COMPARISON OF THE RENAL CLEARANCES OF INULIN AND RADIOACTIVE LABELLED HYPAQUE AS MEASURES OF THE GLOMERULAR FILTRATION RATE IN MAN

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INTRODUCTION

The standard method of estimating the rate of glomerular filtration (G.F.R.) in man has been by the renal clearance of inulin since it was demonstrated that this plant polysaccharide is neither secreted nor reabsorbed by the renal tubules. The criteria which must be satisfied by a substance for its renal clearance to give a valid measure of glomerular filtration rate have been discussed in detail by Smith (1951). Chemical methods for the determination of inulin in plasma and urine have been rather unsatisfactory (Smith 1951) and a variety of substances have been studied in an attempt to find a substitute for inulin. The endogenous creatinine clearance is widely used for clinical purposes but the creatinine/inulin clearance ratio exceeds unity in many human subjects indicating a variable degree of tubular secretion of creatinine (Smith 1951, Berlyne et al 1964) and this clearance is not a reliable measure of glomerular filtration in man.

Recently a number of compounds labelled with radioisotopes have been studied and found to have clearances similar to that of inulin. Allyl inulin labelled with ^{125}I has a clearance virtually identical to that of inulin in the dog (Concannon et al 1964) but is not readily available and is difficult to sterilise. Radioactive ^{57}Co-labelled cyanocobalamin gives a satisfactory measure of G.F.R. if only the free vitamin in the plasma is measured (Nelp et al 1964, Cutler and Glatte 1965). However, even after a large loading dose of unlabelled cyanocobalamin, plasma protein binding of the labelled compound occurs and is variable in extent, nor is it easy to determine the proportion of the labelled/

labelled vitamin which is plasma bound in vivo (Ekins and Sgherzi 1964, Donaldson and Doig unpublished observations). The most convenient inulin substitutes so far available are the radiographic contrast media sodium diatrizoate (Hypaque) and meglumin diatrizoate (Renografin) labelled with ^{131}I or ^{125}I. In man Hypaque appears to fulfil many of Smith's criteria, it is not significantly bound to plasma proteins (Lasser et al 1962) nor does it readily penetrate red blood cells (Denneberg et al 1961), its renal excretion is complete and its extra-renal excretion negligible (Denneberg 1965). Hypaque and Renografin have been shown by various authors to have clearances very similar to the simultaneous inulin or thiosulphate clearance in man and the literature on these compounds has been well reviewed by Denneberg (1965). However some authors have not found the Hypaque and inulin or thiosulphate clearances to be identical (Bianchi and Zampieri 1961, Woodruff and Malvin 1960, Stokes et al 1962, Denneberg 1965) and while some comparisons were carried out during continuous infusion of inulin and Hypaque (Burbank et al 1963), others were made on the basis of a single injection of Hypaque (Bianchi and Zampieri 1961). The latter are difficult to interpret physiologically; the difficulties inherent in all "single injection" clearances have been discussed by Smith (1951) and Robson et al (1949).

It has been variously suggested that Hypaque is neither reabsorbed nor secreted by the renal tubules in man (Burbank et al 1963), nor in the dog (Woodruff and Malvin 1960), that it is reabsorbed in man (Bianchi/

Bianchi and Zampieri 1961) and in dog (Stokes et al 1962) and that it is both secreted and reabsorbed in man (Denneberg 1965).

Morris et al (1965) found that the clearance of ^{131}I Renografin did not alter when the plasma level was raised by large doses of inactive Renografin, suggesting that this compound is not handled by the tubules. However they studied only two subjects in this way. Hypaque would be expected to behave in the renal tubules in the same way as Renografin as it is the diatrizoate ion which is estimated in both cases. However Denneberg (1965) found that the Hypaque/inulin clearance ratio fell, on average, after the administration of the tubular blocking agent probenecid and concluded that Hypaque is secreted and perhaps also reabsorbed by the renal tubules in man.

Because of this confusion in the literature about tubular handling of Hypaque in man, the studies described in this paper were carried out in an attempt to resolve the question of whether or not radioactive labelled Hypaque is handled by the renal tubules and to investigate its clearance as a measure of the rate of glomerular filtration in human subjects.

MATERIAL AND METHODS

Two groups of studies were carried out.

1. PRELIMINARY OBSERVATIONS WITH LABELLED HYPAQUE ONLY.

A total of 91 simultaneous clearances of inulin and labelled Hypaque were carried out during continuous infusion of the test substances, 65 using ^{131}I Hypaque and 26 with ^{125}I Hypaque. Of the total, 46 were clearances on single kidneys carried out during divided renal function studies in hypertensive patients while the remaining 45 were bladder clearances in patients with a variety of renal and non-renal disorders. The bladder clearances were carried out as described below in section 2; the single kidney clearances were carried out similarly, using urine collected from ureteric catheters by a technique similar to that of Stamey (1961). The isotope count rates were determined as described below. Inulin was measured in this series by the method of Hyrovsky (1956). Plasma proteins were precipitated with cadmium sulphate and sodium hydroxide to give a final dilution in the filtrate of 1:15. Urines were precipitated only if protein was present and were appropriately diluted. Five inulin standards were used and all estimations were carried out in triplicate. Clearances were calculated as described below.

2. STUDIES WITH UNLABELLED HYPAQUE.

PROCEDURE:- the subjects were six adult patients, five male and one female. The procedure was carried out in the morning with the subject fasting overnight. Subjects lay supine on a comfortable mattress/

mattress in a warm room, urine was collected by voluntary voiding throughout and with the subject standing or sitting up during bladder emptying. Plastic catheters were inserted percutaneously into forearm veins, two in one arm and one in the other. Through one catheter a 15% solution of mannitol was infused at approximately 10 ml./min. throughout the procedure to maintain an osmotic diuresis. One of the remaining catheters was used for the continuous infusion of a Hypaque/inulin mixture as described below. The other was used for blood sampling. Blood was always taken from the arm opposite to that receiving the Hypaque/inulin infusion, it was withdrawn into heparinised syringes and then expelled into plastic tubes containing a drop of heparin in which it was immediately centrifuged and the plasma separated.

Six clearances were carried out in each subject, three with tracer doses of ^{125}I labelled Hypaque only and a further three after a single dose of unlabelled Hypaque, in accordance with a strict programme which was identical for each subject.

Previous experience had shown that the urine passed at the beginning of the procedure was highly concentrated and had a relatively high inulin-like activity. A blank clearance was therefore carried out under mannitol diuresis to obtain urine of a concentration comparable to that obtained during the inulin and Hypaque clearances. This involved a timed urine collection over 20 minutes during mannitol diuresis with a blood sample which provided the plasma inulin blank taken at the mid time/

time. Under these conditions the urine inulin blank was always less than 0.5% of the average urine level during the clearances.

Following the blank clearance priming doses of inulin and ^{125}I Hypaque were injected and followed by a continuous infusion containing inulin and ^{125}I Hypaque administered at a constant rate of 1 ml./min. by an infusion pump. The priming doses were calculated on the basis of body weight to give a plasma inulin level of about 250 µg./ml. and a total count for plasma ^{125}I activity of not less than 10,000 counts above background under the counting conditions described below. The concentration of the sustaining infusion was calculated on the basis of expected G.F.R. to maintain these levels when infused at 1 ml./min. The total dose of radioactive material varied from 5 to 25 µc of ^{125}I. After 15 to 20 minutes for equilibration the bladder was emptied and the urine discarded and the first clearance period was started. The duration of the clearance periods depended upon the rate of urine flow but was usually 8 to 10 minutes between urine collections with blood withdrawn at the mid clearance time. Three consecutive clearances were carried out in this way. Immediately following the urine collection for the third clearance 40 mls. of a 45% solution of unlabelled Hypaque (Bayer Products) was injected over a period of one minute into the mannitol infusion catheter. No adverse reactions to the injection occurred in any subject. The consecutive clearances continued without interruption until another three had been carried out (making a total of six) when the procedure was stopped and the catheters removed.

The/

The urine volumes for each clearance were measured immediately and were always large enough to allow measurements to the nearest 1 ml. using a measuring cylinder. Rates of urine flow were obtained by dividing these volumes by the clearance times.

ANALYTICAL METHODS

1. Inulin. Inulin concentration in plasma and urine was measured using a modification of the resorcinol method adapted for use on the Auto-Analyser (Technicon) by the Department of Biochemistry, University of Edinburgh. Protein free filtrates were prepared by the zinc sulphate precipitation method of Somogyi (1930) from 2 ml. samples of plasma to give a final dilution of 1:15 for all samples. The urine in this series was found to be virtually protein free and urine samples were simply diluted 1:50 or 1:100 except for the blank (U_0) which was not diluted. Inulin standards containing 10, 20 and 30 µg./ml. of inulin were used. The following method of running the samples and of calculation of the unknown concentrations was developed by Professor R. B. Fisher. The samples were run on the Auto Analyser according to a lattice in which corresponding plasma and urine samples were adjacent (Table 1). This lattice was designed to minimise the effects of base line drift of the instrument and to provide frequent estimations of the standards. As shown in Table 1 the samples were run in groups in forward then in reverse order bracketed between sets of standards run first in ascending then in descending order of concentration. A duplicate set of samples/

TABLE 1

Lattice for Running Order of inulin samples on Autoanalyser

First run $S_1\ S_2\ S_3\ U_1\ P_1\ U_2\ P_2\ P_2\ U_2\ P_1\ U_1\ S_3\ S_2\ S_1$

Second run $S_1\ S_2\ S_3\ P_2\ U_2\ P_1\ U_1\ U_1\ P_1\ U_2\ P_2\ S_3\ S_2\ S_1$

S 1, 2 and 3 are standards of 10, 20 and 30 µg inulin/ml. respectively

P_1 and P_2 are plasma samples from 1st and 2nd clearances

U_1 and U_2 are urine samples from 1st and 2nd clearances

The procedure was identical for all samples from P_0 and U_0 to P_6 and U_6

samples was then run with the order of the groups and the order of samples within each group reversed. The percentage optical transmission at 480 mμ was recorded on a strip chart and the values were read from this to three significant figures. The inulin concentrations were calculated by a digital computer for each group of samples separately. The principles of this calculation are as follows:- the percentage transmissions were converted to optical densities and the best line relating concentration to optical density was calculated from the three pairs of standard determinations which bracketed each group of unknowns. From the coefficients of this line the best estimates of the unknown concentrations were calculated giving a pair of estimates for each sample in each group. The mean of each of these pairs was treated as a single observation of that sample. Thus from each group one mean value was obtained for each of two adjacent pairs of urine and plasma samples. As the whole procedure was run in duplicate but in reverse order the eventual result was two mean values for each plasma and urine filtrate. The average of these two mean values was used as the best estimate of concentration in all further calculations. (Under these conditions the deviation of the two mean values from their average did not exceed 1%). Multiplication by the appropriate dilution factor and subtraction of the appropriate blank gave the inulin concentration in μg./ml. of plasma or urine. Studies of inulin recovery from human serum over the range of 100 to 400 μg./ml. of added inulin gave a mean recovery of 99.54 $\pm$ 0.91% (S.E.) using this method.

2. ^{125}I Hypaque/

was calculated from the urine/plasma concentration ratios thus:-

$$\frac{C\ Hy}{C\ In} = \frac{U\ Hy/P\ Hy}{U\ In/P\ In}$$

which avoids rounding off errors due to multiplying then dividing by the rate of urine flow.

RESULTS

1. Preliminary observations.

The values of the clearances and their ratio in the preliminary series are not tabulated in detail. Figure 1 shows the linear regression of ^{125}I Hypaque clearance against the simultaneous clearance of inulin calculated by the method of least squares and Figure 2 shows the corresponding line for ^{131}I Hypaque and inulin. All statistical calculations were performed by standard techniques as described by Mather (1951) and in each case the values of the clearances from which the line was calculated are plotted. The mean value of the ratio Hypaque clearance/inulin clearance is shown together with the range of the ratio. However these mean values must be interpreted with caution, as discussed below.

The equation of the regression line for ^{125}I Hypaque on inulin is C Hy = 0.95(C In) + 7.35 (Fig.1). The standard error (S.E.) of the slope is $\pm$ 0.0256. Testing the difference of the slope from unity gives a value of t (for 24 degrees of freedom) of 2.072 for which the corresponding value of p is between 0.05 and 0.025. The value of t for the/

FIGURE 1

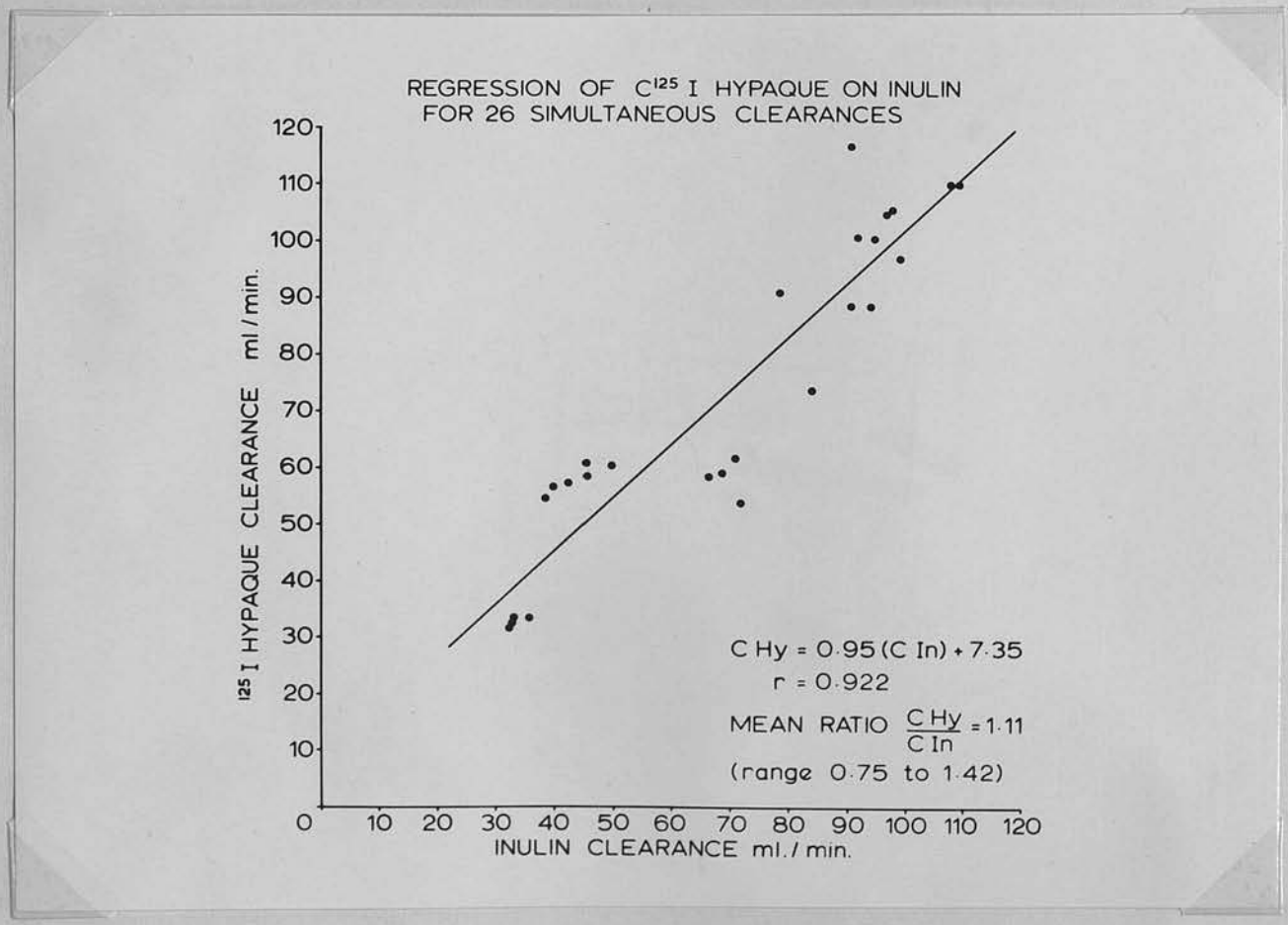
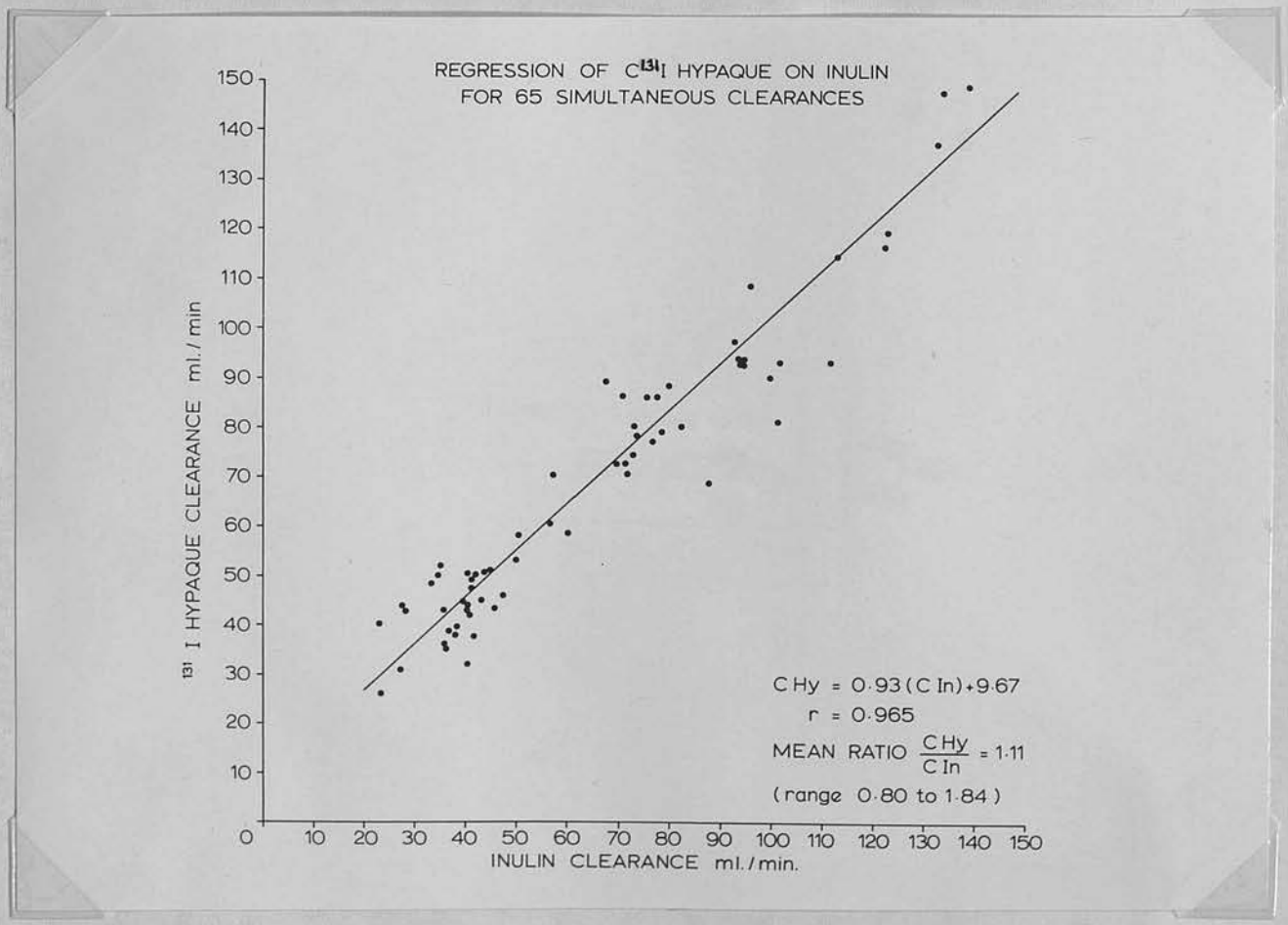

REGRESSION OF C125 I HYPAQUE ON INULIN
FOR 26 SIMULTANEOUS CLEARANCES
125 I HYPAQUE CLEARANCE ml/min.
INULIN CLEARANCE ml./min.
0
10
20
30
40
50
60
70
80
90
100
110
120
C Hy = 0.95 (C In) + 7.35
r = 0.922
MEAN RATIO C Hy / C In = 1.11
(range 0.75 to 1.42)

FIGURE 2


REGRESSION OF C[131]I HYPAQUE ON INULIN
FOR 65 SIMULTANEOUS CLEARANCES
[131] I HYPAQUE CLEARANCE ml./min
INULIN CLEARANCE ml./min.
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
C Hy = 0·93 (C In) + 9·67
r = 0·965
MEAN RATIO C Hy / C In = 1·11
(range 0·80 to 1·84)

the difference of the intercept from zero is 1.226 giving p between 0.30 and 0.20. The correlation coefficient (r) is 0.922 for which $p < 0.001$. The corresponding equation for ^{131}I Hypaque (Fig.2) is C Hy = 0.93 (C In) + 9.67 with standard errors of slope and intercept of $\pm$ 0.0317 and $\pm$ 2.2388 respectively. Testing the difference of the slope from unity and the intercept from zero as before, the values of t (for 63 degrees of freedom) are 2.308 for the slope and 3.768 for the intercept, giving corresponding values of p between 0.02 and 0.01 for the difference of the slope from unity and $p < 0.001$ for the difference of the intercept from zero. The correlation coefficient (r) is 0.965 with $p < 0.001$. The mean value of the ratio was 1.11 for both isotope labels. These mean values of the ratio are derived from groups of patients in each of whom a number of determinations of the ratio was made and the question arises whether the groups of observations for either isotope can be considered as a single population with a mean of 1.11 to which a variance can be assigned. The hypothesis that all of the observations for each isotope have a common variance was tested by Bartlett's test for each group (Bartlett 1937). For the ^{125}I data a value for the heterogeneity χ^2 of 59.72 for 6 degrees of freedom was obtained with a corresponding value of $p < 0.0005$. For the ^{131}I data the results were $\chi^2 = 210.97$ for 15 degrees of freedom giving $p < 0.0005$. Thus in each case the hetetogeneity χ^2 is highly significant and neither group of data can be considered as a single population nor can a variance be assigned to the mean of either group. The significance of the difference of the means from unity cannot therefore be assessed. The implications/

implications of these findings are discussed more fully below.

2. Studies with tracer and unlabelled ^{125}I Hypaque.

The results of these studies are presented in full. In each table "Low" refers to the first three clearances which were carried out with tracer Hypaque only and "High" refers to the last three clearances after injection of unlabelled Hypaque. Table 2 shows the rate of urine flow in each clearance period. In most patients there is no abrupt change in urine flow from period to period but in patients 2 and 6 bladder emptying was probably incomplete on at least one occasion, resulting in sudden changes in the apparent urine flow. Such errors in urine flow affect the values of the clearances but not the ratio of Hypaque clearance/inulin clearance. The plasma and urine inulin concentrations are shown in Table 3 and the total counts above background of ^{125}I, indicating relative concentration of labelled Hypaque, in Table 4. Count rates between patients are not comparable as the counts were not referred to a standard; within each patient all counts are expressed as totals for a common counting period (usually 1,000 secs.). The plasma levels of Hypaque and inulin show slight fluctuation between consecutive clearance periods but this is only approximately 6.5% at its greatest during the first three clearance periods and the levels tend to become steadier in the latter part of the experiment. The clearances of inulin and Hypaque and the ratio Hypaque clearance/inulin clearance are shown for each period in Table 5 a, b and c. There are abrupt changes in both clearances during "Low" in subject 6 and "High" in subject 2 which correspond/

TABLE 2

Rates of Urine Flow ml/min. (mannitol diuresis)

Clearance Period

Subject	Low			High		
	1	2	3	4	5	6
1	15.4	13.3	14.1	18.3	14.7	15.0
2	10.3	9.0	9.6	10.3	6.3	10.9
3	10.0	12.8	12.9	15.5	17.3	17.1
4	13.2	12.9	16.7	16.5	18.7	18.2
5	12.5	10.8	10.9	13.9	11.7	12.3
6	16.1	7.6	18.7	12.5	14.4	11.8

TABLE 3

INULIN CONCENTRATION µg/ml.

Clearance Period

	LOW						HIGH					
	1		2		3		4		5		6	
Subject	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma
1	1902	233	1927	228	1720	229	1407	213	1570	220	1562	212
2	2089	252	2126	246	2230	231	1776	224	1801	221	1750	219
3	1958	231	1606	231	1539	226	1306	219	1169	219	1177	221
4	3060	288	2613	278	2321	262	1830	255	1734	254	1592	252
5	1654	248	1566	249	1549	239	1270	235	1355	231	1371	232
6	1914	254	1682	239	1603	234	1290	221	1221	218	1205	216

All concentrations are average of two mean values after subtraction of blank See text

TABLE 4

HYPAQUE CONCENTRATION, TOTAL COUNTS ABOVE BACKGROUND

Clearance Period

	LOW						HIGH					
	1		2		3		4		5		6	
Subject	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma
1	110748	13466	116816	14521	112866	14340	88022	14640	95379	13736	94536	13485
2	150997	16244	155131	15497	162607	15187	129700	14775	125467	14752	121006	14832
3	119123	13009	102125	13339	95491	12436	77665	12267	65659	12520	67819	12581
4	192015	19263	169696	18354	155778	17533	122419	17539	113240	17773	104452	17630
5	197340	28280	193692	28264	193878	27459	165995	26613	161407	26889	148102	28411
6	182514	24376	180194	23852	167849	21882	139452	20861	126594	21177	127616	21868

TABLE 5

Clearance Period

Subject	LOW 1	2	3	HIGH 4	5	6
	a) Inulin Clearance ml/min.					
1	125.5	111.8	105.6	120.5	104.9	110.1
2	85.2	78.1	92.8	81.8	51.3	87.2
3	84.4	89.3	87.8	92.8	92.1	91.0
4	140.7	120.8	147.5	118.6	127.7	114.9
5	83.6	70.8	72.1	76.6	68.2	72.5
6	121.3	53.4	128.0	73.1	80.6	65.8
	b) Hypaque Clearance ml/min.					
1	126.6	106.6	110.7	109.9	101.9	104.9
2	95.5	90.5	103.1	90.4	53.6	89.0
3	91.3	95.7	98.9	98.3	90.5	92.2
4	131.9	118.9	147.9	115.5	118.9	107.8
5	87.5	74.1	77.2	86.7	70.2	64.0
6	120.5	57.4	143.9	83.6	86.1	68.9
	c) Ratio C Hypaque/C Inulin					
1	1.01	0.95	1.05	0.91	0.97	0.95
2	1.12	1.16	1.11	1.11	1.05	1.02
3	1.08	1.07	1.13	1.06	0.98	1.01
4	0.94	0.98	1.00	0.97	0.93	0.94
5	1.05	1.05	1.07	1.13	1.03	0.88
6	0.99	1.07	1.12	1.14	1.07	1.05

LOW - clearances with tracer ^{125}I Hypaque only

HIGH - clearances after injection of 18 gm. of unlabelled Hypaque

correspond to the bladder emptying errors noted above. However Table 5(c) shows that there were no changes of corresponding magnitude in the ratio at these times. It is also apparent that the ratio remains close to unity at both "Low" and "High" Hypaque levels.

The effect of the injection of unlabelled Hypaque on the clearances of inulin and Hypaque and on the ratio Hypaque clearance/inulin clearance was studied by subjecting the data from this series of experiments to an analysis of variance. The calculations were carried out by standard methods similar to those described by Mather (1951) and the results of the analysis are shown in Tables 6, 7 and 8. The main findings are a significant fall in the mean value of the ratio between "Low" and "High" accompanied by significant falls in the mean clearances of both inulin and Hypaque.

DISCUSSION

Analysis of the results of the preliminary studies with tracer doses of labelled Hypaque showed that there was an excellent linear correlation between the simultaneous clearances of Hypaque labelled with either ^{125}I or ^{131}I and inulin (Figs.1 and 2). If Hypaque were excreted only by glomerular filtration the regression for Hypaque clearance on inulin clearance would be expected to differ from a line of slope unity and intercept zero only by the errors inherent in the methods of making the/

the observations. The slope of the regression for Hypaque labelled with either isotope is close to unity (0.95 for ^{125}I and 0.93 for ^{131}I) though in both cases the difference from unity is suggestively large and is significant at the 5% but not the 1% level of probability. The intercept in each case exceeds zero; the value of 7.35 for ^{125}I is not significant but that of 9.67 for ^{131}I is statistically significant. The overall result is that the Hypaque clearance tends to be greater than the simultaneous inulin clearance suggesting that Hypaque may be secreted by the tubules to a small extent. As the slopes of both regressions are close to unity the ratio of Hypaque clearance to inulin clearance ("the ratio") should provide an adequate measure of the behaviour of the Hypaque clearance relative to that of inulin with the additional advantage of avoiding errors due to inaccuracies in measurement of urine flow. The mean value of 1.11 for the ratio for both isotope labels confirms the tendency to a higher Hypaque clearance than is likely if Hypaque were excreted by filtration alone. Unfortunately these preliminary experiments were not well designed for statistical analysis and as has been shown above, the data cannot supply a valid estimate of the variance of the mean ratio for either label and thus it is impossible to test the significance of the difference of the mean ratio from unity. In summary the results of the preliminary observations show that the Hypaque clearance is, on average, somewhat higher than the inulin clearance and suggest that a small proportion of the excreted Hypaque may be secreted by the renal tubules. A further series of experiments was designed to test the hypothesis that there is some tubular secretion of Hypaque by studying/

studying the effect on the clearance ratio of a large dose of unlabelled Hypaque. If a mechanism for tubular secretion of Hypaque exists it should be possible to saturate this by raising the plasma Hypaque level sufficiently and the Hypaque clearance should then approach that of inulin with a consequent fall in the ratio towards unity. The ^{125}I label was used as this had proved more convenient for storage than ^{131}I and gave a smaller radiation dose to the subject. The Auto Analyser method for inulin is considerably more precise than the manual method used for the earlier studies. However, it is apparent that the variability between subjects is considerable and as it is not practicable to carry out more than about six consecutive clearances on any one subject, a considerable variance between subjects cannot be avoided. The technique of Analysis of Variance allows the desired effect (that of added Hypaque) to be isolated from the effects on the ratio of variance between subjects and any effect due to the order in which clearances are performed. The experiments were designed to be analysed in this way and each of the six subjects was studied in identical fashion before and after an intravenous dose of approximately 18 gm. of unlabelled Hypaque. A single dose was used as it is not essential to know the precise level of unlabelled Hypaque and in any case this would be difficult to measure chemically with the precision necessary to allow clearances of unlabelled Hypaque to be determined. The dose of 18 gm. is sufficient to raise the total plasma level of Hypaque several thousand fold.

The results of the study with unlabelled Hypaque have already been/

TABLE 6

ANALYSIS OF VARIANCE

RATIO: HYPAQUE CLEARANCE / INULIN CLEARANCE (VALUES x 100)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square
Between treatments (high v low)	156.6	1	156.6
Between subjects	890.8	5	178.2
Within treatments	417.5	4	104.4
Residual (error)	347.1	25	13.9
Total	1812	35	

Variance Ratios

Between treatments (high v low)	${}_1F_{25} = 11.27$	$p < 0.01$
Between subjects	${}_5F_{25} = 12.82$	$p < 0.001$
Within treatments	${}_4F_{25} = 7.51$	$p < 0.001$

Mean ratio: Hypaque clearance/Inulin clearance	Low	1.05
	High	1.01

TABLE 7

ANALYSIS OF VARIANCE

INULIN CLEARANCE (VALUES ROUNDED OFF TO NEAREST 1 ml./min.)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square
Between treatments (high v low)	784	1	784
Between subjects	13455	5	2691
Within treatments	2365	4	591
Residual (error)	4040	25	162
Total	20644	35	

Variance Ratios

Between treatments (high v low)	$_1F_{25} = 4.84$	$0.01<p<0.05$
Between subjects	$_5F_{25} = 16.61$	$p < 0.001$
Within treatments	$_4F_{25} = 3.65$	$0.01<p<0.05$

Mean inulin clearance	Low	100.0 ml./min.
	High	90.7 ml./min.

TABLE 8

ANALYSIS OF VARIANCE

HYPAQUE CLEARANCE (VALUES ROUNDED OFF TO NEAREST 1 ml/min.)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square
Between treatments (high v low)	1694	1	1694
Between subjects	8490	5	1698
Within treatments	3886	4	972
Residual (error)	3170	25	127
Total	17240	35	

Variance Ratios

Between treatments (high v low)	$_1F_{25} = 13.34$	$p < 0.01$
Between subjects	$_5F_{25} = 13.37$	$p < 0.001$
Within treatments	$_4F_{25} = 7.65$	$p < 0.001$

Mean hypaque clearance Low 104.5 ml/min.

High 90.8 ml./min.

been described but the analysis of variance performed on them will now be presented in more detail. The total of 36 clearance periods provides 35 degrees of freedom for estimation of the total variance. These are partitioned as follows:- 5 degrees of freedom for the variance between the six subjects, 2 for variance due to order within the three "Low" plasma level clearances and 2 for the corresponding "High" clearances making 4 for the "within treatment" variance; there is 1 degree of freedom for comparison between the treatments (Low and High) accounting in all for 10 degrees of freedom and leaving 25 for the interaction, subjects × treatment × order, which supplies the estimate of error. The total sum of squares is partitioned in corresponding fashion. Table 6 shows the results for the ratio and Table 7 and Table 8 the results of performing the same analysis on the actual clearances of inulin and Hypaque respectively. The effect of unlabelled Hypaque on the ratio is the most important result. Referring to Table 6 it is clear that all the effects are significant at 1% or less, that is the variance between subjects, that due to order, and the effect of Hypaque are all significant compared to the error variance. The mean value of the ratio before added Hypaque is 1.05 while after unlabelled Hypaque it is 1.01 and the change is significant at 1%. Although the data when analysed in this way allows the conclusion that a significant change in the ratio has occurred after Hypaque there is no such satisfactory means of testing the difference of the ratio from unity. To perform such a test the only estimate available of the variance of the lower ratio (expressed here as percentage, 101%) is that derived from the total mean square/

square which includes all the sources of variation. The variance derived in this way of the ratio after Hypaque is ± 1.2% making it clear that a ratio of 101% cannot be distinguished from a ratio of 100% (corresponding to unity) on the basis of the data available. This being so, as it has been clearly established that there is s significant difference in the ratio before and after Hypaque, it is reasonable to conclude that the effect of adding unlabelled Hypaque is to significantly depress the ratio to a value indistinguishable from unity. This result agrees with the prediction on the hypothesis that when only tracer doses of Hypaque are used, a small proportion is secreted by the tubules and that the tubular secretory mechanism can be saturated by elevating the plasma Hypaque level. The hypothesis of tubular secretion provides the simplest and most probable explanation of the results. Mean values of the ratio of less than unity were not found in either of the groups of studies and the present data does not support the suggestion that reabsorption of Hypaque occurs in man.

It is of interest that a similar analysis of the actual clearances (Tables 7 and 8) shows significant falls in both inulin and Hypaque clearances after administration of Hypaque. As it is hardly possible that Hypaque could depress the inulin clearance while the rate of glomerular filtration remained unchanged the G.F.R. must have fallen, on average, during the latter half of the study. This is probably an effect unrelated to the administration of Hypaque but may be related to loss of sodium and water due to the continuous osmotic diuresis maintained/

maintained throughout the experiments.

The small amount of tubular secretion of Hypaque, when tracer levels are used is unlikely to be of significance in most situations where a measure of G.F.R. is required in man, as, on average, it amounts to only about 5% of the simultaneous inulin clearance and in any event inulin clearances are difficult to determine with errors less than 5%. Labelled Hypaque is easily estimated in plasma and urine, no protein precipitation is required and the technique is much less exacting than that required for inulin estimation, and, if necessary, there should be no difficulty in reducing the effect of tubular secretion by adding unlabelled Hypaque to the infusion. It would appear that for most purposes the inulin clearance in man could be replaced by the clearance of radioactive labelled Hypaque without any loss and with a considerable saving in laboratory time.

SUMMARY

1. Studies in man of the simultaneous renal clearances of Hypaque labelled with ^{125}I or ^{131}I and inulin over a total of 91 clearance periods indicated that there was a linear correlation between the clearances and also that the Hypaque clearances tended to be higher than that of inulin suggesting that there was some tubular secretion of Hypaque.

2. The effect of unlabelled Hypaque on the ratio Hypaque clearance/ inulin clearance was studied in 6 subjects.

3. The mean ratio with tracer Hypaque only was 1.05 but after unlabelled Hypaque it fell to 1.01 and this change is statistically significant ($p < 0.01$). This supports the hypothesis that although Hypaque is mainly excreted by glomerular filtration some tubular secretion also occurs resulting in clearances about 5% higher than the simultaneous inulin clearance.

4. There was no evidence to suggest tubular reabsorption of Hypaque.

5. The inulin clearance in man can be replaced for many purposes by the clearance of radioactive labelled Hypaque, in critical studies the effect of tubular secretion can be minimised by adding unlabelled Hypaque to the infusion.

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