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RENAL TOLERATION OF CAFFEIN

HAROLD B. MYERS

From the Laboratory of Pharmacology of the University of Oregon Medical School

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Toleration toward toxic doses of caffein has been produced in cats, dogs and rabbits following the repeated administration of gradually increasing doses of the drug (1). Toleration of toxic doses of caffein suggests the possibility of altered response toward doses of therapeutic degree. Salant and Rieger (2) in a study of chronic intoxication with caffein in dogs, state: "In a large number of experiments . . . . it has been observed that symptoms due to caffein often disappeared when the administration of the same dose of the drug was continued." A measurable decrease in the degree of mental stimulation caused by caffein in human subjects who have received the drug over a period of from four to five weeks has been reported by Wedemeyer (3). An interesting opinion in this regard is that held by many tea and coffee habitués, to the effect that the customary use of caffein containing beverages leads to a moderate degree of toleration of caffein action, especially toward the production of insomnia and diuresis. No data has been found to show whether or not the diuretic action of caffein is altered as a part of the toleration produced.

The cause of the toleration toward caffein has been investigated by Gourewitsch (4). He found large quantities of the drug in the central nervous system and skeletal muscle of caffein tolerant rabbits two days after ceasing injections and concluded that the tissue cells upon which the drug exerts its chief action become less susceptible to that action. It is possible that an increased power of demethylization or oxidation occurs in the body as tolerance is acquired, though no information on this question has been found in the literature.

Measurement of urinary secretion promised a means of definite determination of the production of renal toleration toward caffein, or the lack of it. The rabbit was chosen as the experimental animal. Subcutaneous injections were given six days each week, of a 2 per cent solution of uncombined caffein. The daily dose at first was 50 mgm. per kilogram, a gradual increase being made to 80 and 90 mgm. per kilogram. The rabbits appeared to remain in the best of health with this dosage.

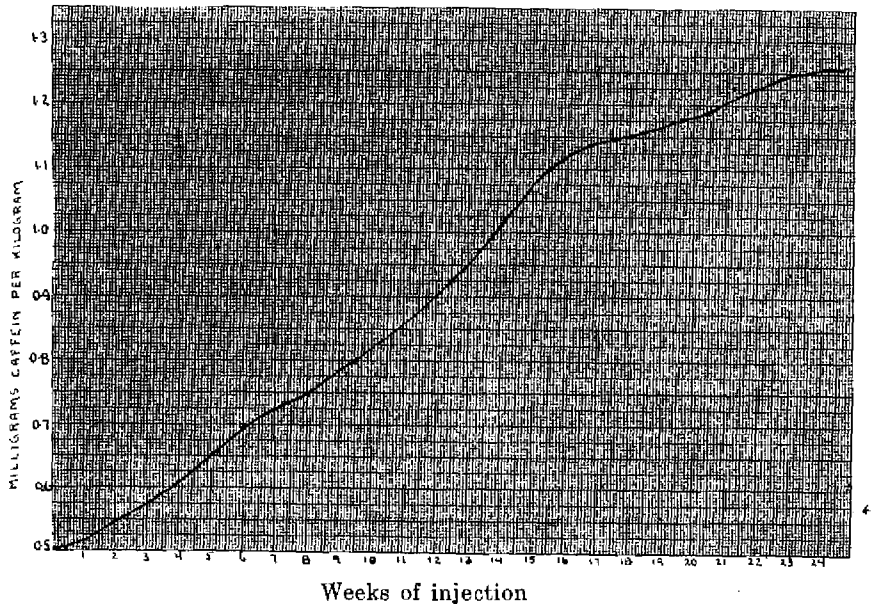

FIG. 1. RATE OF DEVELOPMENT OF RENAL CAFFEIN TOLERATION

DEVELOPMENT OF TOLERATION

The rate of development of renal toleration toward caffein was studied in 9 of 50 rabbits made tolerant toward the drug, by determining minimal effective diuretic doses of the drug at intervals during the continuing administration of the large daily doses of caffein. A curve is shown in figure 1 illustrating the development in time and degree of this toleration. An approximate maximum degree of renal toleration is reached in about four months.

Records of renal response to caffein action in tolerant and control rabbits were obtained from narcotized animals by means of cannulizing the ureters. The doses of caffein whose diuretic action was determined in the narcotized rabbits were later given to rabbits free from the action of any drug other than caffein in order to rule out any possibility of interference from the drugs used to depress the central nervous system in the first series of experiments. The bladders of non-narcotized rabbits were drained by means of a retained catheter having numerous openings near the tip. Results obtained in the catheterized animals duplicated those obtained by cannulizing the ureters.

SOURCES OF ERROR

A possible source of error arises in the effect on urine secretion of the solvent for caffein, injected into the blood stream. Richards and Plant (5) in discussing a study on diuresis, state: "It is questionable whether 0.9 per cent sodium chloride (5 to 6 cc. per kilogram) is capable of causing diuresis when blood flow through the kidney is constant." The caffein was dissolved in isotonic saline solution with pH. = 7.25. It was never necessary to inject more than 1 cc. per kilogram of this solution. Control rabbits injected with 2 cc. per kilogram of this isotonic solution showed no change in the rate of urine flow. It seems improbable that the solvent for caffein played any active part in altering the rate of urine flow following the injection of the drug.

The water content of the tissues was made as uniform as possible by feeding all rabbits the same type of food and withdrawing all food and water from the cage the afternoon of the day preceding the experiment. Caffein was not injected the day preceding the experiment in order to allow for the destruction and elimination of that which had previously been injected in continuing the toleration. It has been shown that the rabbit can destroy and eliminate all but a small fraction of a dose of caffein in twenty-four hours (2).

Interference with renal activity as produced with volatile anesthetics was avoided by narcotizing the rabbits with a solution of ethyl-carbamate in saturated solution of chlorbutanol, given

by stomach tube. Operative procedures were carried out with the aid of a local anesthetic.

The blood pressure of normal mammals is not materially affected by the intravenous injection of caffein in amounts less than 20 mgm. per kilogram weight, as shown by Sollmann and Pilcher (6). The doses requisite for the purposes of this experiment fell considerably below this minimum. The temporary variations in blood pressure due to endocardial irritation from caffein intravenously injected, were minimized by allowing the solution to become highly diluted in blood before reaching the heart, through uniform, slow rate of injection into a lateral ear vein.

An average time of one-half hour was allowed to elapse following the placing of the ureteral cannulas, before starting to record the rate of flow of urine, in order to insure a uniformly regular rate of secretion. The interval in seconds elapsing between succeeding drops was noted in order to measure variations in rate. An average of the time intervals between each five succeeding drops was determined in order to equalize the slight irregularities in time occurring between individual drops, and for greater convenience in charting results. In most of the experiments in which the bladder was catheterized, ten successive time intervals were averaged, for sake of greater uniformity.

Results were kept only on those animals maintaining an apparently normal respiratory and cardiac activity and maintaining a rate of urine flow in keeping with the previous secretion, considering the diuresis, for a period of one-half to one hour after completing the readings.

RENAL RESPONSE

The kidneys of control rabbits show no change in rate of urine secretion when caffein is injected intravenously in amounts less than 0.5 mgm. per kilogram weight. In 11 rabbits injected with 0.5 mgm. caffein per kilogram, there was a negative or questionable response in 7 animals, as illustrated by figure 2. Four rabbits showed a fairly well defined increase in rate of urine flow following the caffein injection, illustrated by figure 3. The in-

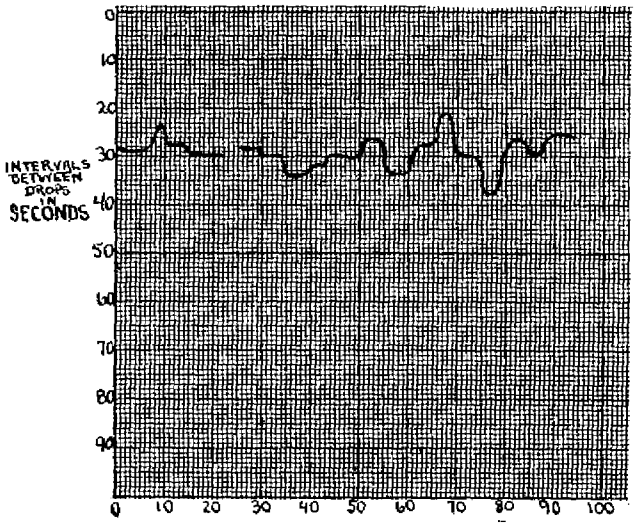

FIG. 2. CAFFEIN, 0.5 MGM. PER KILOGRAM WEIGHT, INJECTED INTRAVENOUSLY DURING BREAK IN CURVE

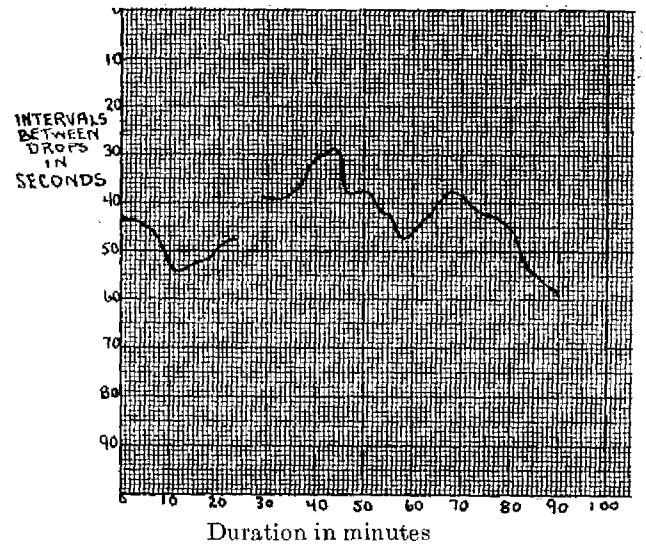

FIG. 3. CAFFEIN, 0.5 MGM. PER KILOGRAM WEIGHT, INJECTED INTRAVENOUSLY DURING BREAK IN CURVE

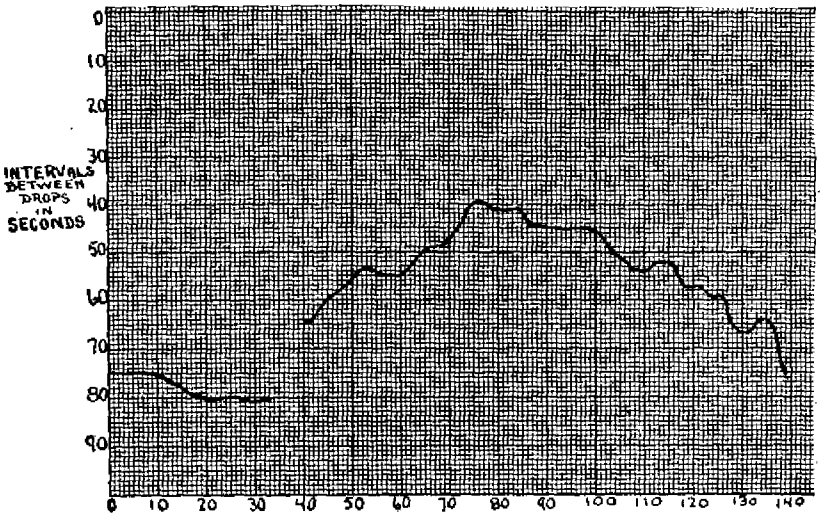

FIG. 4. CAFFEIN, 0.6 MGM. PER KILOGRAM WEIGHT, INJECTED INTRAVENOUSLY DURING THE BREAK IN THE CURVE

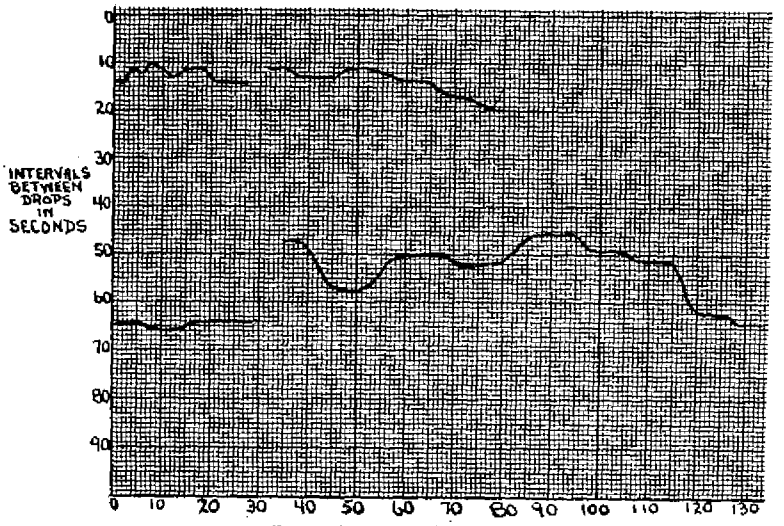

FIG. 5. CAFFEIN, 1.3 MGM. PER KILOGRAM, INJECTED INTRAVENOUSLY (DURING THE BREAK IN THE CURVE) IN CAFFEIN TOLERANT RABBITS

Upper curve shows lack of diuretic action. Lower tracing illustrates minimal action shown by some tolerant animals, with this dosage.

creased flow of urine following the intravenous injection of 0.6 mgm. caffein (per kilogram) as shown in figure 4 serves to substantiate the otherwise questionable alterations in flow resulting from the injection of 0.5 mgm. amounts as being a true minimal response. Figure 4 represents the most pronounced response obtained from 6 rabbits with 0.6 mgm. dose. A marked diuresis always follows 0.7 mgm. caffein per kilogram in a control rabbit.

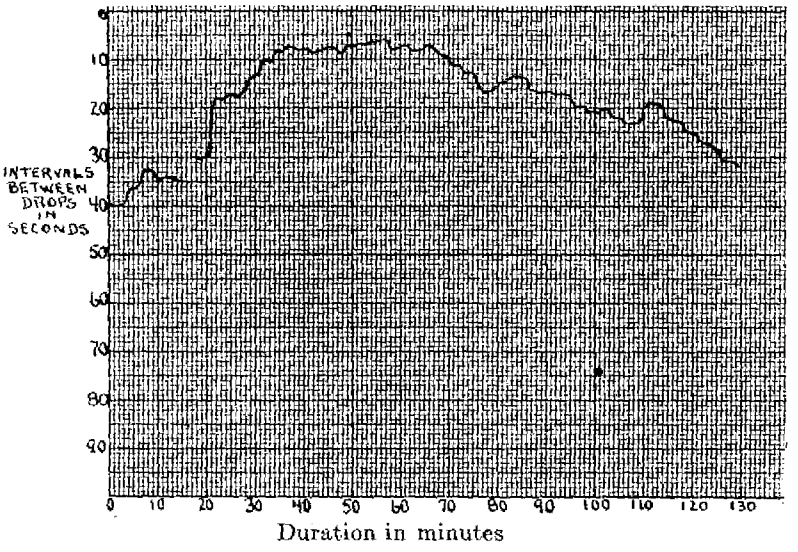

FIG. 6. CAFFEIN, 1.4 MGM. PER KILOGRAM WEIGHT, INJECTED INTRAVENOUSLY (DURING THE BREAK IN THE CURVE) IN CAFFEIN TOLERANT RABBIT

Illustrating moderate diuretic action obtained uniformly in tolerant rabbits with this dose.

A rabbit accustomed to the subcutaneous injection of caffein, six days a week, over a period of four or more months, exhibits no change in volume of urine flow when 1.2 mgm. caffein per kilogram is injected intravenously. Figure 5 shows two curves illustrating responses from tolerant rabbits given 1.3 mgm. caffein. As will be seen, one curve shows no change in rate of flow, the other a minimal response. Other tolerant rabbits given this dosage showed questionable variations or lack of effect. When 1.4 mgm. caffein is injected intravenously into a caffein tolerant rabbit, a moderately developed diuresis always occurs, as shown in figure 6.

The altered renal response exhibited in rabbits accustomed to the injection of caffein, to intravenous injections of doses of that drug definitely diuretic to control rabbits, is evidence of toleration developed toward the diuretic action of caffein, or of impaired function of the kidney. In an attempt to determine whether the latter possibility played a major or minor rôle, or was not concerned, the renal response to the saline type of diuretic was tested. Control rabbits when injected intravenously with sodium acetate in isotonic strength, require between 82 and 86 mgm. per kilogram to produce a minimal increase in rate of urine flow. The kidneys of rabbits that had developed a maximal toleration of caffein action responded as sensitively to these doses of acetate as did those of any control rabbits.

Difference of opinion appears to exist in regard to the effect of large doses of caffein, frequently administered, on the tissue of the kidney. The possibility of pathological changes in the kidney caused by caffein in the large, frequently administered doses used in this experiment, must be considered as a possible factor in causing the decreased response to caffein. The sensitive reaction to sodium acetate in rabbits exhibiting a maximum toleration of caffein makes one somewhat skeptical of any marked pathological changes being present in the kidneys, or responsible for diminished sensitiveness to caffein.

Vinci (7) reports the production of pathological changes in the kidneys of dogs and rabbits by doses of caffein of 40 mgm. per kilogram or over. As referred to in Sollmann's Manual (8), these changes consist of "dilated vessels, cloudy swelling, to vacuolar degeneration and necrosis of the epithelium, and slight interstitial swelling. These histologic changes may be present even when the urine appears normal." The urine usually indicated the tissue damage by the presence of albumin up to 1.4 per 1000; leucocytes, epithelium and casts. The lesions are stated to be "usually only transitory, disappearing a few days after discontinuance."

Cushny (9) states: "Caffein does not injure the kidney even when it is given in large doses and for prolonged periods; it thus

differs from most other diuretics and may be administered in renal disorders without risk of increasing the lesions." The following statement regarding the effect of large doses of caffein on the tissues of the kidney, is found in Meyer and Gottlieb's *Pharmacology* (10) (translation by Halsey): "the drugs of the caffein group cause no pathologic alterations in the kidney, even when they are administered repeatedly in large or even in poisonous doses."

The urine of the rabbits made tolerant toward caffein, reported in this paper, gave negative tests for albumen, by means of heat and acetic acid. The possibility of renal damage being caused by caffein was studied carefully in 7 of the animals during development of the toleration, during its maximum manifestation and by means of pathological study of the kidneys by a pathologist at the conclusion of the experiment.

Occasional faint rings occurred in testing catheterized specimens of urine of the experimental rabbits with Robert's or Mayer's reagents. Similar rings were observed in the urines of control rabbits. Centrifuged specimens of urine from experimental animals showed crystals, epithelial cells and single casts on infrequent occasions. No difference was noted in these respects in the centrifuged specimens of control animals.

The kidneys of the experimental rabbits removed immediately after causing death by means of injecting air into an ear vein, were examined grossly by a pathologist (11) and reported normal in appearance in each instance. Sections of the kidneys were immediately fixed and studied microscopically. Control sections were fixed with similar technique and used for comparison. According to the report of Professor Menne (12) of the Department of Pathology of this school, no pathological changes were apparent in the kidneys of these tolerant rabbits, with the exception of one instance in which the experimental animal developed snuffles and diarrhea in which the pathological diagnosis was "subacute diffuse interstitial nephritis." This particular rabbit had received less caffein than the others after the snuffle infection.