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A Comparison of the Effects of Chlorothiazide, Quinethazone and Placebo on Student Volunteers and on Rats:

A Teaching Exercise

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Medical student participation in a controlled double-blind clinical bioassay provides an effective introduction to clinical pharmacology and perhaps the best stimulus to the future rational evaluation and use of drugs. In one such exercise, 27 volunteers were divided into three groups: one received 50 mg. quinethazone, one 500 mg. chlorothiazide and the third a lactose placebo. Urine was collected for three 90-minute periods, volume and pH being recorded; sodium and potassium were measured with a flame photometer, and chloride by the Volhard technique. Although this study was primarily a comparative bioassay of two established diuretics against a placebo, no previous direct comparisons of these diuretics could be found in the literature. The diuretic activity of chlorothiazide and quinethazone compared to placebo therapy was confirmed in both humans and rats, the use of controls was illustrated, and a higher mean sodium-potassium ratio for quinethazone than for chlorothiazide was demonstrated.

La participation d'étudiants en médecine à des essais cliniques à double inconnue a été une introduction efficace à la pharmacologie clinique et représente peut-être le meilleur stimulant qui soit pour l'évaluation rationnelle des médicaments dans l'avenir. Pour une seule de ces expériences, les 27 volontaires ont été répartis en trois groupes: l'un a reçu 50 mg de quinéthazone, l'autre 500 mg de chlorothiazide et le troisième un placébo à base de lactose. L'urine a été recueillie pendant trois périodes de 90 minutes chacune; leur volume et leur pH ont été notés; on a mesuré le sodium et le potassium au moyen d'un photomètre à flamme et le chlorure par la méthode de Volhard. Cette étude n'était principalement qu'un essai comparatif de deux diurétiques classiques par rapport à un placébo. On ne trouve cependant pas, dans la documentation, de comparaisons directes antérieures de ces deux diurétiques. Cette étude a permis de comparer l'activité diurétique du chlorothiazide et du quinéthazone par rapport au placébo, d'illustrer l'emploi de sujets-témoins et de mettre en évidence l'existence d'un rapport moyen sodium/potassium plus élevé pour le quinéthazone que pour le chlorothiazide.

DURING a laboratory exercise in which the effects of diuretic agents on anesthetized rabbits and conscious rats were being compared, many second-year medical students spontaneously suggested that they should measure the effects of the drugs on themselves. The importance of introducing students to clinical pharmacology, particularly to experimental design and the evaluation of results,[1] is obvious. The criteria for participating in such an experiment were decided upon in consultation with Dr. F. S. Brien, the Professor of Medicine, and were that:

(a) Only healthy *volunteers* would be accepted.
(b) The use of any other medication would disqualify a student.
(c) A history of gout, diabetes, allergy or cardiac, hepatic or renal disease would also lead to disqualification. In addition, a physician was present throughout each experiment and was available, on call, for the remainder of the day.

METHOD

Human Studies

Twenty-seven volunteers were obtained from the 30 students scheduled to perform this exercise on diuretics. The study was double-blind and each student took only one of three treatments; accordingly, nine students took part on each of three consecutive Mondays. The students were assigned to the treatment group (chlorothiazide, quinethazone or placebo) by drawing lots, except that if a student gave a history of hay fever but had no other symptoms of allergy, he was arbitrarily allotted to the placebo group. In this way, at the end of the three-week period, a total of nine students had been studied with each treatment. A coded prescription was written for each student, and the student picked up the medication personally from the pharmacy. The tablets supplied by the pharmacy were as follows: chlorothiazide (Empire Laboratories), 500 mg.; quinethazone (Aquamox, Lederle), 50 mg.; the placebo used was a lactose tablet prepared by Will Pharmaceuticals. The students were instructed to take the various tablets with a glass of water, after voiding, at 8:30 a.m. on the day of the experiment.

Each group reported to the medical school cafeteria at 9:00 a.m. and were supplied with a standard breakfast of cereal, toast and milk. Fruit juice, coffee and chocolate milk were not permitted. Additional water was supplied to provide a mean fluid intake of 800 ml. Three consecutive, 90-minute urine samples were collected from each student; during the collection period, smoking, drinking of water or other fluids, eating or excessive exercise were not permitted. The urinary volume was recorded; the pH was measured using a Radiometer pH meter with a microelectrode, and the sodium and potassium concentrations were determined using a flame photometer. Chloride was measured from 1-ml. aliquots of urine by the Volhard back-titration method. All necessary dilutions were made

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in volumetric glassware with distilled de-ionized water.

Rat Studies

Eighteen female Wistar rats weighing 250-300 g. were randomly divided into two equal groups so that the total weight of each group was similar. The animals were placed in separate metabolism cages on the same rack in a quiet room. About 18 hours before the urine collections were started, the rats were placed on a sugar-cube diet to supply their caloric intake and were allowed water *ad libitum*. Urine was collected under mineral oil to prevent evaporative losses. The 18-hour individual urine samples from each group were then pooled and the total volumes were used as an index of the degree of similarity between the two experimental groups. One group, decided by lot, was given the diuretic and the other an equal volume (1 ml.) of saline intraperitoneally. To dissolve the diuretic drug for intraperitoneal injection it was necessary to add sodium hydroxide; a similar amount of sodium hydroxide was added to the control saline. Urine was collected as a single specimen for a four-hour period after the injection. Samples from each group were pooled and the volumes, pH and ion concentrations measured, as described above. Two diuretics, chlorothiazide* (Diuril) and quinethazone† (Aquamox), were used in this study, and were given on alternate Mondays for six weeks; saline controls were used on each experimental day.

RESULTS

Human Studies

Table I shows the mean output per hour of urine and electrolytes for the nine students on each treatment. Both chlorothiazide and quinethazone caused significant increases in output of fluid and excretion of chloride and sodium over the amounts excreted after administration of the placebo. The difference between the potassium levels of the drug and placebo groups is not significant. The mean pH and the mean sodium:potassium ratio are shown. The lower value of this ratio was calculated as the mean sodium — S.E.M.:mean potassium + S.E.M. and the higher value as the mean sodium + S.E.M.:mean potassium — S.E.M. The mean electrolyte excretion patterns per hour are shown in Fig. 1. The actual values obtained for each of the three 90-minute samples are given in Table II and Fig. 2. It is clear that the fluid outputs following the two drug treatments are comparable. There was also a high urine output in the placebo group, at least during the first three hours of collection; this probably resulted from the water-loading technique employed. Of note are the sustained increased chloride excretion with quinethazone, and the decreased chloride excretion observed in the third 90-minute sample after chlorothiazide treatment. Also worthy of note are the higher mean pH values in Tables I and II for the chlorothiazide-treated subjects than for the quinethazone-treated subjects. Moreover, the mean sodium:potassium ratios at each 90-minute period are higher for quinethazone than for chlorothiazide. Both are higher than the placebo ratios at all times.

TABLE I.—HUMAN STUDIES: MEAN OUPUT OF URINE AND ELECTROLYTES PER HOUR CALCULATED FROM THE TOTAL 4.5-HOUR COLLECTION, MEAN pH, AND MEAN SODIUM:POTASSIUM RATIO AFTER PLACEBO, CHLOROTHIAZIDE AND QUINETHAZONE TREATMENT

Treatment	*Volume ml. (mean)**	*Chloride mEq. (mean)*	*Sodium mEq. (mean)*	*Potassium mEq. (mean)*	*pH (mean)*	*Mean sodium/potassium ratio (and range)***
Placebo.....	96.4 ± 18.37	16.9 ± 2.49	5.94 ± 1.073	3.81 ± 0.686	5.99 ± 0.133	1.56 (1.08 to 2.25)
Chlorothiazide 500 mg....	154.7 ± 17.38	37.8 ± 4.24	17.34 ± 2.121	5.94 ± 0.694	+6.31 ± 0.209	2.92 (2.30 to 3.71)
Quinethazone 50 mg.....	147.8 ± 16.24	41.4 ± 2.26	21.60 ± 1.393	5.01 ± 0.418	+5.63 ± 0.113	4.31 (3.73 to 5.01)

*Mean ± standard error of the mean for n = nine subjects.
**Range of sodium:potassium ratio.
(Mean sodium — S.E. of M) : (Mean potassium — S.E. of M).
and (Mean sodium + S.E. of M) : (Mean potassium — S.E. of M).
+P = 0.02 - 0.01.

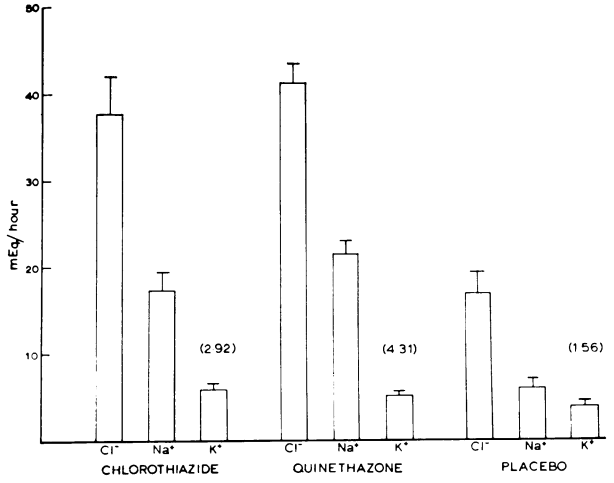

Fig. 1.—Human studies: mean output of electrolytes per hour after chlorothiazide, quinethazone and placebo treatment calculated from the total 4.5-hour collection. Standard errors of the means for n = nine subjects are indicated. Mean sodium:potassium ratios are shown in parenthesis.

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TABLE II.—HUMAN STUDIES: MEAN OUTPUT OF URINE AND ELECTROLYTES, pH AND SODIUM:POTASSIUM RATIO DURING THREE (I, II AND III) 90-MINUTE COLLECTION PERIODS AFTER PLACEBO, CHLOROTHIAZIDE AND QUINETHAZONE TREATMENT.

Treatment	*Number of collection*	*Volume ml. (mean)**	*Chloride mEq. (mean)*	*Sodium mEq. (mean)*	*Potassium mEq. (mean)*	pH *(mean)*	*Mean sodium/potassium ratio (and range)***
Placebo	I	100.7 ± 20.86	20.6 ± 3.57	10.49 ± 2.004	4.60 ± 0.757	6.02 ± 0.153	2.28 (1.58 to 3.25)
	II	209.7 ± 58.12	26.9 ± 4.93	9.32 ± 3.030	8.09 ± 2.604	5.87 ± 0.139	1.15 (0.59 to 2.25)
	III	63.8 ± 19.49	28.5 ± 4.90	7.90 ± 1.539	4.70 ± 0.732	5.96 ± 0.284	1.68 (1.17 to 2.38)
Chlorothiazide 500 mg.	I	257.6 ± 48.87	67.3 ± 10.68	28.80 ± 5.346	7.21 ± 0.945	6.22 ± 0.149	4.00 (2.87 to 5.45)
	II	280.0 ± 35.83	63.0 ± 8.03	25.44 ± 3.460	10.89 ± 1.874	6.49 ± 0.182	2.34 (1.72 to 3.21)
	III	150.0 ± 21.81	39.7 ± 4.64	21.88 ± 3.514	8.78 ± 1.432	6.52 ± 0.304	2.49 (1.80 to 3.46)
Quinethazone 50 mg.	I	234.6 ± 30.82	55.6 ± 1.79	32.59 ± 2.434	6.33 ± 0.450	5.54 ± 0.128	5.15 (4.45 to 5.95)
	II	263.0 ± 42.16	67.7 ± 9.92	33.97 ± 2.842	8.03 ± 0.785	5.79 ± 0.150	4.23 (3.53 to 5.08)
	III	170.8 ± 10.80	62.7 ± 4.11	30.66 ± 3.160	8.37 ± 0.913	5.99 ± 0.236	3.66 (2.96 to 4.54)

*Mean ± standard error of the mean for n = nine subjects.
**Range calculated as in Table I.

Because previous studies[2-5] with quinethazone and chlorothiazide have presented not the absolute values obtained after the diuretics but the increased fluid and electrolyte levels over placebo or control levels, these values were calculated. The increased output per hour was calculated for each drug each day by subtracting the mean value for the three placebo subjects that day from the mean of the three drug-treated subjects on that day. The overall *mean* increased output per hour (Table III and Fig. 3) was thus the average of the results obtained during the three experimental days. Table IV and Fig. 4 show the corresponding mean values for each of the 90-minute collection periods.

The results (Tables III and IV; Figs. 3 and 4) show the similarity in effectiveness of 500 mg. of chlorothiazide and 50 mg. quinethazone, at least as far as fluid output is concerned.

Rat Studies

The results are summarized in Table V and Fig. 5. Although we should have obtained six pooled control urine samples and three pooled samples for each compound, unfortunately one of the samples from the chlorothiazide-treated rats was not analyzed and on two days in all nine of the saline-treated rats no measurable urine was produced during the collection period. However,

TABLE III.—HUMAN STUDIES: MEAN INCREASED OUTPUT OF URINE AND ELECTROLYTES PER HOUR IN THREE SUBJECTS AFTER CHLOROTHIAZIDE AND QUINETHAZONE OVER THE MEAN OUTPUT OF THREE PLACEBO-TREATED SUBJECTS ON THE SAME DAY. VALUES GIVEN ARE THE MEANS ± STANDARD ERROR OF THE MEAN FOR N = THREE DAYS' STUDY, i.e. RESULTS OF NINE SUBJECTS ON EACH TREATMENT. MEAN INCREASED SODIUM:INCREASED POTASSIUM RANGE CALCULATED AS IN TABLE I.

Treatment	*Increased volume ml. (mean)*	*Increased chloride mEq. (mean)*	*Increased sodium mEq. (mean)*	*Increased potassium mEq. (mean)*	*Mean sodium/potassium ratio (and range)*
Chlorothiazide 500 mg...	67.3 ± 1.68	20.87 ± 4.057	*10.93 ± 0.849	**2.11 ± 0.819	5.18 (3.44 to 9.12)
Quinethazone 50 mg....	63.2 ± 15.86	23.50 ± 1.577	*15.62 ± 1.095	**1.19 ± 0.994	13.13 (6.65 to 85.28)

*P = 0.01 - 0.001.
**P = 0.5 - 0.4.

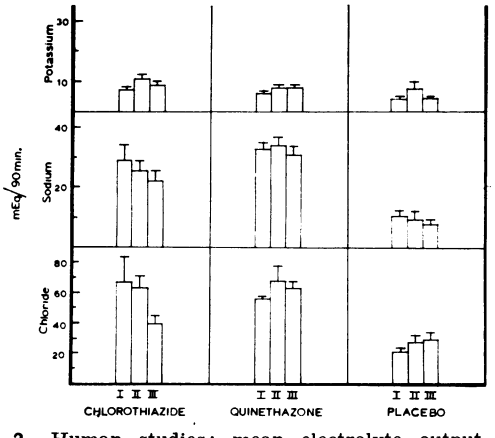

Fig. 2.—Human studies: mean electrolyte output after chlorothiazide, quinethazone and placebo treatment during each of three (I, II and III) 90-minute collection periods. Standard errors of the means for n = nine subjects are indicated.

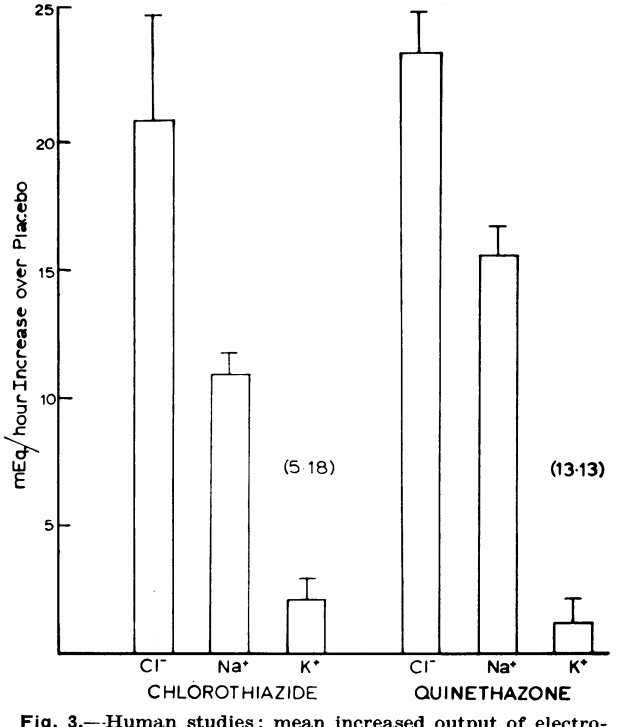

Fig. 3.—Human studies: mean increased output of electrolytes per hour in three subjects after chlorothiazide and quinethazone over the mean output of three placebo-treated subjects on the same day. Values given are the mean ± standard error of the mean for n = three days' study, i.e. results of nine subjects on each treatment. Mean increased sodium-increased potassium ratios are shown in parenthesis.

TABLE IV.—HUMAN STUDIES: MEAN INCREASED OUTPUT OF URINE AND ELECTROLYTES IN EACH OF THREE (I, II AND III) 90-MINUTE COLLECTION PERIODS IN THREE SUBJECTS AFTER CHLOROTHIAZIDE AND QUINETHAZONE, COMPARED WITH THE MEAN OUTPUT OF THREE PLACEBO-TREATED SUBJECTS ON THE SAME DAY. VALUES GIVEN ARE THE MEANS ± STANDARD ERROR OF THE MEAN FOR N = THREE DAYS' STUDY, i.e. RESULTS OF NINE SUBJECTS ON EACH TREATMENT.

Treatment	*Number of collection*	*Increased volume ml./90 min. (mean)*	*Increased chloride mEq./90 min. (mean)*	*Increased sodium mEq./90 min. (mean)*	*Increased potassium mEq./90 min. (mean)*
Chlorothiazide 500 mg.....	I	156.9 ± 44.71	45.96 ± 7.637	18.32 ± 2.874	2.62 ± 0.748
	II	70.3 ± 57.01	36.10 ± 5.348	16.14 ± 2.724	2.80 ± 3.712
	III	75.5 ± 3.08	11.19 ± 5.839	14.74 ± 0.742	4.08 ± 1.768
Quinethazone 50 mg.....	I	134.0 ± 29.81	34.98 ± 2.726	22.11 ± 2.714	1.74 ± 0.734
	II	53.3 ± 62.63	36.57 ± 7.500	24.65 ± 4.265	—0.05 ± 4.690
	III	97.2 ± 18.65	34.21 ± 1.374	23.52 ± 2.282	3.67 ± 0.817

these results clearly demonstrate the marked diuretic activity of chlorothiazide and quinethazone on rats: there was a significantly increased flow of urine and output of sodium, potassium and chloride. Too few experiments were performed to detect any significant difference that might exist between the two drugs.

The greatest difference between the two treatments was in chloride excretion, but even here the student 't'-test gave a P value of only 0.1 to 0.2. As in the human studies, the mean pH after chlorothiazide is higher than after quinethazone. In the rats, however, the saline control group had a pH similar to the chlorothiazide group.

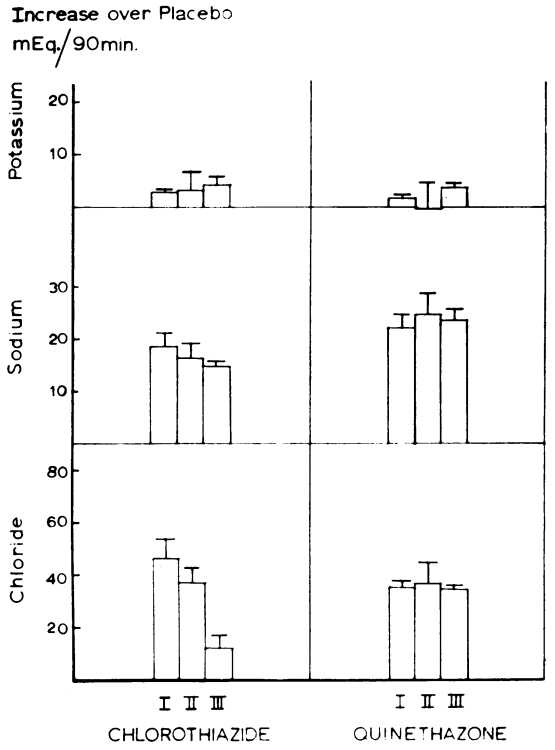

Fig. 4.—Human studies: mean increased output of electrolytes per each of three (I, II and III) 90-minute collection periods in three subjects after chlorothiazide and quinethazone over the mean output of three placebo-treated subjects on the same day. Values given are the means ± standard error of the mean for n = three days' study, i.e. results of nine subjects on each treatment.

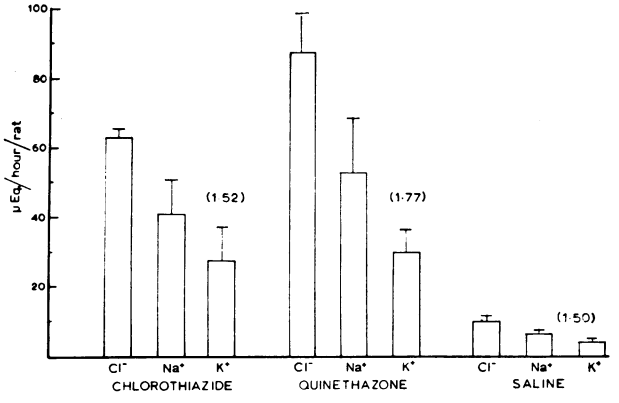

Fig. 5.—Rat studies: mean output of electrolytes per hour per rat after chlorothiazide, quinethazone and saline treatment calculated from the pooled four-hour collections from nine rats. Values given are the means ± standard error of the mean for n = two to five days' study (as in Table V). Mean sodium:potassium ratios are shown in parentheses.

DISCUSSION

These experiments demonstrate the parallel qualitative diuretic activity of chlorothiazide and quinethazone in human subjects and rats. No clear-cut statistical significance can be attached to such a small-scale study, but the trend of the results

TABLE V.—RAT STUDIES: MEAN OUTPUT OF URINE AND ELECTROLYTES PER HOUR PER RAT AFTER SALINE, CHLOROTHIAZIDE, AND QUINETHAZONE, CALCULATED FROM THE POOLED FOUR-HOUR COLLECTIONS FROM NINE RATS. VALUES GIVEN ARE THE MEANS ± STANDARD ERROR OF THE MEAN FOR *n = TWO TO FIVE DAYS' STUDY AS SHOWN IN PARENTHESES. MEAN pH AND SODIUM: POTASSIUM RATIO ARE GIVEN

Treatment	*Volume of urine (ml.)*	*Chloride (µEq.)*	*Sodium (µEq.)*	*Potassium (µEq.)*	*Mean pH*	*Mean sodium/potassium ratio (and range)*
Saline.....	0.11 ± 0.039 (4)*	9.77 ± 1.41 (3)	6.08 ± 1.528 (4)	4.05 ± 1.252 (4)	7.51 ± 0.345 (5)	1.50 (0.86 to 2.72)
Chlorothiazide 10 mg.....	0.37 ± 0.001 (2)	63.30 ± 2.70 (2)	41.15 ± 9.950 (2)	27.30 ± 9.800 (2)	7.65 ± 0.350 (2)	1.52 (0.84 to 2.92)
Quinethazone 1 mg.....	0.46 ± 0.078 (3)	88.20 ± 11.48 (3)	53.0 ± 16.030 (3)	30.0 ± 6.570 (3)	6.63 ± 0.775 (2)	1.77 (1.01 to 2.95)

indicates agreement with those in other relevant publications and demonstrates both similarities and differences between the agents employed.

The importance of controls and of a double-blind technique was clearly demonstrated by the record of side effects on the first day. At 11:00 a.m. one subject, B.H., complained of headache. He had no previous history of headaches and for this reason was told by the attending physician that he was free to take fluid and electrolyte replacements and to terminate the experiment. However, he asked to be allowed to continue until the final collection was completed (1:00 p.m.). After 1:00 p.m. another subject, B.G., complained of "dizziness and uneasiness" and was permitted to leave the class and report back if necessary. When the code was broken (1:15 p.m.), it was found that both subjects had taken a diuretic compound (B.H., quinethazone; B.G., chlorothiazide), and it is conceivable that these were true side effects. However, a number of factors should be considered in assessing the importance of these reported side effects: both students could have guessed that they had taken one of the diuretic drugs because their urine output was more than 1 l. in the four and one-half hour period; moreover, because of the timing of the announcement of the code, B.G. knew he had taken chlorothiazide before reporting symptoms. Even more important, there were pyridine fumes in the laboratory (from a biochemical experiment) and one of the placebo-treated group, as well as two students not involved in the diuretic experiment at all, complained of headache. During a discussion before the second experimental day, we purposely expressed the view that the sickness and headaches were all caused by the pyridine and that more adequate ventilation would be supplied henceforth. It is interesting that no side effects were reported on the second or third experimental days. This experience emphasizes the importance of considering environmental influences and stimuli during the assessment of clinical studies.

Although our primary object was to introduce medical students to practical clinical pharmacology, data concerning two established diuretic agents and a placebo in apparently normal human volunteers were obtained and can be contrasted with the findings of other reports on these compounds, keeping in mind the problems of such comparisons.[6] Results may vary depending upon several factors other than duration of action and dosage frequency. Our study, like some others,[1, 7] was carried out on normal subjects. Seller *et al.*[2] used patients who had no evidence of cardiovascular or renal disease, whereas other studies used compensated cardiac patients who had no detectable edema at the time of study,[4] or bedfast digitalized patients with chronic congestive heart failure.[8]

From reviewing the literature on chlorothiazide and quinethazone, it became apparent that these two agents had not been compared previously in a single study. We also noted that most of the useful studies in which electrolyte excretion had been recorded involved as few subjects as did our own study, e.g. Seller *et al.*[2] used only eight patients in their studies on quinethazone. Their results that quinethazone is a clinically useful diuretic are corroborated by our work, but their values are for 24 hours whereas ours are only for a four and one-half hour period. Furthermore, we used 50 mg. instead of 200 mg. of quinethazone. Nevertheless, their fig. 3, which gives the sodium-potassium ratio as a measure of the increase in excretion over control levels, may be compared to the values in our Table III and Fig. 3. Our estimates of 5.18 and 13.13 (mean sodium/potassium ratios) for 500 mg. chlorothiazide and 50 mg. quinethazone, respectively, obtained in the same study may not represent significant differences. It is thus unlikely that Seller's ratios of 2.5 for 1000 mg. chlorothiazide and 4.5 for 200 mg. quinethazone in apparently separate bioassays are significantly different. Our higher average for nine subjects on 50 mg. quinethazone is in keeping with the calculated value of 9.1 for the three patients on 50 mg. quinethazone in Seller's table I.

Ford[3] used a dose of 50 mg. quinethazone in a study of 10 non-edematous patients with hypertensive cardiovascular disease; from his results a mean sodium:potassium ratio of 6.22 may be calculated for the 24-hour samples. In addition, measurement of his histograms[3] from five patients allows calculation of a sodium:potassium ratio of 5.7 for the two- to four-hour samples and 3.8 for the four- to six-hour samples.

In another study Ford, Moyer and Spurr[4] gave 500-1000 mg. chlorothiazide to 10 men with previous congestive heart failure. From their results[4] we calculated sodium:potassium ratios of 2.33, 1.74 and 2.84 for the increased nil to two-hour, two- to four-hour and four- to six-hour outputs. And from yet another study of Ford's,[5] using 1000 mg. chlorothiazide in 10 cardiac patients with mild edema, we calculated a mean ratio of 2.44 for the increased sodium:potassium excretion over 24 hours.

Without regard to drug dosage or duration of urine collection, we have calculated from all sources, sodium:potassium ratios of 5.18, 2.4, 2.33, 1.74, 2.83 and 2.44 (mean of 2.8 $\pm$ S.E.M. 0.49) for chlorothiazide, and ratios of 13.13, 4.5, 9.1, 6.22, 5.7 and 3.8 (mean of 7.1 $\pm$ 1.42) for quinethazone. Such a misuse of statistics gives a P value (t-test) of less than 0.02. Although our own values of 5.18 and 13.13 are not significantly different, if the possible ranges are considered (Table III), there is a distinct possibility that the ratios would become significantly different if the number of subjects were to be increased. If this be the case, it is important to know what is responsible

for the higher ratio. Does the diuretic for which a manufacturer claims a high sodium:potassium excretion ratio achieve the higher ratio by causing excretion of less potassium (in absolute terms) or is the potassium loss about the same and the sodium much greater? Hypokalemia has been a worrisome consideration with intensive diuretic therapy; in the past it has been the practice to compensate for excessive potassium loss by administering potassium chloride *per os*. Recently, however, it has been reported that enteric-coated potassium chloride supplements have given rise to small-bowel ulceration in patients,[9] dogs[10] and monkeys.[11] This fact emphasizes the importance of careful selection of a diuretic agent.

In the present experiments and in the other studies cited, there is no statistical evidence that less potassium is lost after quinethazone than after chlorothiazide. In our experiments, however, during the second 90-minute collection after administration of quinethazone the mean output of potassium was less than that of the placebo group. In a larger study, or after a longer period of collection, some statistical difference between the sodium/potassium ratios of these two drugs may be uncovered. What was evident from our study was that, in the doses given, quinethazone produced a significantly (P=0.01 - 0.001) greater natriuretic effect than did chlorothiazide, suggesting that if the aim of therapy is to increase the excretion of sodium, quinethazone will be about 50% more effective than chlorothiazide for the same amount of potassium loss, even in conditions in which their effect on urine is not significantly different. This agrees with Ford's published results of studies done in 1960[5] and 1962,[3] but in the latter paper Ford, Moyer and Spurr claimed that when they compared their earlier results after chlorothiazide[4] with those after quinethazone,[3] there was significantly less potassium loss after quinethazone. This apparent confusion is not unexpected because the two diuretics were not compared at the same time or even in the same type of patient. Other work[2] has shown that doses of 200 mg. quinethazone given to 26 patients for six weeks did not significantly alter the serum potassium level, suggesting that with this drug hypokalemia is not a danger.

In our study the mean urinary pH for the chlorothiazide-treated group, 6.31, was significantly higher (P=0.02 - 0.01) than that of the quinethazone-treated group, 5.63, which implies that the mechanism of action of the two compounds is not identical. Ford, Moyer and Spurr[4] obtained pH values of 7.1 and 6.6 for the four- and six-hour samples after 500 to 1000 mg. of chlorothiazide, and show that the urinary pH after 25 mg. quinethazone did not rise above 6.0.[3] If the students in the present study had measured bicarbonate excretion, they would probably have found that the higher urinary pH after administration of chlorothiazide was due to increased loss of this ion.[4] It may be that the excretion of less chloride during the third collection period after chlorothiazide was coincident with a rise in the excretion of bicarbonate similar to that noted by Ford, Moyer and Spurr.[4] If one accepts that increased excretion of bicarbonate and of potassium is evidence of carbonic anhydrase inhibition,[12] less potassium loss would be expected after quinethazone than after chlorothiazide.

It is of interest that quinethazone appears to have comparable diuretic effects on normal human subjects and on rats, whereas another new clinically effective diuretic, ethacrynic acid, is apparently ineffective in rats.[13]

As a teaching exercise this assay might have been more elegant had: (a) the sodium and potassium contents of the standard breakfast been calculated, (b) the loss of body weight following the diuretic agents been measured, *cf.* Bloomfield and Tetréault,[1] or (c) sodium chloride been administered[1, 14] in an amount which did not lead to retention of salt relative to water or water relative to salt.[15] However, it is interesting to note the close agreement between our results with chlorothiazide and those of Martz *et al.*,[14] who gave chlorothiazide with sodium chloride supplements. When nine normal subjects given 500 mg. chlorothiazide were compared with nine placebo-treated subjects, we found a mean increased excretion of sodium of 10.93 ± 0.85 (S.E.M.) mEq./hr. during a 4.5-hour period. Calculations from the results of Martz *et al.* show that after the same dose of chlorothiazide but with added sodium chloride the mean urine output increased by 13.3 ± 2.13 mEq./hr. over that measured during placebo therapy in the same eight normal subjects during a five-hour period.

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