The results presented here with dextroamphetamine sulfate in mice pretreated with reserpine solution are in agreement with those reported by Smith (3) who used reserpine solubilized with ascorbic acid to pretreat his mice. The results obtained with both dextroamphetamine sulfate and benzphetamine hydrochloride in mice pretreated with the reserpine solution do not agree, however, with those of van Rossum *et al.* (1, 2) who used acetic acid to solubilize the reserpine (personal communication). Only when the mice were pretreated with a reserpine suspension could the results reported by van Rossum *et al.* be repeated.

Brain amine levels after pretreatment with either the reserpine solution or suspension for 3 days at 1 mg./Kg./day do not explain the differences in results obtained on locomotor activity. These preparations both depleted measurable brain amine levels about the same extent.

The results shown in Fig. 3 indicate that whether the reserpine is in solution or suspension and the pH of the diluent influence the initial rate of reserpine uptake and the level of reserpine attained in the brain. This in turn is thought to influence the rate of decline in the brain amine levels. The pH of the reserpine solution used to pretreat the mice for the locomotor activity studies was 3.5 and therefore gave higher and more rapid reserpine levels in the brain and more rapid decline of brain amine than did the reserpine suspension which had a pH of 5.8. If the reasoning of Stein (12) is correct that reserpine pretreatment may cause supersensitivity to amines in the brain as well as in the periphery, and if the sensitivity is increased with time then the increased locomotor response in the mice pretreated with the reserpine solution may be due to the increased length of time these mice had to develop the supersensitivity.

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Keyphrases

Reserpine activity—diluent effect
Methylcellulose—reserpine activity
Citric acid solution—reserpine activity
Dextroamphetamine SO_4 activity—reserpine–diluent effect
Benzphetamine HCl activity—reserpine–diluent effect
Brain, mouse—analysis
Locomotor activity—analysis

Potassium Absorption—A Comparison of *In Vitro* and *In Vivo* Studies

By A. J. JOUHAR, E. S. GARNETT, and J. S. WALLINGTON

Some ^{42}K was released from a slow release preparation within 15 min. of ingestion and further potassium release continued for approximately 200 min. A similar absorption pattern was found from enteric-coated tablets of potassium chloride although a considerable initial delay occurred before potassium was released from this preparation. The *in vivo* behavior of the slow release preparation and enteric-coated tablets was similar to that seen in static solution experiments but differed considerably from that found in the B.P. disintegration apparatus. There was no evidence that sudden release and absorption of potassium took place from enteric-coated tablets.

LONG CONTINUED ADMINISTRATION of thiazide diuretics reduces exchangeable potassium, and the need for potassium supplements is well recognized (1, 2). These may be given separately or in a compound tablet, such as the film-coated, two-layered slow release preparation.[1] In this preparation crystals of potassium chloride (KCl) are coated with a polymeric material and compressed to form one layer, and the other layer contains bendrofluazide. The KCl layer is intended to dissolve gradually and so release potassium ions (K^+) continuously. Since it has been

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[1] Tradename of Neo-NaClex-K.

shown radiologically that this preparation passes down the gut (3), localized high concentrations of K^+, which induce gastric irritation and are believed to cause intestinal ulceration (4–6), may be prevented. Such slow release preparations are formulated with the help of *in vitro* control methods which should ensure uniform behavior in the gastrointestinal tract. In this investigation, the authors have compared the results of such *in vitro* experiments with *in vivo* ^{42}K absorption studies in man.

MATERIALS AND METHODS OF MANUFACTURE

Tablets for *in vivo* studies were made containing ^{42}KCl, after it was shown by microscopy that irradiation to give a specific activity at the time of administration of 70–80 μc. per dose did not produce any change in the crystals of KCl. The radiochemical purity of ^{42}KCl was 100% at the time of administration.

The usual production process for the slow release preparation of granulating KCl crystals to form a damp mass was not possible because of difficulty in manipulating 2 ft. 6 in. long hand-operated tongs behind a screen of 1 in. perspex. Instead, a remotely controlled microstirrer was used to mix the ^{42}KCl crystals into a slurry with a granulating solution of coating materials dissolved in excess solvent.

The slurry was dried over an electrically heated mantle at 70° and the solid residue was forced through 8- and 16-mesh sieves with a large glass stopper. Weighed quantities of granules were fed into a hand-operated tablet machine, with a small glass weighing boat designed so that it could be easily picked up with the tongs. After adding inert granules in place of bendrofluazide to form the second layer, the tablet was ejected from the machine. The tablets were film-coated by dipping in warm film-coating solution, and allowing them to dry on a pin bed in a warm air stream.

Enteric-coated tablets of ^{42}KCl were made by directly compressing the weighed crystals in the hand-tablet machine. Each tablet was coated by dipping into cellulose acetate phthalate/vinyl acetate copolymer/solvent solution several times. The slow release tablets and the enteric-coated tablets both contained 630 mg. KCl (8.4 meq. K^+).

Similar quantities of ^{42}KCl were made into solution for the *in vivo* studies. Nonradioactive preparations were used for the *in vitro* work.

In Vitro Studies—(*a*) *Static Solution*—The extent of release of KCl from the tablets was measured at intervals of 20 min. for 7 hr. by the following method: 120 ml. of the double-distilled water or buffered solutions (pH 7.9 and 8.9) at 20° were placed in each of 40 polythene pots. One tablet of the slow release preparation or an enteric-coated KCl tablet was placed in each of two pots at 20-min. intervals for 400 min. Twenty minutes after the last tablets had been placed in the pots, all the tablets were removed. The potassium chloride concentration of the solution remaining was then measured with a Pye conductance bridge, and the rate of release plotted.

(*b*) *Solution in the B.P. Disintegration Apparatus*—Three tablets of the slow release preparation or three enteric-coated KCl tablets were placed in a B.P. disintegration apparatus and agitated according to the B.P. monograph (8). The amounts of KCl released in periods of 5 min. were measured with a Pye conductance bridge.

In Vivo Studies—Male volunteers were fasted overnight and then given a solution of ^{42}KCl, two radioactive slow release tablets, or two enteric-coated ^{42}KCl tablets. Blood samples, each of 20 ml., were taken from an uncuffed arm vein at intervals of 5 to 100 min. for the next 320–540 min.

The plasma from each sample was separated at once and the ^{42}K content of 10-ml. aliquots was measured in a M64 Geiger-Müller liquid counter connected to an EKCO Automatic Scaler Type N530F through an EKCO N558 probe unit with the dead time set at 300 μsec. The background count rate of this system was 12 counts per minute (c.p.m.). The plasma samples were counted for 50 min. or until 3,000 counts had been accumulated (whichever was the shorter). Samples were recorded as containing a significant amount of ^{42}K provided they gave a count rate of at least twice background. The maximum count rate achieved in any one experiment was approximately 50 times background. After subtracting background count and correcting for radioactive decay using a ^{42}K half-life of 12.5 hr., the c.p.m. from each sample were expressed as a percentage of the c.p.m. obtained from that sample containing the maximum radioactivity in each subject.

RESULTS

In Vitro Studies—Figure 1 shows the rate of release of KCl from each of the formulations studied.

After 200 min. in static distilled water, 50% of the available KCl had been released from the slow release preparation and after 420 min. 70% of the KCl had been released.

Because the rate of release of KCl from enteric-coated preparations depends upon pH, tests were carried out in static solutions at various pH levels. Thus at pH 7.9, the rate of release of KCl became maximal after 150 min. and was complete after about 420 min. At pH 7.0 and at pH 8.9 the solution rates were faster than at pH 7.9 and were immediately maximal, slowing after 120 min. The release of KCl was complete after about 420 min.

In the B.P. disintegration apparatus all the potassium was made available from the slow release preparation within 80 min. in acid pepsin solution and within 50 min. in alkaline pancreatin solution.

Release of KCl from the enteric-coated preparation in the B.P. disintegration apparatus in alkaline pancreatin solution was delayed for 10 to 15 min. but was complete at 30 min.

In Vivo Studies—Fig. 2 shows the appearance of $^{42}K^+$ in the plasma after ingestion of a solution ^{42}KCl. In all cases, peak activity occurred within 35 min. Figure 3 shows the appearance of $^{42}K^+$ in the plasma from the radioactive slow release preparation. ^{42}K was detected soon after ingestion, but a peak plasma level was not obtained until about 200 min. Figure 4 shows the appearance of $^{42}K^+$ in the plasma after giving enteric-coated ^{42}KCl; no radioactivity was detected in the plasma for at least 120 min. Thereafter, the pattern of absorption paralleled that obtained from the slow release preparation.

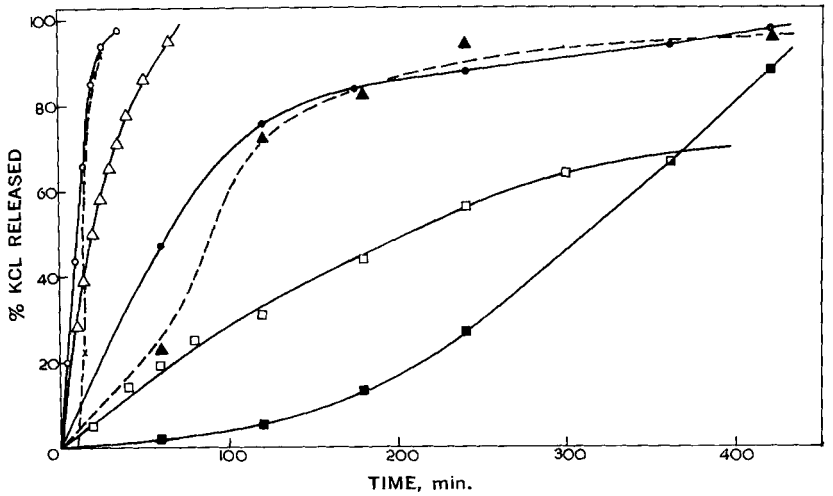

Fig. 1—Release rate in vitro *of KCl from slow release tablets and enteric-coated KCl tablets. B.P. disintegration apparatus held at 37°, and static solutions at 20°. Slow release tablets: ○, B.P. disintegration apparatus—alkaline pancreatin solution; △, B.P. disintegration apparatus—acid pepsin solution; □, static solution at pH 7.0. Enteric-coated KCl: ×, B.P. disintegration apparatus—alkaline pancreatic solution; ▲, static solution at pH 7.0; ■, static solution at pH 7.9; ●, static solution at pH 8.9.*

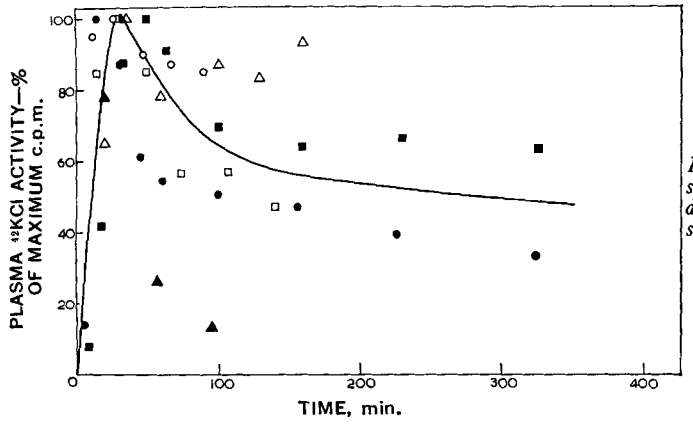

Fig. 2—Plasma ^{42}KCl activity—potassium chloride solution. Mean plot drawn from series of six results. Each symbol represents the results obtained from one human volunteer.

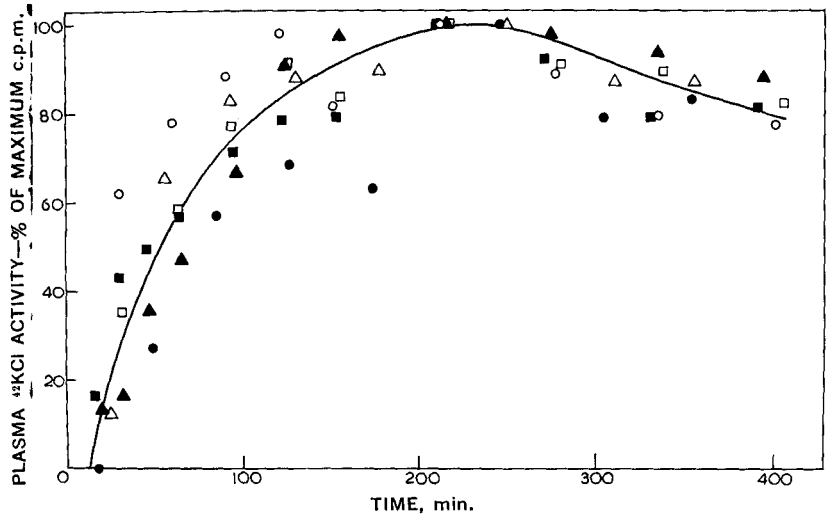

Fig. 3—Plasma ^{42}KCl activity—slow release tablets. Mean plot drawn from series of six results. Each symbol represents the results obtained from one human volunteer.

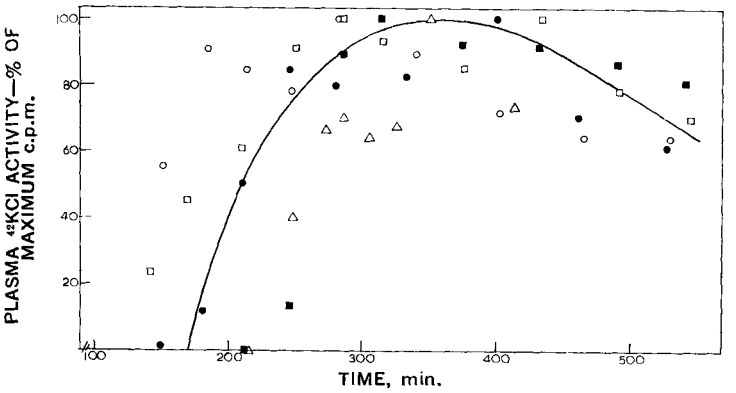

Fig. 4—Plasma ^{42}KCl activity—enteric-coated potassium chloride tablets. Mean plot drawn from series of five results. Each symbol represents the results obtained from one human volunteer.

DISCUSSION

The use of radioactive materials in pharmaceutical formulations presents manufacturing problems as only a few tablets must be specially produced for each investigation. Nevertheless, it has been possible to make radioactive potassium chloride tablets with high specific activity at the time of preparation by using special handling and screening procedures, and with these radioactive preparations it has been possible to study the *in vivo* behavior of a variety of formulations.

The first studies showed that ^{42}K is rapidly absorbed after oral administration of ^{42}KCl in solution. It is assumed that this early absorption occurs in the upper small bowel since activity appeared in the blood within 5 min. of ingestion and peak activity occurred within 30 min. It is unlikely that gastric absorption of ^{42}K contributes significantly to the early uptake curves as Code *et al.* (7) have shown that potassium is absorbed only slowly from the stomach.

When ^{42}KCl was given as the slow release preparation, activity appeared in the plasma within 20 min. indicating that some ^{42}K had been absorbed from the upper small bowel. These results might be expected from the B.P. disintegration experiments which showed that potassium is released early from the slow release preparation both in acid pepsin and alkaline pancreatin solution. They do not, however, give any information about the possible release of ^{42}K from the formulation in the stomach with subsequent absorption from the jejunum.

Following the early appearance of ^{42}K in the plasma after administration of the slow release preparation, the concentration of ^{42}K rose slowly to a maximum at about 220 min. This suggests that ^{42}K was slowly released into the gut, since ^{42}K in solution produced an early peak in plasma activity. The apparent pattern of release is quite different from results obtained in the B.P. disintegration studies in which all of the potassium had been released by 60 min. The pattern accords more nearly to that seen in the static solution experiments in which potassium was released for about 300 min.

The pattern of absorption, once established, from enteric-coated ^{42}KCl tablets was very similar to that seen with the slow release preparation and again, static solution studies seem to give a more realistic measure of tablet breakdown in the intestine. It will be noted however that although the pattern of absorption of ^{42}K from enteric-coated tablets and the slow release preparation is similar, ^{42}K does not appear in the plasma for at least 100 min. after the administration of the enteric-coated preparation. The observed variation in delay probably results from such factors as gastric emptying time and the pH and motility of the small bowel. Thus in one study ^{42}K appeared in the plasma only after 212 min.

It is appreciated that serial measurements of plasma concentration of ^{42}K may not necessarily give a true impression of intestinal absorption since the plasma is only one of at least three compartments between which ^{42}K is distributed. However, since there was no evidence of electrolyte imbalance in any of the subjects, the pattern of ^{42}K redistribution from plasma can be assumed to be qualitatively similar in all of the subjects; thus the pattern of ^{42}K appearance in the plasma reflects uptake from the intestinal tract.

In conclusion, it has been shown that the *in vivo* behavior of the slow release preparation and the enteric-coated tablets is similar to that seen in static solution experiments, and differed considerably from that found in the B.P. disintegration apparatus. Further, there was no evidence for an explos've release of potassium from the enteric-coated tablets.

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Keyphrases

Potassium absorption
In vitro–in vivo absorption compared
^{42}K-Potassium chloride solution and tablets—*in vivo* testing
Liquid scintillation counting—analysis
Potassium chloride tablets—*in vitro* testing
Conductance bridge—analysis
Enteric coating effect—potassium release