.}$ ammonium sulphate standard in the proportions 9:1. Thus sufficient ammonium was added to the plasma to increase its ammonium level by 100 $\mu\text{g. of ammonium nitrogen}$ per 100 ml. Allowance was made for the dilution of the plasma samples in calculating the recoveries of ammonium.

The results are presented in Table I.

Recoveries by the ion-exchange method are adequate and consistent, as was found previously (Acland and Strong, 1965). Recoveries by the microdiffusion method are inconsistent and unsatisfactory.

TABLE I
AMMONIUM LEVELS IN WHOLE BLOOD AND PLASMA
DETERMINED BY MICRODIFFUSION AND ION EXCHANGE

Sex and Age of Subject	Clinical State	Ammonium Level ($\mu\text{g. of NH}_4^+ - \text{N}/100 \text{ ml.}$)				
		Plasma (Ion Exchange)		Plasma (Microdiffusion)		Whole Blood (microdiffusion)
		Un-treated	Recovery of 100 $\mu\text{g./100 ml.}$	Un-treated	Recovery of 100 $\mu\text{g./100 ml.}$	
♂ 29	Normal	33	—	42	—	100
♂ 39	Hypertensive	28	—	25	—	227
♂ 22	Normal	36	—	66	—	86
♀ 15	Atrial septal defect (pre-operative)	23	—	46	—	53
♀ 60	Hypertensive	30	93	56	97	—
♀ 32	? Thyrotoxic	20	96	55	81	—
♀ 61	Hypertensive	44	94	93	85	—
♂ 42	? Hypothyroid	44	94	101	65	—
♂ 21	Atopic eczema on steroids	46	95	32	144	—

Since the ammonium levels in whole blood and plasma, as determined by microdiffusion, do not appear to bear a constant relation either to each other or to the plasma ammonium level determined by ion-exchange, it is not possible to adjust the result of a determination by microdiffusion to correspond to the value which would be obtained by ion exchange. In our hands, the microdiffusion method has proved to be valueless, whereas the ion exchange method has been satisfactory.

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