THE PHARMACOLOGIC ACTIONS OF THEOPHYLLINE AS RELATED TO BLOOD LEVELS

By

Edward Byrd Truitt, Jr.

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P.O. Box 1346
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E. B. T.

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FOREWORD

Because of their early discovery before the turn of the century and their long use by clinicians in a variety of conditions, the available literature on the methylated xanthine drugs is ponderous. Added to this, the controversial nature of their mode of action and of their therapeutic value has made an analysis of the pharmacologic action of these drugs a lengthy and difficult task. Furthermore, the tendency of some textbooks of pharmacology to continue to accept early established explanations for their action without frequent revaluation on the basis of newer concepts has added to the difficulty. The first section of this thesis is an effort to call attention to some of the contradictions existing and to point out several possible explanations of the disagreement.

Owing to the uncertainty of the mechanism of the therapeutic action of theophylline, and the lack of quantitative data on its effects which are applicable to man, the administration of this drug has developed on a more or less empirical basis. The second purpose of this thesis is to describe the development of a method for measuring blood concentrations of theophylline and its application to therapy with the drug. Part of this investigation undertakes to compare the efficacy of various routes of absorption of the drug in terms of blood levels. Other parts show a correlation of blood concentrations of theophylline with its pharmacologic action. Several investigations bearing on the controversial action of theophylline on the circulation are included.

Fortunately, a large portion of the work was possible in human subjects, which obviates the transposition of values obtained in experimental animals. This has been made possible through collaboration with

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CHAPTER I

THE THERAPEUTIC USES OF THEOPHYLLINE

I. The Role of Theophylline in Disease of the Coronary Arteries

The value of the methyl xanthines, particularly theophylline, in the treatment of disease of the coronary arteries has been a subject of much controversy during the past twenty years. On the basis of experimental results in animals, which will be reviewed in the following chapter, theophylline and other methyl xanthines have been recommended in conditions where a vasodilatation of the coronary arteries would be of value. The two most important conditions for which their use has been advocated by some and rejected as useless by others is in angina pectoris and coronary thrombosis. Their use in other types of coronary artery disease, such as acute coronary failure, congestive heart failure following myocardial infarction and paroxysmal cardiac dyspnea (cardiac asthma) will be discussed in later sections.

1. Angina Pectoris

Angina pectoris is a clinical entity which is characterized most dramatically by attacks of paroxysmal pain or discomfort in the substernal region which are induced by exertion as well as other more obscure causes. A more exact name for the syndrome would be arteriosclerotic or syphilitic coronary stenosis with angina pectoris, depending on the etiology. In true angina the pain develops gradually and characteristically radiates to the inner aspect of the left arm and even at times to the wrist or fingers. There are many variations in the pattern or intensity of the pain in different patients. It usually forces the patient to stop all

activity. The pain disappears upon rest or administration of a prompt-acting vasodilator such as nitroglycerin or amyl nitrite.

The incidence of this disabling disease is apparently increasing. Because of the frequency with which it strikes active energetic males in their later forties and fifties, usually at the peak of their careers, it is of great economic importance. Obviously the need for a drug available by the oral route which would markedly reduce the severity and intensity of the attacks is great. Such a vasodilator which produced neither distressing side effects nor developed tolerance to its action would permit a large portion of these patients to resume their normal activities in whole or in part.

While there is no single specific organic cause of angina pectoris and the various hypotheses to explain its mechanism are very divergent, the invariable mechanism is thought to be cardiac anoxia regardless of origin. This explains the frequency of angina pectoris in arteriosclerosis of the coronary arteries, and also its occurrence in some cases of anemia. Some cases are due to other conditions, such as syphilitic aortitis producing narrowing of the coronary orifices, rheumatic or syphilitic disease of the aortic valve with free aortic regurgitation and other rarer causes. The disease is considered by some to be a spasm of constriction in the coronary vascular bed. Others explain the pain only on the basis of accumulated metabolites.

The trigger mechanism which precipitates the attack can be a variety of causes. Levine (1) believes that these can all be explained through the endocrine system, mainly the adrenals or thyroid glands. As evidence he cites the precipitation of angina symptoms by subcutaneous injection of adrenalin.

The treatment of angina pectoris may be divided into two separate problems. The first is the treatment of the individual attacks with fast-acting vasodilators such as nitroglycerin, amyl nitrite, or octyl nitrite. The second problem is the general care of the patient directed toward a reduction in the severity and the frequency of the attacks. It is for this latter role that the use of theophylline and theobromine compounds has been advocated.

The first use of xanthine compounds as a coronary vasodilator is attributed to Askanazy (2), 1895, who used theobromine sodium salicylate (Diuretin). He obtained favorable results in cases of angina and cardiac asthma. These results were confirmed by Breuer (3), 1902. Guggenheimer (4), 1925, obtained favorable results from the use of Euphyllin (theophylline-ethylene-diamine) in arteriosclerotic disease without edema which he thought attributable to coronary vasodilation. Marvin (5), 1926, and Smith, Miller and Graber (6), 1926, claimed the action of the xanthine diuretics to be of value in the cardiac failure associated with arteriosclerosis. Following this, a large number of clinical reports have appeared claiming a beneficial effect in anginal patients by Musser (7), 1928; Smith, F. M. (8), 1928; Gilbert and Kerr (9), 1929; Coogan (10), 1934; Smith, Rathe and Paul (11), 1935; Brown and Kiseman (12), 1937; Massel (13), 1939; LeRoy (14), 1941; and Paul and Montgomery (15), 1948. However, in direct antithesis to these results are the studies of Evans and Hoyle (16), 1933; Gold, Kwit and Otto (16), 1935; and Master, Jaffe and Dack (18), 1939, who found patients with anginal pain were unable to differentiate between doses of theophylline-ethylenediamine and a placebo. The majority of these studies, both pro and con, were made with out-patients and the drug was given by the oral

route. In most cases the dosage employed was 0.2 Gm. (3 grs.) of theophylline-ethylenediamine four times daily. Evidence of a more objective nature was provided by the studies of Levy, Bruenn and Williams (19), 1940, and Riseman and Brown (20), 1937. Levy and co-workers obtained a 83% prolongation of the appearance of pain in patients breathing 10% O_2 and 90% N_2 as well as a 58% diminution in the deviation of the RS-T segment following the intravenous administration of the usual intravenous dose (7-8 mg./kg. or 0.5 Gm.) of theophylline-ethylenediamine. They also obtained a 26% delay in the time of appearance of pain and a 32% diminution of the effect on the electrocardiogram following oral therapy. Riseman and Brown presented data supporting a claim that theophylline preparations are valuable in increasing the amount of standard exercise a patient can perform. Whereas their data indicate an increased ability to perform exercise, the improvement is apparently not 100% as they claimed in a later paper (Boyer (21), 1943).

Even today opinion is still unsettled on the value of these drugs in anginal patients. DeGraff (22), 1942, concluded that while theophylline preparations may not be harmful, it is doubtful whether they are of any marked benefit. Boyer (21), 1943, in reviewing the allowable claims, was unable to see clear-cut evidence supporting the clinical use of theophylline preparations in angina. In general, those favoring the use of xanthine drugs in anginal pain attribute the failure of those investigators who reported no significant action to: (1) the use of patients with advanced arteriosclerotic disease who are beyond help of any kind, (2) insufficient dosage, (3) the use of subjective symptoms reported by the patients, and (4) the variability of the disease itself. On the other hand, individuals who do not believe in the vasodilating properties

of the drug by the oral route attribute the success of these favorable reports to a placebo-like action. More recently Steinberg and Jensen (23), 1945, failed to show any significant clinical value in orally-administered theophylline. They used theophylline aminoisobutanol, which they claimed overcomes the gastric distress caused by many other theophylline preparations. Their dosage was only 0.18 Gm. three times a day of a preparation containing 67% theophylline.

It is noticeable, upon review of these controversial papers, that in many there is a lack of adequate control both in placebo medication and in large numbers of patients. The medication is not given under medical supervision, and the subjective nature of the symptoms reported by the patient could be subject to quantitative differences. It seems that the whole problem needs a re-evaluation in large numbers of patients by neutral investigators using the electrocardiogram or other objective criteria and a more thorough application of statistical methods to the results. Also a correlation of blood levels with vasodilator action would give uniformity to the picture.

2. Coronary Occlusion and Myocardial Infarction

A discussion of coronary occlusion and resultant myocardial infarction should properly follow angina pectoris because approximately one half of all patients having acute attacks have had earlier attacks of angina. Owing to the descriptions of Herrick, coronary occlusion is now diagnosed more clearly than it was before 1912.

The majority of cases of coronary occlusion are the direct result of arteriosclerotic changes in the coronary arteries. In some, the formation of atheromatous placques, fibrosis, and calcification may lead to a complete occlusion, but the majority of cases are caused by sub-intimal

hemorrhage of the thin-walled giant capillaries which develop in the walls of the arteriosclerotic vessels. These sub-intimal hemorrhages may block the vessel by bulging into the lumen of the vessel. In other cases they may damage the endothelium and cause thrombosis. Retrograde thrombosis may occur in the occluded vessel and may even block other arteries. Coronary occlusion may be caused by a variety of less common causes, such as embolism in cases of subacute bacterial endocarditis or fat emboli from fractures. Syphilitic aortitis can block the coronary ostia, but this is usually a gradual process.

Coronary occlusion has the associations similar to arteriosclerosis of age, diabetes, and hypertension. Its onset is usually without apparent cause, but severe exertion, intense emotion, and many other causes have been reported. Coronary occlusion is noted for being a cause of sudden death, yet the immediate mortality varies from 16% to 33%, with some estimating as low as 10%. The prognosis varies with the size of the myocardium occluded, the particular area involved, the blood pressure, and other factors.

The most characteristic symptom of the onset of coronary occlusion is the pain, which is described as the most excruciating agony. It is toward the immediate relief of this pain through coronary vasodilatation that the administration of theophylline intravenously is primarily directed. Theophylline compounds are also administered with the purpose of (1) improving the surrounding myocardium by increasing the collateral circulation and antagonizing the reflex coronary spasm in vessels surrounding the occluded area and (2) reducing the occurrence of arrhythmias and reducing the dyspnea which may be present. A large dose (0.5 Gm.) should be given intravenously, slowly and with care. The drug should be avoided

if the blood pressure tends to fall precipitously. Because of the accompanying nausea, continuation of therapy must often be by the rectal or parenteral route.

The basis for the immediate administration of theophylline is mainly empirical. However, recent work has tended to establish its use on a more scientific basis. Le Roy, Fenn and Gilbert (24), 1942, found that the injection of theophylline-ethylenediamine (Aminophylline) reduced the immediate mortality rate from 70% to 56% following ligation of the circumflex branch of the left coronary artery. A combination with atropine sulfate further reduced the mortality to 33%. However, theobromine-sodium salicylate alone caused a reduction to 23%.

Fowler, Hurwitz and Smith (25), 1935, studied the effect of repetitious doses of methyl xanthines on experimentally-induced myocardial infarction produced by ligation of the coronary arteries. They claimed that theophylline-ethylenediamine causes a reduction in the size of the infarcts as compared with control animals. This study was open to criticism because of the uncertainty of their method of estimation of the size of the infarcts. Also the number of animals used (19 in all) was not as many as could be desired. Wiggers and Green (26), 1936, were unable to demonstrate any improvement on collateral circulation to the ischemic area in an isolated heart preparation following ligation of the descending branch of the left coronary artery. Gold, Trevell and Medell (27), 1937, repeated this work, using cats instead of dogs. They measured the infarcted area more carefully with a planimeter and came to the opposite conclusion, i.e., that no significant difference could be found between the size of the area in the control and treated animals which had been given 25 mg./kg. of aminophylline once a day. Contrary to LeRoy et al. (24),

they noted a larger number of fatal ventricular arrhythmias in the treated animals than in the controls.

More recently Mokotoff and Katz (28), 1945, repeated Fowler's work in dogs with a very careful method of measuring the size of the infarcts. They were careful to tie off at the same point in each dog. Theophylline-ethylenediamine 15 mg./kg. was injected intravenously immediately after the operation, twice daily subcutaneously for seven days, and then once daily for the remainder of an eight-week period. The hearts were carefully injected under uniform pressure with a radio-opaque mixture and the area of the infarction measured from the X-ray picture with a planimeter. They found a small but significant decrease in the size of the myocardial infarction in the aminophylline-treated animals.

This latter work is very heartening to those who believe the methyl xanthines to be of value. The question is whether the action in man is the same as in the dog in view of the different method of demethylation of the methyl xanthines in canines (see section on metabolic fate of theophylline). The problem also needs extensive clinical evaluation. The difficulties involved in the clinical assay of the effectiveness of theophylline in coronary occlusion are great and will probably be met only by experience in a very large number of cases.

II. The Use of Theophylline as a Diuretic

The xanthines were developed principally as diuretic drugs. Caffeine was first studied scientifically by von Schroeder (29, 30), 1886, 1887, along with the then newly-prepared theobromine. Because of its central stimulation, caffeine was soon discarded in favor of theobromine and theophylline-ethylenediamine, later introduced by Dessauer (31) in 1908. Theophylline has the greatest diuretic power of the three, but theobromine is preferred in most cases because higher doses can be given with less gastro-intestinal disturbances and less cerebral stimulation.

The investigations leading to the understanding of the mechanism of xanthine diuresis will be discussed more fully in a later section. The theory that these drugs produce a brief increase in renal blood flow and a sustained increase in glomerular filtration rate with the principal effect being a decreased reabsorption of sodium chloride and water seems to be the best explanation of their actions. The excretion of sodium chloride seems to be the principal beneficial action, since the amount of water eliminated will vary with the degree of hydration (Newman (32), 1947).

In spite of the fact that the mercurial diuretics are far more effective, the xanthine diuretics have still maintained a value in therapeutics because of their low toxicity. Their advantage is evident when one considers that with the mercurials one is mildly poisoning the renal tubules, whereas the xanthines will be limited by the side effects produced before renal damage can occur. The xanthines can be used in cases of limited renal function, whereas mercurials are contraindicated. They can also be taken orally and rectally by the patient, whereas the more toxic mercurials, even in the recent oral tablets, require close supervision.

In general, the xanthine diuretics exert their best action in decompensated cardiac patients when edema is present. According to Hayman (33), they are effective in about two thirds of the cases. The rapid effect of the intravenous injection of theophylline in pulmonary crisis associated with left ventricular failure will be discussed in the following section.

The xanthines are not very efficacious in cases of acute glomerulonephritis. They can be used in subacute and chronic cases. They are relatively inefficient in edemas associated with nephroses of various types. Tolerance develops rapidly to their action in this type of disease. They exert a diuretic effect in the chronic ascites associated with cirrhosis of the liver, chronic adherent pericarditis, etc. (Goodman and Gilman (34), 1941).

One of the main difficulties in the use of the xanthine drugs as diuretics is the development of tolerance to their action. This can be avoided somewhat by alteration of the dosage form. Scherf and Boyd (35), 1947, recommended the giving of relatively large doses (0.3 Gm.) three times a day every fourth day. They found this "jolt" treatment more effective than continuous administration, which ceased to have effect in a few days.

The common limiting factor in xanthine medication is the appearance of nausea, vomiting, severe headache, and restlessness with excitability. The first two difficulties can be overcome to some extent by administration with a meal. The use of the newer combinations of theophylline with sodium aminoacetate, sodium aminoisobutanol and other combinations exhibiting better gastric tolerance decreases nausea upon taking but not that owing to high concentrations of the xanthine. The addition of

sedatives such as phenobarbital can overcome the central stimulating effect.

Theophylline is also valuable in promoting the absorption of the mercurial from the site of injection, making intramuscular injections possible. It also relieves pain and reactions at the site of injection (DeGraff, Batterman and Lehman (36), 1938).

Recent studies (Davis and Shock (37), 1949, and Sinclair-Smith et al. (38), 1948) on the renal function changes associated with heart failure indicate that theophylline has a specific effect on the kidney in producing the desired responses in decompensated left ventricular failure and other edemas. It would be of value in the successful administration of this drug if better control were available of absorption and better correlation with blood concentrations of the drug.

III. The Therapeutic Use of Theophylline in Asthma and Other Dyspneas

It has been stated that very few therapeutic applications of drugs provide as immediate and dramatic relief to a patient as the intravenous administration of 0.5 Gm. of theophylline-ethylenediamine in the relief of Cheyne-Stokes respiration. Theophylline finds its most secure place in therapy in the relief of the dyspnea of cardiac asthma (paroxysmal non-exertional dyspnea) and other dyspneas, Cheyne-Stokes respiration, and more recently in the treatment of bronchial asthma.

Theophylline was first used in treatment of the dyspneas of cardiac origin. It was used in these cases primarily as a diuretic, and its effect on Cheyne-Stokes breathing was only mentioned occasionally (Guggenheimer (4), 1923) until stressed by Vogl (39, 40), 1927, 1932. The mechanism of action of theophylline in the relief of periodic breathing of the Cheyne-Stokes type is not completely understood, although it is thought to be a central effect. Guggenheimer (41, 42), 1932, 1933, believed the effect to be due to the vasodilatating action of theophylline. However, nitroglycerin is ineffective. Greene, Paul and Feller (43), 1937, believe that the relief is directly related to the falls they observed in venous and intrathecal pressure. Marais and McMichael (44), 1937, favored the theory that the action was by a direct effect on the center, due mainly to the ethylenediamine portion of the drug and in the absence of any significant changes in circulation. However, they measured the circulatory functions of only two persons and were looking for evidence of increased function rather than a decrease in cerebral blood flow which would cause the fall in intrathecal pressure. Scherf and Boyd (35), 1947, point out that theophylline and sodium acetate is also effective in Cheyne-Stokes breathing.

The recent study of Wechsler et al. (45), 1950, lends support to this theory, showing that a fall in intrathecal pressure could be due to cerebral vasoconstriction.

As stated before, dramatic relief is obtained in certain conditions following intravenous use of the drug. The injection of 0.5 Gm. theophylline-ethylenediamine in the acute pulmonary dyspnea accompanying left ventricular failure is rapidly effective and is often helpful in raising the morale of the patient. Relief of paroxysmal nocturnal dyspnea from cardiac failure can sometimes be obtained in mild cases by the use of suppositories of the drug. An undesirable side effect is the central stimulation which may cause sleeplessness in spite of respiratory relief. Marais and McMichael (44), 1937, provide a review and discussion of the use of theophylline-ethylenediamine in Cheyne-Stokes breathing. Freud (46), 1933, used theophylline-ethylenediamine in infants by suppository in periodic breathing associated with capillary bronchitis, pneumonia of childhood, and encephalitis.

Theophylline may give relief in cases of cardiac asthma both by its diuretic action and by its action on respiration. Unfortunately it is regarded as only symptomatic treatment in the foregoing cases to provide temporary and emergency relief. It is not preferred in place of more active drugs, such as digitalis and the mercurial diuretics for the relief of these conditions.

The first use of theophylline in bronchial asthma is generally credited to Herrmann and Aynesworth (47), 1937, although prior publication is claimed by Efron (48), 1936. Herrmann and Aynesworth reported that as early as 1931 they found the intravenous use of 0.24 to 0.48 Gm. of theophylline-ethylenediamine to be very useful in cases of acute

asthmatic attacks which were refractory to epinephrine. In a series of 41 injections in 16 cases of asthmatic bronchitis or chronic bronchial asthma presenting attacks of "status asthmaticus," 31 gave complete and persistent relief. In some failing to get relief it restored sensitivity to epinephrine. They stressed the importance of slow and careful injection in reducing the incidence and severity of side effects. Efron and Everett (49), 1939, reported the relief of two thirds of their epinephrine-fast cases with the intravenous use of the drug. Tablet medication seems to be ineffective in acute attacks. Since the first paper by Herrmann and Aynesworth a large number of papers have appeared confirming the value of theophylline in the treatment of both acute and chronic cases of asthma: Hajos (50), 1936; Baldwin (51), 1938; Mitchell (52), 1938; Halperin (53), 1938; Brown (54), 1938; Hyman (55), 1939; and Carr (56), 1940. Recent discussion on the subject has concerned the best form of administration and the principles underlying its spectacular action. Barach (57), 1944, found rectal instillation of 0.6 Gm. in water almost as immediately effective as the intravenous route and having less side effects. Dees (58), 1943, found rectal suppositories to be effective even in cases of severe asthma. They provide a satisfactory means of dosage in pediatrics. Prigal et al. (59), 1946, found that the addition of pentobarbital overcame anxiety and other cerebral effects of theophylline. The more recently introduced methods of aerosol and rectal administration are discussed thoroughly by Hartman (60), 1949.

Bubert (61), 1948, underlined the basic principles in the treatment of acute "status asthmaticus" as being first, "the widening of the bronchial lumena so that gelatinous infected exudate can be discharged and second, the introduction into the finer bronchi and into the alveoli

of anti-infective agents through the widened lumen." Theophylline is a valuable adjunct to the accomplishment and maintenance of both of these objectives. Bubert and Cook (62), 1948, recommend the aerosol method of administering theophylline along with antibiotics with the oral use as maintenance therapy.

IV. Other Therapeutic Uses of Theophylline

Theophylline appears to be very effective in biliary colic. The intravenous injection of 0.25 to 0.5 Gm. of theophylline-ethylenediamine often brings rapid relief. Butsch et al. (63), 1936, showed that aminophylline and atropine lowered intra-biliary pressure. Walters et al. (64) showed this action in human subjects who had "T"-tubes in the biliary tracts for drainage. Theophylline lowered intra-biliary pressure after it was raised by morphine. The action seems to be a specific antispasmodic action on the sphincter at the end of the biliary tract. Mears and Delor (65), 1938, recommended it as the drug of choice in biliary colic and suggested its use directly into the cystic duct to aid in the passage of small stones. Gladstone and Goodman (66) reported good results in eight patients. Cole (67) found the intravenous injection very useful in emergency cases and capable of affording complete relief in some cases.

One report on the use of aminophylline in the treatment of migraine has appeared. Marin (68), 1946, reported relief in all of 10 subjects by intravenous administration followed by oral dosage. If migraine is caused by a spastic vasodilatation of the cerebral vessels, the action of theophylline can be explained by its reduction of cerebral blood flow (Wechsler et al. (45), 1950).

Epstein (69), 1946, found the intravenous injection of theophylline-ethylenediamine to be valuable in relieving pruritus in various dermatoses. This action can be related to the results of Stewart and Jack (70), 1946, who observed an increase in peripheral blood flow and increase in skin temperature. However, the drug does not seem to be of value in peripheral vascular diseases (Boyer (21), 1943).

For use in cerebral stimulation, the action of caffeine is more effective. Theobromine has the greater activity on skeletal muscle.

CHAPTER II

HISTORY AND PHARMACOLOGIC ACTIONS OF THEOPHYLLINE

I. The Action of Theophylline on the Circulation

The early work on the elucidation of the action of theophylline is overshadowed by the interest of the early investigators in caffeine. Because of the similarity of action of the three best-known members of the methylated xanthine group, most of the early work involved the use of caffeine. A comparison with the other two, theophylline and theobromine, was sometimes, but not always, made. Two classifications might be used to discuss the action of the methyl xanthines on the circulation. A very complete comparison of the effects of stimulant (small and moderate doses up to 20 mg./kg. of caffeine), depressant, and fatal doses can be found in Sollman (71), 1948. Perhaps a better classification might be a discussion of the action of this class of drugs, with particular reference to theophylline, on the components of the circulatory system which they affect. Following this arrangement, the pharmacologic actions of the methyl xanthines are divided into the following categories: (1) the myocardial stimulating action, (2) the action on coronary blood vessels, (3) the action on peripheral blood circulation, and (4) the action on the blood itself. However, before reviewing in detail the experimental evidence on each classification, it would be best to discuss the general effect of therapeutic and moderate physiologic doses on the circulation. The data of Starr et al. (72), 1937, are the most complete available. Starr's data show a slight bradycardia, slight, but equal, increases in systolic and diastolic blood pressure, an increase in cardiac output

accompanied by an increased stroke volume and increased left ventricular work per beat and per minute. Heart volume decreases only slightly, and the metabolic rate is increased. There is a decrease in the arteriovenous oxygen difference. Finally, there is a decrease in peripheral resistance. Although these data represented averages for only seven patients, they are in general agreement with other experimental findings.

1. The Action of Theophylline on the Heart

The resultant action of the methyl xanthines on the heart is due to their action via the vagus nerve and directly on the myocardium. Thus the response of an isolated preparation varies from that of the intact animal in that it is unhampered by vagal influences. The action of the vagus is thought to be due to vagal stimulation by the action of the drug on the vagal center in the medulla. There is an increased vagus excitability (Fredericq (73), 1913). This effect occurs in the turtle, and its mechanism may be a potentiation of the effects of acetylcholine (Lamalle (74), 1941), (possibly an anticholinesterase action -- see Section IV).

The stimulant effect of the methyl xanthines was noted by early investigators using excised preparations with both amphibian and mammalian hearts. Hedbom (75), 1899; Bock (76), 1900; Loeb (77), 1903; and Plant (78) noted increases in rate and amplitude of contraction, as well as the force of contraction. The majority reported theophylline to exceed the other methyl xanthines in its stimulant action on contraction. Flaum and Kossler (79), 1933, observed an increase in the cardiac output of the dog heart-lung preparation experimentally damaged by chloroform, CO_2, and barbiturates. Bock and Buchholtz (80), 1920, found no change in cardiac output in dogs from caffeine. Other investigators have also noted

increased minute volume, Mahaim and Rothberger (81), 1936; Chandlier (82), 1939; and others. Smith and Jensen (83), 1936, ascribed the beneficial action of theophylline-aminoisobutanol in a heart-lung preparation with experimentally-induced heart failure to myocardial stimulation. Electrocardiograms of excised heart muscle showed an increase in rate of contraction for all three xanthines, but conduction was depressed by theobromine and increased by caffeine and theophylline (Sakai (84), 1918). Krop (85), 1944, found that theophylline was the most effective of the three methyl xanthines in increasing the force of contraction in the isolated cat papillary muscle preparation. It was effective in concentrations as low as 1:20,000, and the duration of effect was longer than with caffeine and theobromine. However, he concludes that this concentration (1:20,000 or 5 mg.-%) is above that usually obtainable in man. Although this is conceded in view of the blood concentrations found later by this author, it seems very improbable that an excised and progressively failing preparation as is shown in his data would react as sensitively as the intact organ. Krop noted an immediate cessation of stimulation upon replacement of the drug solution with fresh Locke's solution as compared to the continued effect of digitalis glycosides after washing (Cattell and Gold (86), 1938). He also noted spontaneous contractions with high concentrations. Cattell and Gold (87) have emphasized that the force of contraction increases with an increase in rate. Thus the action of caffeine on the pacemaker of the heart reported by Clark (88) may be a factor.

The myocardial stimulation occurs in the intact animal, but is manifested more by an increase in cardiac output than by a change in rate. This increase in minute volume occurs in spite of a decreased rate owing to vagal action, and a lowered blood pressure which sometimes may occur

owing to peripheral vasodilatation.

The effects of the myocardial stimulation have also been studied in man. Grollman (89), 1932, concluded that small doses of caffeine have no effect on the cardiovascular system. Larger doses (0.5-1.0 Gm.) cause a rise in oxygen consumption, an increase in arteric-venous oxygen difference, and a slight rise in cardiac output. The data of Starr et al. (72), 1937, which have already been mentioned showed an increased cardiac output caused by theophylline. Increases in cardiac output and decreased peripheral resistance following theophylline were also noted by Neuthard and Hoen (90), 1937. This increase has been observed by many others: Chandlier (82), 1939; Howarth and associates (91), 1947; Escher et al. (92), 1948; and Green et al. (93), 1949. The increase in cardiac output is usually brief, lasting only 10-20 minutes after the intravenous injection of 0.5 Gm. In patients with congestive heart failure Howarth and associates reported small but sustained increases in cardiac output. An increase in cardiac output will take place following a lowered venous pressure, but theophylline causes a greater increase than that produced by cuffs on the thighs. Berséus (94), 1944, claimed that theophylline in doses of only 0.2 Gm. had no effect on cardiac output and stroke volume in congestive heart failure patients or in normal patients.

An interesting speculation as to the underlying mechanism of action of theophylline is that of Zak (95), 1943. On the basis of experiments with various dyes he concluded that the mechanism of xanthine action on the myocardium was that of causing an increased permeability. In conformity with this theory Goutier (96) found an increased sensitivity of the heart to the inhibiting effects of potassium after the xanthines.

Thus it appears that theophylline and the related methyl xanthines

have an effect described by Boyer (21) as an "adrenalin-like" action on the heart. It is probable that it acts directly on the contractile mechanism to increase the force and the rate of contraction. It could also effect the rate and thus the force of contraction by an action on the pacemaker in the auricle. An effect on the auricle could be easily explained by its vasodilating action, causing an increase in temperature in this region. The possibility that its whole action on the heart is through dilatation of the coronary arterioles is also possible. The direct effect on the heart in the intact animal is modified by its action via the vagus nerve. It would be desirable if more investigators studying the effects in the intact animal would compare the effects of the drug with and without this modifying action of the vagus nerve.

2. The Effect of Theophylline on Coronary Blood Flow

As yet, the action of theophylline on the coronary blood flow of the mammalian heart has not been completely and conclusively elucidated. The reason for this is the complexity of the factors regulating coronary blood flow. When one learns that the coronary blood flow is controlled by the cardiac rate, the cardiac output, the mean arterial blood pressure, the caliber of the coronary vessels, the vigor of contraction of the myocardium (extravascular support), and right auricular pressure (venous pressure), as well as by the character of the blood itself, it is not surprising that the action of a drug on all of these factors has not been fully appraised.

The evidence supporting claims to an action of theophylline on the coronary flow in mammalian hearts can be best examined by classifying the numerous investigations according to the type of preparation. The earliest evidence that the methyl xanthines had a direct action on the

vascular musculature was through observations of the effect on surviving arteries. Eppinger and Hess (97), 1909, observed that strips of coronary artery lengthened in a solution of caffeine. Preparations using isolated arteries are very unphysiologic and do not necessarily reflect the effect of the drug in the intact animal.

Other early investigators in this field used the isolated perfused mammalian heart after the method of Langendorff (98), 1895. They all agreed that the methyl xanthines increased the rate and amplitude of contraction. The majority also claimed an increase in coronary flow occurred, caused by vasodilatation (Hedbom (75), 1899; Loeb (77), 1903; Meyer (99), 1912; Sakai and Saneyoshi (100), 1915). This preparation, although it measures the coronary vascular bed changes, is a denervated and progressively failing preparation. Furthermore it is altered from the normal in that the chambers of the heart are not performing normal work and the venous pressure at one end of the vascular bed is not the same as in the intact preparation. However, this preparation should give indications of gross changes in the caliber of the coronary arteries.

Heathcote (101) reviewed the early literature of the action of the methyl xanthines on the heart and compared caffeine, theobromine, and theophylline on the isolated frog and rabbit heart. It is of interest that he noted the increase in coronary flow usually occurred after the increase in frequency and the augmentation of the beat. It is also earlier in disappearing. He ascribed the action of theobromine and theophylline salts to vasodilatation. A concentration of caffeine (1:10,000) which caused the same increase in rate and amplitude of contraction as a lesser concentration of theobromine (1:20,000) did not cause an increase in perfusion rate. This same concentration of theobromine caused a 36% increase in rate of

flow through the coronary arteries. Theophylline he found to be even more active on the coronary vessels. A 1:30,000 dilution caused a 30% increase in flow. Smith, Miller, and Graber (102), 1925, reported a 20% to 45% increase in flow with concentrations of theophylline of 1:25,000 and 1:50,000 and that the combination with ethylene-diamine caused an increase of 40% to 90% in the coronary flow for the same concentrations. Other investigators have routinely used this type of preparation even up to the present time for evaluating and comparing coronary vasodilation: Iwai and Sassa (103), 1923; Guggenheimer and Sassa (104), 1923; Wedd (105), 1931; Winder and Kaiser (106), 1948; Paul and Montgomery (15), 1948, and Beccari (107), 1948. In order to obviate the effects of increased amplitude of contraction and changes in heart rate on coronary flow, Katz and Lindner (108), 1939, used an isolated fibrillating dog's heart perfused at constant pressure with blood rendered incoagulable. They obtained increases in flow with aminophylline.

A more complicated preparation that was early used for the determination of the effects of theophylline on coronary flow is the Starling isolated heart-lung preparation (Starling and associates (109, 110, 111), 1910 to 1914). This preparation may be adapted to measure coronary flow by use of the Moravitz-Zahn cannula in the coronary sinus, returning the outflow to the perfusion system. This preparation, like the isolated rabbit heart, is a denervated and progressively failing preparation. However, it enables the experimenter to reproduce better the pressures which exist in vivo. Blood rendered incoagulable is preferred for perfusion of this preparation. Peripheral resistance can also be maintained constant or altered at will. Thus the variations of coronary flow are partially dependent upon the strength of the contraction, the cardiac output, and blood

pressure. However, the measurement of coronary blood flow by means of coronary sinus outflow is subject to errors which will be discussed later.

Many workers have used this type of preparation and reported increases in outflow after injections of theophylline compounds (Sakai and Saneyoshi (100), 1915; Bodo (112), 1928; and Fisher, Guggenheimer and Muller (113), 1922). Stoland, Ginsburg, Loy and Hiebert (114), 1934, found the duration of the increased flow in both this type preparation and in the intact dog to vary from about 9 minutes for 2 mg./kg. to 32 minutes with 8 mg./kg. of the theophylline-ethylenediamine. Kountz and Smith (115), 1937, obtained increases in coronary flow in the heart-lung preparation using pathological human hearts. They were able to obtain a vasodilatation even in hypertrophied hearts.

A most commonly used experimental preparation for the determination of coronary flow is the insertion into the coronary sinus of an anesthetized animal the cannula first described by Moravitz and Zahn (116), 1914. The validity of this method as an interpretation of coronary blood flow has been seriously questioned by Johnson and Wiggers (117), 1937, and Katz, Jochim and Weinstein (118), 1938. The effect of right auricular pressure on coronary flow is altered by cannulation. The coronary sinus outflow does not constitute the entire drainage of the coronary vascular bed nor a constant percentage thereof. Therefore changes in intraventricular pressure by myocardial stimulating drugs will cause an increased outflow by this avenue of least resistance because the outflow by the anterior cardiac veins, the thebesian vessels, and other coronary-ventricular avenues is impeded. The effect of the xanthines has been tested many times in this type of preparation (Smith and Miller (119), 1928; Gilbert and Fenn (120); Leroy and Speer (121), 1940; and Krantz

et al. (122), 1947).

In order to demonstrate changes in the animal's circulation without altering its intactness, the use of the Rein thermostromuhr (123), 1928, and (124), 1928 to 1935, or the later direct-current types of Saldes and Herrick (125), 1937, was at first advocated. Theophylline-ethylenediamine has been shown to cause an increase in coronary artery flow in the anesthetized (Negria et al. (126), 1940) and in the trained unanesthetized dog (Essex et al. (127), 1940) with the latter instrument. The validity of thermostromuhr readings as indicative of drug changes in coronary flow has been questioned by Shipley et al. (128), 1942, and by Gregg et al. (129), 1942.

Experimental measurement of coronary inflow is possible by the use of several similar instruments. Among them are the differential manometer, the bubble flow meter, the rotameter, and others similar in principle to the differential manometer. Some of those instruments; namely, the rotameter and the differential manometer, are capable of recording the phasic variations in coronary flow with each contraction of the heart. However, the use of these instruments has the disadvantages of a traumatizing open-chest operation, artificial respiration, anesthesia, plus trauma to the coronary vessels themselves, and a short occlusion of coronary flow during cannulation. Often the position of the heart is changed by suspension in a pericardial cradle. Bayly et al. (130), 1943, showed that the trauma involved in exposing the coronary artery just for ligation was enough to flatten or invert the T-wave of electrocardiograms from the myocardium supplied by this branch. Boyer and Green (131), 1941, used the differential monometer technique to measure the action of the xanthines on coronary blood flow. They noted an increased coronary flow

in diastole, but they also observed a stimulation of the myocardium which caused a decreased flow during systole. Boyer (21), 1943, in a later review of the allowable claims for xanthine drugs, states, "Unless it can be shown that the increase in coronary flow is greater than the increase in cardiac metabolism, these drugs cannot be considered effective in increasing the relative blood supply to the heart." Eckenhoff and Hafkenshiel (132), 1947, obtained increases in coronary blood flow both upon intra-arterial and intravenous injection. The intra-arterial injection of 1.0 mg. caused a 76% increase in flow, accompanied by a slight increase in mean arterial blood pressure and a slight increase in heart rate. However, the intravenous injection of 6 to 7 mg./kg. caused a fall in mean arterial blood pressure of 15% but an increase in heart rate of 24% with an increased coronary flow of only 11%. Thus it appears that, while the intra-coronary injection of the drug in concentration may be effective in causing vasodilitation, the usual dose administered intravenously caused only a small increase in coronary flow, which could be the result of the myocardial stimulation. It is unfortunate that these authors did not study theophylline-ethylenediamine as they did nikethamide to show whether the increase in coronary flow results in an increased cardiac efficiency. This is evidenced by an increase in the ratio of the oxygen supplied to the demand, while resulting in no decrease in the ratio of the amount of work produced to the amount of oxygen consumed. Foltz, Rubin and Steiger (133), 1949, measured the changes in oxygen saturation of coronary sinus blood in dogs by coronary sinus catheterization. They noted a fall in coronary venous oxygen saturation with an increase in the arterio-venous oxygen difference. While there was no change in blood pressure, there was an increase in heart rate. They believe that the increase in oxygen utilization by the

heart indicates a failure of the coronaries to deliver more than enough oxygen to the myocardium to supply the stimulated contraction. Thus they claimed that theophylline-ethylenediamine intensifies the myocardial anoxia. It seems that in order to assay the value of the action of theophylline on the heart and circulation, it must be proved whether the increase in coronary flow causes the increased rate and amplitude of contraction, or whether the initial action is on the myocardial fibers, causing a stimulation of contracture and producing a vasodilatation by the increased accumulation of metabolites.

Other evidence of an indirect nature has been reported. Hanzlik and Moy (134) reported that theophylline-ethylenediamine antagonized the circulatory depression of posterior pituitary. Theophylline-ethylenediamine reduced the pressor action of posterior pituitary in animals with normal or high blood pressure and reversed low blood pressure due to weakened heart action.

Bayly et al. (129) found that the intravenous administration of 0.12 Gm. of theophylline-ethylenediamine caused an improvement in the T-wave pattern of the local electrocardiogram following a temporary occlusion of the coronary artery supplying the area. They state that the drug is capable of reducing the intensity and/or the extent of the local ischemia following occlusions up to 90 seconds. Leslie and Mulinos (135) were unable to show a significant improvement on the sum of the deviations of the RS-T segment in the three standard leads of the electrocardiogram, both before and after induced anoxemia. However, they admit the dosage might be insufficient (15 mg./kg. maximum), among other reasons for the lack of effect.

An interesting theory of the action of theophylline is that

it may act by reducing venous pressure and increasing cardiac output. Steinberg and Jensen (136) noted a fall in venous pressure not accompanied by a change in plasma volume and lasting 60 to 90 minutes. This was in cases of congestive heart failure. Howarth, McMichael and Sharpey-Schafer (91), 1947, observed a lowering of right auricular pressure (venous pressure) and a rise in cardiac output. They showed that the rise in cardiac output is due to the drug and not to the lowered venous pressure. The increase in cardiac output disappears if the lowered venous pressure persists. Smith and Jensen (83), 1946, studied the effects of theophylline-aminoisobutanol in an isolated heart-lung preparation with experimental heart failure produced by chloral hydrate. They decided that in this preparation, where the coronary vascular system is already dilated, the only beneficial action could be attributed to direct myocardial stimulation."

In summary, it seems that irrefutable evidence is lacking to show that theophylline and other methyl xanthines exert a direct action on the coronary arteries to produce vasodilation and thereby improve the circulation in the heart muscle. Instead it seems to exert an adrenalin-like action to produce increased vigor of contraction, an increased heart rate in denervated preparations, and a secondary increase in coronary blood flow which may be in part due to a direct action on the smooth musculature and in part to the accumulated metabolites from increased cardiac activity.

3. <u>The Effect on the Peripheral Blood Circulation</u>

Many of the early investigators of the action of caffeine on the circulation were led to the erroneous conclusion that the drug caused

a vasoconstriction (Wagner (137), 1885; von Schroeder (29, 30), 1886, 1887). This was probably because of the bradycardia which occurs through vagal action and the increase in blood pressure which is the result of myocardial stimulation. Sollman and Pilcher (138), 1911, reinvestigated the many actions of caffeine. This extensive study did much to clarify the confusion surrounding the early literature on the effects of caffeine on the circulation. However, analogy cannot always be drawn as to the action of theophylline and especially theobromine. Many of these early-established conclusions were made in morphinized, curarized, and chloralized animals, which drugs are definitely depressive to the heart and circulation. Therefore, where possible, recent available references will be used in preference to older literature, which is often unobtainable directly.

The immediate effect of rapidly-injected intravenous doses is to cause a sharp drop in blood pressure, which rises again quickly. This is thought to be due to the depressant action of concentrations of the drug on the heart.

Starr et al. (72), 1937, reported the usual effect of the drug to be a slight rise in blood pressure (5-10% after intravenous injection of 0.48 Gm. of theophylline. Lequime and Heerswynghels (139), 1939, observed a hypotensive phase following the rise in blood pressure and that hypertensive subjects were longer than the usual three minutes in returning to normal blood pressure. A fall in blood pressure may also occur, but not usually. The effect on blood pressure is to some extent dependent upon the dose, the rapidity of administration, and the initial blood pressure. Doses up to 20 mg. of caffeine cause a fairly constant rise,

whereas higher doses cause a progressive fall, dependent upon the amount given. In animal experiments with low initial blood pressures, caffeine more often causes some recovery (Sollman and Pilcher (136), 1911). With smaller doses and by other routes than by vein there is frequently no change in blood pressure. A fall in blood pressure and cardiac acceleration occurs in anesthetized dogs (Stoland et al. (114), 1954; Gilbert and Fenn (120), 1929).

Much interest has recently been directed toward the effect of theophylline on venous pressure or, more exactly, right auricular pressure. An early study by Capps and Mathews (140), 1913, showed no effect by caffeine on femoral vein pressure. Gunther et al. (141), 1942, also found no change in venous pressure. This is conceded as far as peripheral vein pressure is concerned, for the drug would have to lower intramuscular pressure to have this effect. However, it seems that the increase in cardiac output has some action in lowering right auricular pressure (Howerth et al. (91), 1947) and venous pressure (Davis and Shock (37), 1949).

Because of the use of theophylline in certain respiratory conditions, its effect on pulmonary hemodynamics is also of interest. Lacht (142), 1914, showed that 0.1% of caffeine could relax isolated pulmonary rings. Berezin (143), 1914, claimed that the lung vessels were at first constricted and then dilated. A more recent study of the effects of theophylline on the pulmonary circulation has overcome the objections of an open-chested study and the use of anesthesia (Freidberg et al. (144), 1945). They used doses of theophylline-ethylenediamine which had little effect on respiration and caused a small fall in systemic arterial pressure (in their anesthetized animals) and cardiac acceleration. Pulmonary

systolic pressure and pulse pressure rose in all cases. In one case pulmonary diastolic pressure rose. They interpreted the increase in pulse pressure in both the pulmonary and arterial circuit as indicating an increased cardiac output and stroke volume despite the cardiac acceleration. The elevation of pulmonary pressure does not indicate that theophylline's beneficial action clinically is due to relief of pulmonary congestion. The quick return of pulmonary diastolic pressure to normal they explained either by pulmonary dilatation or, more likely, by an adjustment of the output of the right and left ventricle.

The peripheral effect of theophylline in the blood vessels is one of vasodilatation. This overcomes a stimulant effect on the vasomotor center which causes a vasoconstriction. The effect on the vasomotor center has been demonstrated by perfusion (Sollman and Pilcher (138), 1911) and in decerebrate cats (van Eswald (145), 1930). It is generally agreed that the drug causes a peripheral vasodilatation, as shown by oncometric studies of organ volume (see Sollman for early work (71). A study by Hanzlik and Moy (134), 1945, showed that theophylline antagonized the pressor action of posterior pituitary. In dogs with low initial levels theophylline-ethylenediamine caused some elevation in pressure and an improvement in cardiac function. Stewart and Jack (70), 1946, observed a peripheral blood flow increase by skin temperature increases which lasted only 20 to 30 minutes. They ascribe the action to the increased cardiac output, which is of about the same duration. Nakasima and Hasiguti (146), 1938, reported that theophylline antagonized adrenalin constriction in the isolated perfused rabbit's ear. Yano (147), 1937, observed an increase in capillary pressure in the subcutaneous tissues of the frog after theophylline.

Although this vasodilatation is generally conceded, no definite proof exists as to whether it is due to the increased cardiac output or to an active dilatation of the blood vessels except that it appears to outlast increases in blood pressure (Sollman and Pilcher (138), 1911).

The early studies of Phillips and Bradford (148), 1887, and others to follow indicated a definite vasodilatation in the kidney after caffeine. This interpretation of the oncometric measurement has been questioned by Chasis et al. (149), 1937, as the over-all effect on the kidney. However, they and others have reported short increases in renal blood flow followed by decreased flow.

Roy and Sherrington (150), 1887, reported an increase in the cerebrum from oncometric observations. Raphael and Stanton (151) noted no change in brain volume by plethysmograph measurements. Noell (152), 1942, claimed theophylline and caffeine to cause a vasodilatation of the cerebral vessels. Contrary to a vasodilatation, theophylline seems to cause a vasoconstriction in the head and is effective in cases of migraine (Marin (68), 1946). Caffeine has long been used as a headache remedy. Recently Dumke and Schmidt (153), 1943, observed an increase in cerebral blood flow in monkeys after intra-arterial injection of theophylline-ethylenediamine. However, their results following intravenous injection were partly negative. Wechsler et al. (45) investigated the effects of 0.5 Gm. intravenous doses of theophylline-ethylenediamine in human subjects as to cerebral blood flow and cerebral oxygen consumption. They found a definite decrease in cerebral blood flow and oxygen uptake. These data correlate with the observation of Greene et al. (154), 1937, that aminophylline causes a fall in cerebrospinal pressure since cerebral vasoconstriction causes such a decrease and cerebral vasodilatation an

increase. This cerebral vasoconstriction also can explain the action of theophylline on the respiratory center and the vasomotor center by causing an accumulation of CO_2.

In summary, the evidence available seems to indicate that most of the peripheral effects of theophylline on the circulation are related very closely to its action on the heart. The increase in cardiac output appears to explain partly, if not wholly, (1) the increase in pulmonary pressure, (2) the increase in renal blood flow, (3) the rise in skin temperature, and (4) dilatation in the splanchnic and other organs. This increase in cardiac output causes the slight elevation in blood pressure that occurs with moderate doses. It also appears to be responsible for the slight fall in venous pressure. Theophylline appears to cause a decrease in cerebral blood flow. A peripheral vasodilatation by direct action of the drug on the blood vessels may take place, although direct evidence is lacking. The proof of this action seems to be in the fact that vasodilatation usually, but not always, predominates over the vasoconstriction caused by stimulation of the vasomotor center. It seems that the possibility that this vasodilatation is passive and directly related to the changes in cardiac output and blood pressure should be investigated for a better understanding of this phase of xanthine action.

4. The Effect of Theophylline and Other Methylated Xanthines on Blood Coagulation

Because of the widespread and repetitious use of theophylline and other methylated xanthine drugs, any evidence pointing to an acceleration of the blood-clotting mechanism by these drugs should be thoroughly investigated. The earliest recorded observation of this effect was by Klemperer (155) in 1896, who thought that the action of caffeine evoked

the development of a coagulative ferment. Other reports by Nonnenbruch and Szyszka (156) in 1920; Meissner (157), 1921; and Addicks (158), 1922, caused the preparation of theophylline with ethylenediamine to be classified as a hemostatic agent by Morawitz (59), 1926, and Pickering (160), 1928. Tobitani (165) in 1941 claimed that substances containing the guanidine nucleus decreased blood clotting times by increasing the formation of thrombin. Sirasako (162) reported in the same journal that caffeine caused a decreased bleeding time in rabbits.

Critical attention was attracted to this action of xanthines in 1944 when Field, Larsen, Spero, and Link (165) claimed a decreased prothrombin time in dogs, rabbits, and rats following administration of various methyl xanthines. Most of their studies involved dogs taking single oral doses of 10 to 400 mg./kg. or repeated oral doses of 4, 8, and 12 mg./kg. three times daily. They measured prothrombin times using a one-stage method with 12.5% plasma. They concluded that this effect was not due to hemoconcentration, a vitamin K-like action, or to the release of methyl groups in the liver. Furthermore, they stated that these drugs counteracted the effects of 3,3' methylene bis (4 hydroxycoumarin) (dicumarol) in producing hypoprothrombinemia.

Quick (164), 1945, however, was unable to corroborate these findings in dogs and rabbits. He suggested that their use of a less active thromboplastin which gave longer normal prothrombin times plus their wide range of normal values obtained for a 12.5% plasma urges a caution in drawing positive conclusions from their data. He noted that the prothrombin level can fluctuate within a wide range and still not affect the clotting time of blood. Field et al. (163) did not present evidence that the blood was hypercoagulable, but assumed that it was

from an increased prothrombin level, as evidenced by a shortened prothrombin time. Quick believed that the most important factor was the rate of thromboplastin release that governed the coagulation time. He was also unable to find any evidence that the methylated xanthines counteracted the action of dicumarol in producing hypoprothrombinemia.

Scherf and Schlachman (165) in 1946 claimed the observation of similar changes to that of Field et al. in humans. They gave single intravenous doses (0.5 Gm.) or repeated oral doses (0.5-1.0 Gm.) of aminophylline, theocin, or other xanthines. The changes they observed reached a maximum in four to five hours and sometimes persisted for 24 hours.

Following these papers there have been a large number of investigations which, like Quick's, did not substantiate any contraindication to the use of theophylline-type preparations. Rieben (166), 1946, was unable to confirm the work of Field and co-workers. However, Field, Sveinbjornsson and Link (167), 1945, found an increased plasma fibrinogen induced by the methylxanthines. Holland and Gross (168), 1948, in a careful study in dogs and humans, were unable to reproduce any of the changes, using several different preparations of theophylline and two methods of measuring prothrombin time. Other clinical studies in humans by Poindexter and Meyers (169), 1946; Breytspraak and Greenspan (170), 1946; Gilbert, Dey and Trump (171), 1947; Blood and Patterson (172), 1948; and Overman and Wright (173), 1950, have all found no significant variation in the prothrombin times of patients receiving theophylline preparations in single or multiple intravenous or oral doses.

A recent investigation by McCormick and Young (174), 1949, based on newer findings in the mechanism of prothrombin liberation by plasma

Ac-globulin has opened the question anew. Ware et al. (175), 1947, described plasma Ac-globulin as a proenzyme type of plasma protein. When acted upon by some of the first thrombin formed from prothrombin by thromboplastin, calcium, and a platelet factor, it is converted to serum Ac-globulin. This form actively catalyzes the interaction of thromboplastin and prothrombin and accelerates the rate of formation of thrombin. McCormick and Young observed in dogs following large doses of Aminophylline (100 mg./kg.) a transient elevation of prothrombin followed by a hypoprothrombinemia with a return to normal in two to three weeks. However, they found simultaneously a more marked and persistent rise in Ac-globulin activity.

Honorato (176), 1949, also observed a shortening of the prothrombin time of rabbits which he believed to be due to an increase in the thromboplastin co-factor (Ac-globulin of Seegers). It can be seen that an increase in Ac-globulin activity accompanying a hypoprothrombinemia would have little or an uncertain effect on whole blood-clotting time. Also it would be possible to have a hypercoagulability without a hyperprothrombinemia if the increase in Ac-globulin activity were accompanied by no sizable change in prothrombin level. It has been found also that far more dicoumarol is necessary to change the prothrombin time in patients receiving aminophylline (Olwin (177), 1950).

The whole picture of the true effect of the methylated xanthines is confused and contradictory. Possibly newer methods of measuring the clotting time of whole blood, as in silicone-coated containers where the clotting time is prolonged to 20-30 minutes, may demonstrate significant changes not observable with presently-used methods. Margulies and Barker (178), 1949, noted changes in whole blood coagulation time of

patients receiving dicumarol in silicone-coated tubes (Dri-Film 9987 - General Electric Co.) that were not observable in ordinary glass tubes. Certainly, when one considers the widespread use of the xanthine preparations, a hypercoagulability of clinical importance would have been observed long ago.

II. The Effects of Theophylline on the Secretion of Urine

In contrast with the controversial question of whether or not theophylline has a beneficial effect on the heart, it is generally conceded to have a diuretic effect on the kidneys. However, even more extensive than the literature discussing the action of the xanthines on the heart is that concerning the exact mechanism of their action on the kidney in producing this diuresis. The theories concerning the mode of action of xanthine diuresis can be divided into two periods: (1) the older literature up to 1930, which was highly controversial and based upon many unphysiologic experiments, mostly in lower animals such as amphibians, whose renal excretory mechanism has been shown to be not as highly developed as that of mammals, and (2) the more recent concept of renal function based upon the clearance concept of Rehburg (179), 1926. Many reviews of this older literature are available: Schmitz (180), 1932; Bartram (181), 1932; and others. Whereas it would be impractical to give space to all the early experimental work on the action of the methyl xanthines on urine production, it might be useful to mention several of the early theories and how modern renal clearance techniques have questioned some and substantiated others. The earliest theory was one of direct cellular stimulation independent of circulatory changes first advocated by von Schroeder (29, 30), 1887, 1888. Other theories of direct cellular action include that of Sobieranski (182), 1903, who attributed caffeine diuresis to diminished reabsorption of the glomerular filtrate. Cushny and Lambie (183), 1921, held a theory that the site of action was on the glomerular membrane, since they observed the diuresis to outlast the renal vasodilatation. A large number of investigators believe that the diuretic

effects can be wholly explained on the basis of vascular changes in the kidney. This view was first advocated by Lowei, Fletcher and Henderson (184), 1905, based on an earlier observation of Phillips and Bradford (148), 1887. Other theories have claimed the action of the xanthines to be extra-renal, but have not received much support since the diuresis occurs in isolated organs. Veil and Spiro (185), 1918, and Ellinger and co-workers (186, 187), 1921-22, proposed that the xanthines reduced the affinity of proteins for water. Curtis (188), 1929, based an extra-renal theory of action upon a slight but definite rise in the total chlorides in the blood. He also was able to inhibit xanthine diuresis by intraperitoneal injection of distilled water. He believed the drug to cause a shift of chlorides to the plasma which caused a hyperemia.

More recent investigations, especially those by interpretation of clearances on non-metabolites in the untraumatized kidney in un-anesthetized humans, have done much to disprove many of these theories, but much confusion still remains. The vitalistic or active secretion theory of von Schroeder (29, 30) has not been confirmed by the studies of Tamura and Miwa (189), 1919; Hayman and Schmidt (190), 1927; and Van Slyke et al. (191), 1934.

Sobieranski's (182) theory was based on decreased staining of tubular epithelium by indigotate dye and could be caused by the increased glomerular filtration which is generally conceded. Also Cushny's theory (192), 1926, of alteration of the glomerular membrane has received little support.

Most of the controversy surrounding the renal action of xanthines has centered around the effect on renal hemodynamics. The conclusion that they cause a renal hyperemia has been questioned by many but not wholly

disproved. The study of Chasis et al. (149), 1938, points out many of the uncertainties upon which this theory rests. The only evidence from untraumatized unanesthetized animals is that of Janssen and Rein (193), 1928, in dogs and by Walker et al. (194), 1937, but the duration or consistency of the increase was not such that it could wholly explain the diuresis. This confirms the conclusions of Cushny and Lambie (185) that the diuresis outlasts the increase in renal blood flow. The point that has not been disproved is that the drug might increase effective glomerular filtration pressure by local vascular adjustments such as constriction of the efferent arteriole in the kidney or vasodilatation of the afferent arteriole. This was suggested by the observation of Richards and Schmidt (195), 1924, that the number of functioning glomeruli in the frog kidney was observed to increase upon the injection of caffeine. However, caution must be observed in transposing observations from amphibia to man. Also such a highly specific action on one of two similar arterioles is doubtful.

With the advent of the clearance technique a method of measuring renal function without trauma or anesthesia was provided. Complete discussion of the experimental basis of this method may be found by reference to Smith (196).

The earliest renal clearance studies on theophylline were done by the use of creatinine and urea, both of which have been abandoned in favor of inulin (Shannon (197), 1935). Davenport et al. (198), 1934, and Chrometzka and Unger (199), 1931, reported diuresis in dogs with no changes in creatinine clearance. However, Schmitz (180), 1932, using large doses of Euphyllin in dogs, reported consistent increases in glomerular filtration, but no constant change in tubular reabsorption. Fulton et al. (200), 1934, and Page (201), 1933, reported no consistent effect on urea clearance

by the methyl xanthines. Other studies have also found no significant alteration of renal hemodynamics in rabbits and dogs (Foster (202), 1947; Walker et al. (194), 1937; and Newman (32), 1947). Dicker and Heller (203), 1945, have shown renal function in the rat to be similar to dogs and man. Dicker (204), 1946, found increases in glomerular filtration, renal plasma flow, and tubular water reabsorption following oral and subcutaneous doses of theophylline.

In man the effects of theophylline on renal hemodynamics have been much more uniform. Blumgart et al. (205), 1934, reported no increase in creatinine clearances in man. However, other studies using inulin and diodrast or p-amino hippurate have shown repeatedly alterations in renal hemodynamics. There is still some variation in the magnitude and duration of the responses. Chasis et al. (149), 1938, reported increases in glomerular filtration and in filtration fraction, but a decrease in renal plasma flow following a brief increase. This was confirmed by Escher et al. (92), 1948. They observed that in chronic congestive heart failure the increase in renal plasma flow is small but sometimes sustained. This they thought agrees with the observation of Howarth, McMichael and Sharpey-Schafer (91), 1947, that, in patients with congestive heart failure, increases in cardiac output are more sustained than in normal subjects. Green et al. (93), 1949, found similar increases in renal function plus increases in sodium output. They found aminophylline to have more effect than bromo- and chloro- derivatives both on cardiac output and diuresis. The most thorough study of the effect on renal function found was that of Davis and Shock (206), 1949. In 25 control subjects they report these results following the intravenous administration of 0.48 Gm. theophylline-ethylenediamine:

1. Effective renal plasma flow was increased only during the half hour following injection. It then declined to control levels or below.
2. Glomerular filtration was increased for 2½ hours. Continuous infusion of 0.96 Gm. of the drug produced a longer rise for a sustained period (55 or 60 minutes or longer).
3. Filtration fraction remained the same for one hour, but then increased significantly for the remaining three half-hour periods.
4. Urine flow was trebled and returned to normal after about one hour.
5. Sodium excretion also trebled and remained elevated. The concentration of sodium rose as the urine volume fell.
6. Venous pressure declined slightly.

In view of the retention of salt and water in cardiac decompensation, this action of theophylline in increasing sodium excretion is interesting. This effect on electrolyte excretion was noted some time ago. Grunwald (207), 1900, and Kerpel-Fronius and Butler (208), 1935, found marked losses of sodium chloride and potassium from rabbits with theobromine. They noted a serum concentration, weight loss, a concentration of serum non-protein nitrogen, and death with an ascending paralysis of the limbs. The latter attribute this paralysis to hypokalemia. Sinclair-Smith et al. (38), 1949, found a similar increase. They attributed the action of theophylline-ethylenediamine in decompensated cardiac patients to two mechanisms: (1) a direct effect on the circulation, presumably due to the increase in cardiac output which increases glomerular filtration rate and renal plasma flow, and (2) a specific tubular effect causing diminished reabsorption of sodium chloride. They noted variations in response, depending on the degree of decompensation. These data are partly in accord with the earlier conclusion of Smith (196), 1937, that the essential factor in xanthine diuresis is diminished tubular reabsorption of

water. This may be due to either a local action on the tubule cells or to the increased sodium and potassium load presented by the action on the glomerular filtration. Also the tubular action may be a local effect, a neurogenic effect considering the central stimulating action of the xanthines, or owing to a hormonal effect mediated either directly or indirectly by vascular changes in either the adrenals or the pituitary. Green and Farah (209), 1949, made a study in adrenalectomized and hypophysectomized dogs which points to a tubular control of sodium excretion. Further developments on control of sodium and probably potassium excretion should bring interesting facts concerning kidney function.

The possibility of an extra renal action was mentioned previously. This theory was probably based upon two changes in the blood which have been shown to be secondary to the diuresis (Dechard et al. (210), 1946). These are: (1) an increase in the water content of the blood, a hydremia, followed by hemoconcentration, and (2) decreases in the chloride content of the serum followed by a return to normal or an increase. This could be explained on the basis of excess sodium chloride loss by the kidney and a return to normal by replenishment from the tissues. Kirstein (211), 1947, thoroughly reviewed the contradictory evidence on these changes. He found no statistically definite variations in the chloride or water content of the blood in a large number of humans who showed definite diuresis after injection with theophylline-ethylenediamine and theophylline-diethanolamine. He found a correlation between dose of theophylline and urine output. Lipschitz et al. (212), 1943, found a linear relationship between log. dose of theophylline and log. action in the rat only between certain limits and that above this dose there was a decrease in diuresis. However, it is probable that this linear relationship may occur in laboratory

animals with uniform salt intake and the same pre-hydration. Results in cardiac patients are very variable.

In general, these drugs produce a marked response in hydrated animals and in normal animals an effect probably limited by the amount of salt that can be removed without disturbing normal limits of osmotic balances.

In experimental glomerulo-nephritis the xanthines are ineffective, but they do act in tubular nephritis until the anuric stage is reached (Hellin and Spiro (213), 1897, and confirmed by others).

Caffeine and theophylline in very large doses (40 mg./kg.) over long periods will cause definite nephritic changes, but they usually disappear a few days after discontinuance of the drug.

Haas (214, 215), 1943-44, made a study of the methyl xanthines as to the chemical structure to diuretic and other activity. He rated theophylline > para-xanthine > theobromine > caffeine > methyl xanthine > 1 methyl xanthine > 3 methyl xanthine. (> = greater activity than). Blood pressure responses paralleled diuresis. Formation of 1,3 dimethyl dimethyl xanthosin (theophylline-riboside) decreased activity, whereas esterification with phosphoric acid increased the diuretic and other effects. Lipschitz et al. (212), 1943, rated the diuretics in comparison with urea as unity; salyrgan - 400, bismuth and potassium tartrate - 219, theophylline - 119, caffeine - 32, theobromine - 7.2, and potassium nitrate - 3.9. Eddy and Downs (216), 1928, gave the equivalent doses for equal diuretic effect of caffeine, theobromine, and theophylline as 1:1.2:0.4 respectively.

In summary it may be said that, although the diuretic action of the theophylline is definitely established, the exact mechanism of its action on the kidney is not fully known. Most of the more recent evidence

points to the interaction of several factors: (1) an increase in cardiac output which causes a rise in renal blood flow, (2) an increase in glomerular filtration which outlasts the increase in renal blood flow, probably owing to vascular changes in the glomerulus, and (3) a diminished reabsorption of sodium chloride and water. Of these three factors, the effect on tubular reabsorption of electrolytes is most probably the main basis of theophylline action as a diuretic.

III. The Effects of Theophylline on Respiration

Similar to the action of theophylline on the circulation and the secretion of urine, the early work in determining the action of the drug was done by experiments on caffeine. Like the use of theophylline as a diuretic, its use in the stimulation of respiration was and still is, to some extent, empirical. This review will attempt to trace briefly the development of knowledge concerning the mechanism of action of the methyl xanthines on respiratory function. It will also try to compare the various theories of the basic mechanisms underlying this action.

The early investigators concerned themselves with observations of the effect of caffeine upon the rate (Binz (217), 1878), depth (Heinz (218), 1890), and rhythm of breathing more than the effect of the drug on the respiratory center. Sollman and Pilcher (219), 1911, noted respiratory stimulation of morphinized animals. Cushny (220), 1913, with therapeutic doses observed an effect lasting only 5 to 10 minutes. He showed the response to carbon dioxide was greater after caffeine than before.

Other early investigators concerned themselves with the fall in alveolar CO_2 tension as an index of the state of the respiratory center. Higgens and Means (221), 1915, used this as an indication that the action was on the respiratory center. Grabfield and Means (222), 1917, however, were unable to show that caffeine caused an increase in the response of the center to increasing concentrations of CO_2. However, by their method the maximum response to each concentration of CO_2 was not obtained. Smith (223), 1928, observed the decrease in alveolar CO_2 to last one to two hours, indicating an increased respiratory volume, whereas the observable hyperpnea lasted only 2 to 10 minutes. In animals with cerebral compression

which caused a constant medullary anemia and stimulated an increase in cerebrospinal pressure, caffeine caused little or no stimulation to respiration. This showed it was not a reflex respiratory stimulant (Loevenhart et al. (224), 1922). Hanzlik (225), 1923, showed that caffeine did not act as a reflex stimulant to respiration on subcutaneous injection.

After elimination of the sino-aortic mechanism by section of the vagi and extirpation of the carotid sinuses, caffeine still caused an increase in the respiratory minute volume. On the basis of this, LeMessurier (226), 1936, concluded that its action is mainly, if not entirely, central. Theophylline has also been shown to have an analeptic action in animals depressed by morphine or barbiturates (Hazard and Jequier (227), 1938; and Heerswynghels (228), 1938).

Richmond (229), 1949, reinvestigated the early works on the sensitivity of the respiratory center to CO_2 and found that, after equilibration of the subject to breathing 3% and 5% CO_2 in oxygen, a subcutaneous dose of 0.25 Gm. of caffeine and sodium benzoate caused a further increase in respiratory minute volume in both cases. A slight increase was noted when breathing only air. This, he concluded, indicated that caffeine rendered the respiratory center more sensitive to CO_2. However, with aminophylline in only five subjects, the same dose (but not on the basis of xanthine content) caused an increase in minute volume with 3% CO_2 but not with 5% CO_2.

Several other explanations of the mode of action of the methyl xanthines on the respiratory center have been proposed other than increased sensitivity to CO_2. Paul and Greene (230), 1936, and later Greene, Paul and Feller (43), 1937, believed that the favorable action of the drug

in dyspneas of various origins was due to its effect in reducing intrathecal and venous pressure. Marais and McMichael (44), 1937, in an investigation of the mechanism of action of the drug in Cheyne-Stoke respiration, decided that no vascular changes were involved but that the action was directly on the respiratory center and due mainly to the ethylenediamine portion of the molecule. In support of Greene, Paul and Feller's theory is the observation of Wechsler et al. (45), 1950, that a cerebral constriction takes place in the majority of cases with aminophylline. A decline in cerebrospinal fluid pressure follows cerebral vasoconstriction. Thus the action of theophylline might very well be due to an increase in the relative hypoxia of the respiratory center over and above that caused by CO_2. Wechsler and associates also noted a decreased cerebral O_2 consumption except in apprehensive subjects. This may well explain the lack of increased effect of aminophylline with 5% CO_2 in one of Richmond's subjects who, incidentally, was female.

While these investigators looked to a central explanation of the action of the respiratory effect, others looked for an action directly on the lung itself. Macht and Ting (231), 1921, reviewed the early work on the effect of purine derivatives on isolated bronchial muscles. He noted little effect of caffeine on the normal preparation but observed that the drug exerted a profound effect in relaxing pig bronchial strips which had been contracted with muscarine. He found a greater effect with purines containing fewer substituants and that the series exerted a greater effect in the following order: adenine and guanine > xanthine and hypoxanthine > theophylline and theobromine > caffeine. Young and Gilbert (232), 1940, showed that aminophylline (1:1000) counteracted histamine contracted bronchial rings, whereas the drug had little apparent effect on normally relaxed

sections. They also found it to inhibit histamine and anaphylactic-induced bronchial spasms in guinea pigs in doses of 30 mg./kg. Gilbert and Goldman (235), 1940, found a similar antihistamine action on constricted puppy bronchiolar sections. Confirmation of this antispasmodic action against histamine has been found repeatedly (Emmelin et al. (234), 1941; Loew et al. (235), 1946; and Lehmann and Young (236), 1945) and has been extended to pilocarpine, barium, and anaphylactic-induced spasms in both the isolated and intact lung (Ludueña (237), 1942) and in the isolated guinea pig ileum (Grandjean (238), 1948). Curry and Loard (239), 1948, found that aminophylline antagonized the respiratory but not the systemic effects of pilocarpine. The methyl xanthines do not antagonize the effect of histamine on gastric secretion but rather potentiate it (Robertson and Ivy (240), 1949). Hamburger and Halpren (241), 1948, believe that theophylline acts directly on the bronchial musculature, since they observed decreased resistance to artificial respiration under an aerosol of theophylline.

An altogether different theory of the action of theophylline in dyspneas is supported by Osgood and Ehret (242), who believe the primary effect is due to pulmonary vasodilation increasing the blood flow and any bronchodilator effect is of secondary importance.

The most widely accepted theory of action of the methyl xanthines as respiratory stimulants is that the action is mainly, if not entirely, central by an action on the respiratory center to increase the sensitivity of the center to CO_2. Others believe this effect may be a cerebral vasoconstricting action of the drug increasing CO_2 in the center. The evidence for a direct action in relaxing the bronchial musculature does not appear convincing, although the drug seems to have a decided action in antagonizing

certain bronchoconstrictors; namely, histamine, pilocarpine, barium chloride, muscarine, and anaphylactic reactions. In fact, theophylline could better be classified as a bronchial-antispasmodic than a bronchodilator. The theory that the effect in the relief of dyspneas by theophylline is mediated by a pulmonary vasodilation warrants consideration, but data on changes in pulmonary hemodynamics do not point to such an effect.

IV. The Effects of Theophylline on the Central Nervous System and Skeletal Muscle

Central Nervous System

The central stimulating action of the methylxanthines, particularly caffeine, is well known, as they are useful drugs in relieving drowsiness and "mental" fatigue. In moderate doses these drugs stimulate cerebral function, producing a quicker and clearer flow of thought, somewhat quicker reaction speed, and some decrease in the accuracy of delicate movements. Larger doses give rise to nervousness, insomnia, restlessness, tremor, and palpitation. Toxic doses are capable of producing convulsions of the strychnine type (Sollman (71), 1948). The effect of caffeine on brain activity is reflected in attacks of rapid potential variations in brain action currents (Fischer (243), 1932). Rapid injection of large doses of theophylline will cause grand mal-like cortical seizures, but 8-chloro-theophylline requires almost ten times this dosage to produce the same effect (Johns and Hinwich (244), 1950).

In recent years increased knowledge concerning the mechanisms of drug action at the synapse has thrown more light on the mode of action of xanthines in producing their stimulatory action. It now appears that their effect is mediated through an effect on acetylcholine concentration at the synapse. Bernheim and Bernheim (245), 1936, reported that caffeine caused inhibition of the hydrolysis of injected acetylcholine. Torda and Wolff (246), 1945, have shown an increased acetylcholine production by brain in vitro by all three methylxanthines. The activities of the three were quantitatively similar. Caffeine and theobromine (Zeller and Bissegger (247), 1943, and Nachmanson and Schneeman (248), 1945) and more recently

theophylline (Bounameaux and Goffart (249), 1949) have been shown to have an anticholinesterase activity on the true or specific cholinesterase. Thus the effect observed by Torda and Wolff could be due to decreased acetylcholine hydrolysis. Although the anticholinesterase action of these drugs may not be the fundamental explanation of their stimulation in the central nervous system, it is suggestive of a relation to the breakdown or synthesis of acetylcholine.

Skeletal Muscle

The actions of the xanthines on muscle have been observed since the first use of the drug. However, only recently has the action been correlated with the more intimate knowledge of the mechanism of muscular contraction. The early observations all lead up to two conclusions: (1) that small concentrations exerted a stimulant or defatiguing action on muscular contraction, and (2) higher concentrations produced a depression, a rigor, a fatigue of the muscle which appears first by lengthening of relaxation time and then by decreased amplitude of contraction. Until recently there has been no clear evidence to show whether the effects of the drug were peripheral or central. Recent studies by Huidobro and Amenabar (250), 1945, and Huidobro (251), 1945, have clarified much of the confusion. They explain the action of the methylxanthines on a neuromuscular preparation by a double mechanism. First they found an action on the neuromuscular junction which lowers the excitatory threshold to acetylcholine. Secondly, the xanthines have a direct action on denervated muscles which is also stimulatory. The first effect is most likely due to the anti-cholinesterase action of the drug. It is probably not caused by vasodilatation, since other vasodilators do not give such an action. The action on the neuro-muscular junction is related

to the frequency of stimulation, since low frequencies produce greater increases in contraction than high frequencies. He also observed that the xanthines exerted a decurarizing action and that they added to the depressant action of prostigmine at the neuromuscular junction. Torda and Wolff (252), 1945, found that the xanthine drugs increased the sensitivity of muscle to potassium as well as acetylcholine. Lower concentrations of the drug were required to increase the sensitivity to potassium. Gemmill (253), 1947, found increases in oxygen consumption and glycolysis of rat diaphragm with concentrations of 50 mg.-% of the various methylxanthines.

Torda and Wolff (254), 1948, have reported that caffeine inhibits the adenosine triphosphatase of skeletal muscle. They obtained this effect with higher than therapeutic concentrations. The exact mechanism of muscular contraction is not as yet understood well enough to speculate as to whether this might be the fundamental mechanism of these drugs on striated and even smooth muscle.

V. The Absorption, Excretion, and Fate of Theophylline in the Body

1. Absorption

Theophylline is readily absorbed from the gastro-intestinal tract. Thus, it is available by oral and rectal administration. The lungs are also capable of absorbing theophylline when administered in the form of aerosols and dusts. In addition, theophylline is given by parenteral administration, usually intravenously. Caffeine is completely absorbed upon oral administration, as none occurs in the feces and it is presumable that the same occurs with theophylline. No comparison in terms of blood levels of the efficacy of these routes has been reported.

The primary problem in the administration of theophylline is its solubility. Theophylline itself is soluble only 1 part in 120 parts of water. Dessauer (31), 1908, first introduced the combination of theophylline with ethylenediamine (Aminophylline) covered in a German patent in 1907 (255). Theophylline is also solublized by other aqueous amines, as well as with many other compounds, such as salts of organic acids (sodium acetate, calcium salicylate, etc.). Theophylline forms soluble complexes or double salts with these compounds. This combining power is due to its very slight acid properties (dissociation constant 1.62×10^{-11} at 25°). Leuallen and Osol (256), 1949, give a partial review of the combination of theophylline with aqueous amines and similar organic complexes.

It is believed that the gastric distress and nausea are caused by decomposition of these complexes by the hydrochloric acid of the stomach into the original components. Krantz et al. (122) introduced a combination of theophylline with sodium aminoacetate (Theoglycinate). The amino

group of the glycine gives the compound an increased acid-buffering capacity. Paul and Montgomery (15), 1948, showed the resistance of Theoglycinate to precipitation by hydrochloric acid to be more than that of Aminophylline. The drug was tolerated in doses up to 4 Gm. (i.e., 2 Gm. of theophylline) in 24 hours without nausea and vomiting. Other similar combinations of theophylline with the sodium and potassium salts of p-aminobenzoic, glutamic and anthranilic acids provide in the case of the potassium salts even more soluble forms of theophylline (257). Theophyllineaminisobutanol (258) has also been claimed to less irritating to the gastric mucosa and to be available in larger doses (23). Theophylline with isopropanolamine has also received therapeutic trial (259).

Most combinations of theophylline, especially Aminophylline, are unstable in liquid preparations except sealed ampoules. The majority of the oral therapy is performed with tablet medication. The plain tablets are more readily soluble than those with enteric coatings, but have the disadvantage of causing gastric irritation. They are best given with or immediately following a meal. Enteric coatings vary in their rates of disintegration, and the patient is unable to know when relief will be forthcoming.

Whereas the most satisfactory results are obtained with the administration of theophylline preparations intravenously, use of this route requires administration by a physician or a specially trained nurse. Although several deaths have been reported following the intravenous administration of Aminophylline (260, 261), an analysis shows that either a severe cardiac or a renal disease already existed which usually contraindicates the use of such a dose. Injection must be made slowly and in 10 to 20 cc. dilution. The reason given for this precaution varies.

Goodman and Gilman (54) state that it is to avoid untoward reactions due either to a sudden concentration of the drug in the heart, or to precipitation of the slightly acidic xanthine in the alkaline medium of the blood. Considering the precipitation of theophylline by the acid of the stomach, it is doubtful that the latter explanation is correct. Goodall and Unger (262) have described a continuous intravenous drip method, but it requires special attention, along with hospitalization. Thrombophlebitis also occurs. Part of the benefit probably comes from the glucose, salt, and fluids given.

Intramuscular administration is generally dissatisfactory to the patient, and the effect is not rapid. The alkalinity of aminophylline causes the choice between pain from high alkalinity in a small amount of water, or pain from a large amount of water and moderate alkalinity. There is danger from fat necrosis and sterile abscesses.

Inhalation of theophylline is frequently valuable in asthmatics. Richards, Barach and Cromwell (263), 1940, described a method of producing an aerosol of Aminophylline with a nebulizer supplied by compressed air or oxygen. Prigal (264), 1947, utilized steam to volatilize Aminophylline. The problems attending this route of absorption are: interference from mucus plugs, inefficient absorption (50% or less), and definite pulmonary irritation. More recently Taplin and associates (265), 1949, have reported the use of micropowdered Aminophylline and theophylline by inhalation. It is probable that bronchoirritation would occur in this form as well.

The rectal route of absorption has several advantages. Absorption is rapid, and the drug is well tolerated. Gastric irritation is avoided, as well as the disadvantages of injection. A difficulty encountered

in this route is the inability of some patients to retain suppositories or enemas. Sedatives and local anesthetics can be easily added. Rectal use of theophylline has largely been through the use of rectal suppositories. Suppositories are sometimes uncertain and slow in melting. Absorption from a retention enema described by Barach (57) gives results midway between oral and intravenous administration in effectiveness. Hartman (60) has proposed continuous rectal instillation in saline or dextrose with inclusion of a local anesthetic if desired for the use of unhospitalized severe asthmatics. He also recommended the administration of rectal jellies and hydrophilic ointments containing the drug.

Of the several routes available, the intravenous route is best for promptness of action, but is of course not available for self-medication. Oral absorption is uncertain and not sufficiently rapid in most cases. The rectal route has many obvious advantages over the oral route, but patients usually prefer oral to rectal medication. Inhalation absorption is sufficiently rapid to provide relief even in severe respiratory conditions.

2. Excretion and Fate in the Body

Because of the ingestion of substantial amounts of the methylated xanthines in coffee, tea, and other beverages, early German investigators were interested in their fate in the body. These investigations were reviewed by Myers and Wardell (266) in 1928. After the ingestion of theophylline, a small amount is excreted in the urine unchanged, similar to caffeine (267). Plummer (268) found an excretion of only 1.8% of the administered theophylline recoverable in the urine in the first ninety minutes, during which time it had completely disappeared from the blood stream. Schack and Waxler (269) found theophylline to be distributed

throughout the body fluids after intravenous administration. No concentration in any particular organ could be demonstrated. In the blood, theophylline is restricted to the plasma and is only slightly bound by the plasma proteins. Tissue concentrations of theophylline were observed to fall parallel to blood levels.

A portion of the drug undergoes demethylation to form monomethylxantnines (270). Kruger and Schmidt (267) established the order of decreasing stability of the methyl groups in man to be $7 > 1 > 3$. In rabbits the 7 and 1 groups are about equally stable (271). Thus in man and rabbits 1 methylxanthine appears in the urine following ingestion of theophylline, whereas in the dog the principal monomethyl excretion product is 3 methylxanthine. No evidence of further degradation of the monomethylxanthines to xanthine is reported.

A larger portion of theophylline is oxidized at position 8 to form 1,3 dimethyluric acid which may also undergo demethylation to form 1 or 3 monomethyluric acids. Many investigations have been conducted to determine whether the methylxanthines were capable of increasing the uric acid excretion. Both positive and negative results were reported. Development of a specific enzymatic method of measuring true uric acid has shown beyond doubt that, whereas the true uric acid excretion is unchanged, there is a large increase in the total phosphotungstic acid reducing substances excreted which directly result from the ingestion of the theophylline (272, 273). Myers and Hanzal (274) found, on the basis of the expected oxidation of theophylline at position 8, that the increase in phosphotungstic acid reducing substances in the urine indicated that theophylline was excreted largely as 1,3 dimethyluric acid with a small amount of 1 methylxanthine. They also found crystals in the urine apparently

identical with 1,3 dimethyluric acid. Presumably in the dog and other lower animals these methyl uric acids are capable of further degradation to allantoin.

Goodman and Gilman (34) and Sollman (71) give a statement concerning caffeine that the remainder of the drug -- up to 80% -- is oxidized to urea. Goodman and Gilman further state that theophylline and theobromine share the same fate except that a larger portion of the drugs may appear unchanged in the urine. This idea was drawn from the work of Salant and Rieger (275), 1912, who were unable to find any increase in uric acid output and could account for only 8% excreted unchanged. It was known also that 10 to 40% was excreted as mono- and dimethylxanthines.

The conclusion that a part of these drugs is oxidized to urea has not received further substantiation. Bernheim et al. (276), 1946, found the injection of theophylline, as well as the other methylxanthines, into rabbits caused a decrease in the total and the urea nitrogen of the urine, with no constant variation of the other nitrogen compounds. They also noted a decrease in the non-protein nitrogen and urea nitrogen of the blood. This confirms a previous in vitro study which showed a decreased formation of urea from ammonium salts by liver and kidney slices agitated in Krebs bicarbonate solution. Cohen and Hayano (277), 1947, also reported an inhibition of the conversion of citrulline to urea. Martin (278), found an increased excretion of allantoin following a maximal dose of theophylline, but not caffeine or theobromine. Uric acid excretion was also increased. This shows the influence of the lack of a methyl group in position 7 on the oxidation of theophylline to a methyl uric acid, which in animals (rats in this case) is capable of further metabolism to allantoin.

In summary, it seems most probable, from the majority of evidence,

that the metabolism of theophylline takes place in the following manner in man. After absorption and distribution throughout the body fluids, a small portion is excreted unchanged in the urine. If the blood level is elevated enough to cause renal vasodilitation and an increase in renal blood flow, an increased amount will be filtered into the urine. A small portion undergoes demethylation at the number 3 position and is excreted partly as 1 methylxanthine or may undergo oxidation at position 8 to form 1 methyl uric acid. A larger portion is oxidized without demethylation and excreted as 1,3 dimethyl uric acid. Theoretically some theophylline is demethylated at both the 1 and 3 positions to form xanthine, which is capable of forming true uric acid, but this amount is so small as to be as yet undetected. Evidence for further degradation has not been substantiated.

CHAPTER III

EXPERIMENTAL DATA

I. Methods for the Quantitative Estimation of Theophylline

Although theophylline has been used as a drug for over fifty years and its chemistry has been well established, no suitable method applicable to biological fluids for the quantitative estimation of its concentration has been made until recently. Truitt et al. (295), 1947, published the first of the two methods described herein. Plummer (288), 1948, published a method applicable to blood and urine which was based on the iodometric titration of a theophylline-copper complex precipitated by copper acetate. More recently Schack and Waxler (269), 1949, developed a spectrophotometric method applicable to blood, urine, and tissues for both theophylline and theobromine. A similar method has also been presented for caffeine by Fisher et al. (279), 1949.

The need for a simple and rapid analytical method of measuring theophylline blood concentrations is well demonstrated in the empirical administration of the drug, both in experimental investigations and in therapy. Doses are seldom given on a weight basis and without respect to blood levels and rate of disappearance from the blood. Very little is known about the effect of conditions such as nephrosis and other kidney conditions, as well as deficiencies of the liver which may cause prolongation of the time required to dispose of theophylline.

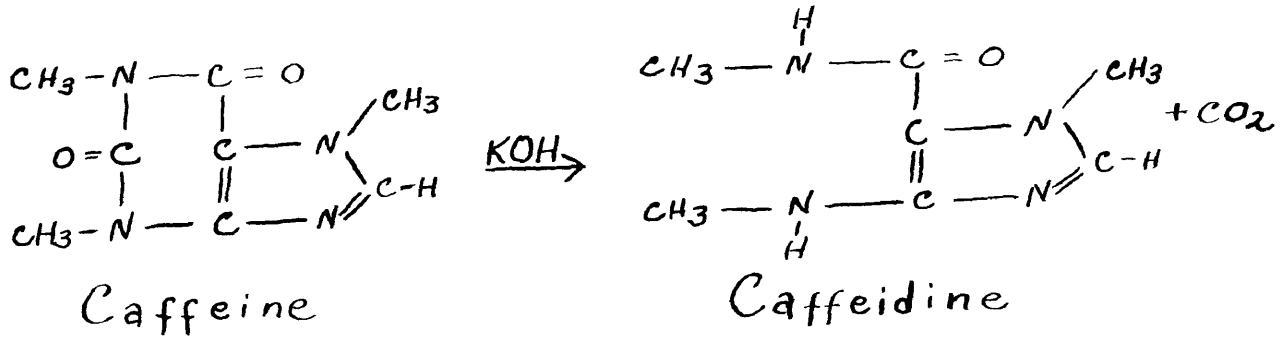
Preliminary attempts to measure theophylline quantitatively were made, using the ultraviolet spectrophotometer. Difficulty was had with interfering substances having absorption peaks near to that of

theophylline (277 mμ in N/10 NaOH).

Many oxidative reactions for xanthine bases are known, but the majority are unspecific and of low sensitivity. A method utilizing Gibbs reagent (2,6 dichloro-quinone-chloroimide) was also found to be lacking in specificity.

Theophylline will couple with diazotized sulfanilic acid (280) but not with adequate sensitivity. Sanchez (281) showed that oxidation of the methylated xanthines with 33% NaOH enabled them to couple easily with diazo-p-nitro aniline. Theophylline in concentrations expected after therapeutic doses and extraction from 5 cc. blood samples did not give sufficient color for a colorimetric procedure. Among a number of stable $ZnCl_2$ complexes of diazonium salts supplied by Dr. W. Minnis of the National Aniline Division of the Allied Chemical and Dye Corporation, Fast Blue 2B Salt, which is the zinc chloride complex of the diazotized 5 amino-2 benzoylamino-1,4- diethoxy-benzene, was found to couple with a product of the alkaline oxidation of theophylline in very high dilution.

Maly and Andreasch (282) give the following degradation of caffeine with fixed alkali to form caffeidine:



Caffeine Caffeidine

Further heating will decompose caffeidine into methylamine, ammonia, sarcosine, formic acid, and CO_2. Theophylline, which lacks the methyl group of caffeine at the 7 position, is assumed to react in a similar

manner to form the analogous "Theophyllidine." This oxidation product of theophylline readily couples with the diazotized salt in alcoholic solutions, probably by coupling at the active hydrogen at position 8. The soluble azo derivative formed is moderately stable and has an absorption peak of 535 mμ in the visible range when measured in the spectrophotometer. The reaction is sensitive to 5μg. of theophylline. The rate of formation of color is dependent upon temperature and reaches a maximum in 5 to 7 minutes under the conditions of the method.

Caffeine, but not theobromine, gives some color by this reaction, but not the intensity obtained with theophylline, as seen in Table 1.

Table 1. Comparative Reactivity of Methyl Xanthines

Drug	Concentration	Optical Density Per Cent of Theophylline
Theophylline	1 mg. %	100
Caffeine	1 mg. %	8
Theobromine	1 mg. %	0

Uric acid, creatinine, creatine, urea, and glycine do not interfere. Blood of Nembutalized animals, as well as blood containing heparin, was found not to interfere with the method. Sulfonamide drugs, however, will produce color by this reaction and must be avoided. The various methyl uric acids which result in the metabolism of theophylline were not available for experimental trial, but are known to be unstable in hot alkali. A further study of the specificity of the reaction by means of phase distribution ratios has been made after the method of Brodie et al. (284), 1947 (see Appendix, Section I).

Procedure, as applied to theophylline solutions:

1. Solution of known concentration is placed in a 25 x 100 mm. thin glass pyrex test tube. A glass bead is added and the solution evaporated to dryness.
2. Add exactly 2.0 cc. of 50% V/V potassium hydroxide solution and boil for 15 to 20 seconds over a Bunsen flame.
3. Immerse tube in a beaker of ice water for 1 minute (time and heat of boiling should not be sufficient to cause solidification of KOH at this point). Add exactly the quantity of 50% V/V acetic acid to neutralize to pH 5.5 - 6.0 as previously determined, using methyl red as an indicator.
4. Cool in an ice bath for 1 minute.
5. Transfer to a 10 cc. stoppered graduate and make up to 5 cc. with water. Add 5 cc. of the diazo reagent.[1] Mix.
6. Read color intensity after 5 to 7 minutes in a Fisher Electrophotometer, using a 525 mμ filter and a semimicro absorption cell.

Color versus Concentration

Several concentrations of anhydrous theophylline were subjected to the above determination and a straight line relationship between optical density as measured on the logarithmic scale of the Fisher Electrophotometer and the concentration of theophylline in milligrams-per cent, using the specified 5 cc. sample, was obtained, as seen in Fig. 1.

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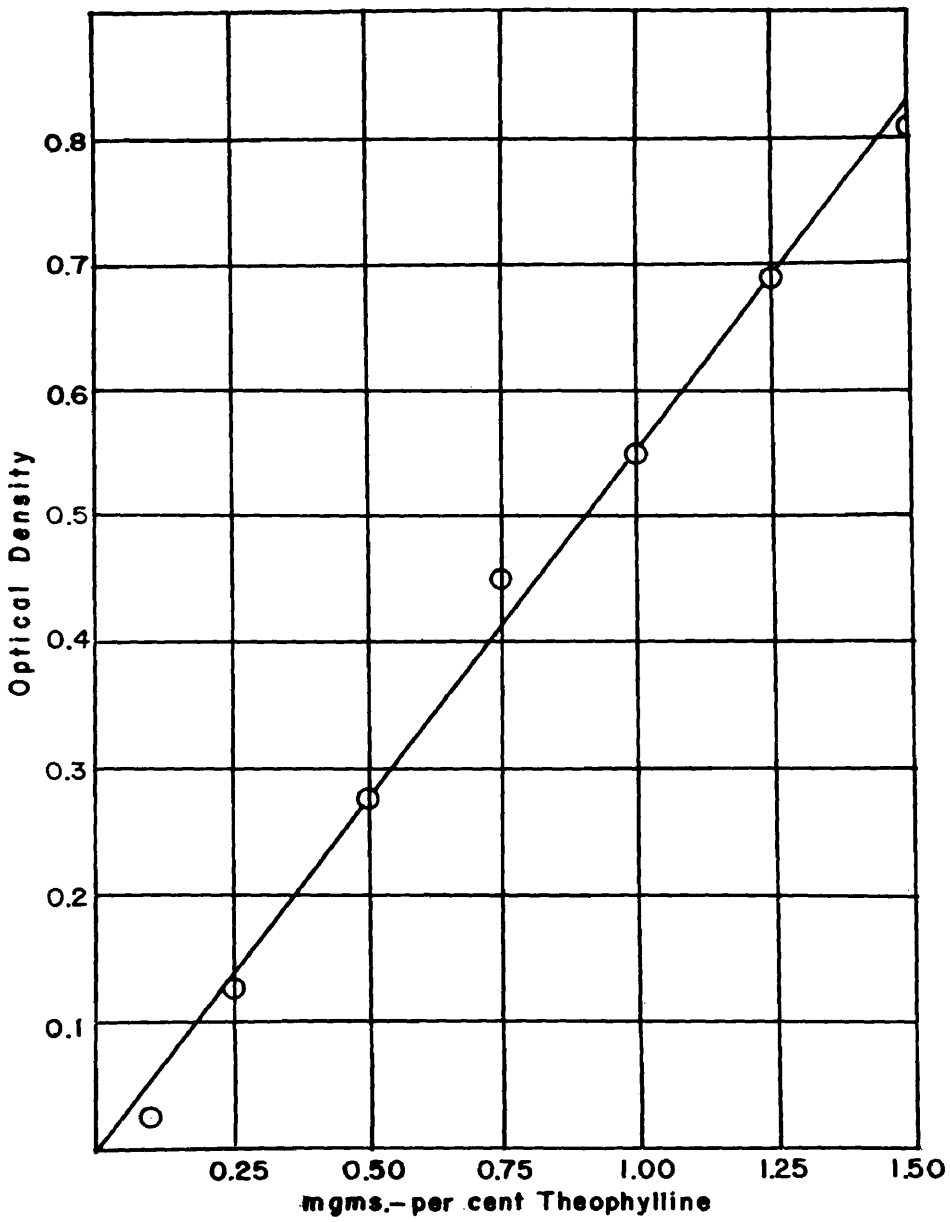
[1] Dissolve 100 mg. of Fast Blue 2B Salt in 100 cc. of isopropanol (Merck's Reagent Grade) and filter through a dry filter. Preserve at 0° C. and remake after 2 hrs.

Fig. 1



CONCENTRATIONS OF THEOPHYLLINE

Application to Blood

The above procedure was adapted to blood as follows:

1. Add 5 cc. of oxalated blood to a mixture of 10 cc. of water and 5 cc. of 2/3 N sulfuric acid contained in a 50 cc. centrifuge tube. Mix thoroughly.

2. When the blood is laked, add 5 cc. of 10% sodium tungstate

(Baker's Special according to Dr. Folin) solution and mix thoroughly.

3. Centrifuge for 5 minutes at 2500 R.P.M.
4. Decant the supernatant liquid and filter through a 11.0 cm. No. 2 Whatman filter paper.
5. Evaporate 10 cc. of the clear filtrate to dryness on a steam bath aided by a current of air.
6. Extract the residue with 3 successive 5 cc. portions of $CHCl_3$.
7. Filter the combined $CHCl_3$ extracts through a pledget of cotton into a 25 x 100 mm. thin glass pyrex test tube and continue as above.

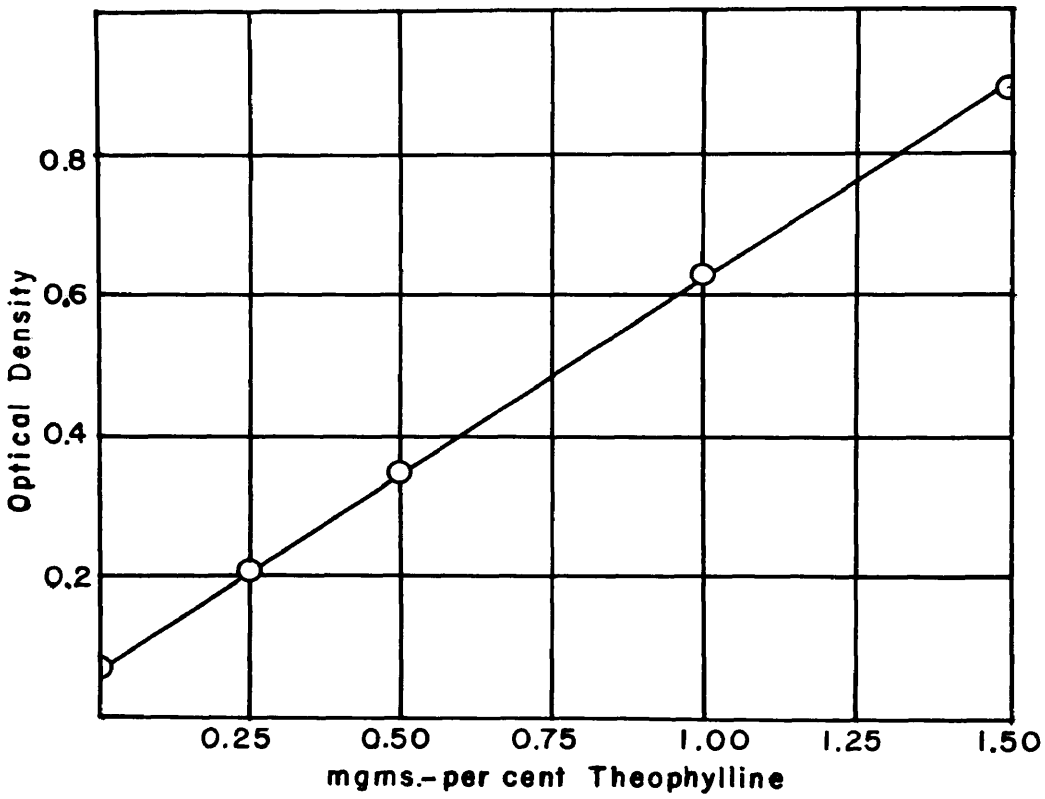
Standard Curve of Theophylline in Blood

A standard curve shown in Fig. 2 was prepared by addition of known amounts of anhydrous theophylline to oxalated pooled human blood samples. Each point on the curve is the result of 10 or more determinations.

Revised Method for Theophylline Blood Levels

Because of the length of the procedure and the difficulty in step 6 of extracting the dry residue of some blood filtrates, as well as the inaccuracies involved in several of the transfers, it was decided to simplify and, if possible, improve the accuracy of the method. In their studies on theophylline and theobromine, Schack and Waxler (269) demonstrated that theophylline is restricted to the plasma of blood. Based on experience by Reimers (283) and Plummer (268), it was decided to employ a mixture of isopropyl alcohol and chloroform as the organic extracting solvent. Schack and Waxler found a mixture of 20 parts of chloroform to 1 part of isopropyl alcohol to be satisfactory. Using this extraction solvent, the following procedure was adopted and found to correlate very

Fig. 2



BLOOD LEVELS OF THEOPHYLLINE

closely with values obtained by the first method:

Reagents:

1. Chloroform-Isopropyl Alcohol (20:1) C. P.
2. Acetic Acid 50% W/V
3. Potassium Hydroxide 50% W/V
4. Hydrochloric Acid 1.0 N

5. Solution of Fast Blue 2-B Salt in Isobutyl Alcohol containing 0.25 mg./cc. Filter and keep in an ice bath at 0° C. Remake fresh every two hours.

Procedure:

1. Into a 50 cc. glass stoppered flask place 30 cc. of the Chloroform-Isopropyl Alcohol (20:1) mixture, 0.1 cc. of 1.0 N HCl, and 2.0 cc. of plasma.
2. Shake for 30 minutes on a shaking machine.
3. Transfer to a 50 cc. centrifuge tube and centrifuge at 2000 R.P.M. for 3 minutes.
4. Remove exactly 25 cc. of the extraction mixture and place in a 25 x 100 mm. pyrex test tube. Add a glass bead.
5. Evaporate to dryness on a steam bath.
6. Add 2.0 cc. of 50% KOH and boil for 15 seconds over a Bunsen flame. (Flame should not be so hot as to cause solidification upon cooling.) Cool in an ice bath.
7. Neutralize to pH 5.8-6.0 with 50% Acetic Acid. Cool in an ice bath to remove heat of neutralization.
8. Add 7.0 cc. of the Fast Blue 2B Salt Solution and mix well until color develops.
9. Pipette off 5 cc. of the alcohol phase and place in the micro cuvette of the Fisher Electrophotometer.
10. Read after 15 minutes, using a 525 mμ filter.

Using the above procedure, a standard curve was developed similar to Fig. 2. Blood samples containing no theophylline were analyzed as blanks and the average reading of 10 or more determinations was subtracted from each of the average readings of the known concentrations of theophylline

in blood.

Comments:

In step 4, removal of the lower phase is accomplished by pushing aside the plasma layer, which is quite thick, with a stirring rod and inserting the pipette. Evaporation of the extraction solvent in step 5 is speeded by bubbling air through the liquid. This also avoids boiling over. The amount of acetic acid to neutralize 2.0 cc. of KOH solution is previously calculated, using Methyl Red as an indicator. The rate of development of the red color is dependent upon the temperature at which it develops. Thorough mixing of the two phases is important. Removal of the alcohol phase in step 9 is simplified by complete removal of the lower clear aqueous phase with a pipette. Maximal absorption by the reaction product takes place at 535 mμ, as measured in the Beckman Ultraviolet Spectrophotometer.

II. Absorption of Theophylline by the Tissues

In the past the comparative evaluations of the efficacy of the various routes of administration of theophylline was based mostly upon the observations of clinicians and the reactions of patients. The reactions of humans to theophylline are so uncertain and variable that no quantitative data are available to prove the reliability of these observations. Dosage of theophylline has been mostly empirical, and little has been known of the amount necessary per kilogram of body weight to produce certain responses of the drug. Neither has the maintenance dose of the drug been known with certainty.

The following studies were carried out first of all to provide a standard of reference for expressing the activity of theophylline. With accurately measured blood levels for comparison, the administration of the drug by various routes could be evaluated as to completeness, rapidity, and constancy of absorption. Also from these data the rate of disappearance of the drug from the blood stream could be computed. The development of tolerance to the diuretic action of theophylline is well known, but a statement could not be found as to whether this might not have been due to failure of the patient to absorb the drug well after several days.

The purpose of the following experiments is thus two-fold: first to establish quantitatively the blood levels obtainable in man and dogs by the administration of therapeutic doses by various routes, secondly to correlate as well as possible these levels with some activity of the drug.

Analytical Method

The first half of these theophylline blood levels was done by the first method described in Section I of this chapter. The latter half was analyzed by the modified method which permitted a larger number of blood samples per study. Blank samples were drawn from each subject. All samples were analyzed in duplicate.

Selection of Subjects

The human subjects for this study (with 3 exceptions) were young male patients, with normal renal and cardiac function, from the United States Marine Hospital in Baltimore. Xanthine-containing beverages and medication were removed 12 hours prior to administering theophylline. Theophylline-ethylene-diamine (Aminophylline) studies were made using the Searle brand throughout. Theoglycinate (Brayten Co.) was used for the study involving theophylline-sodium aminoacetate.

The Intravenous Route of Administration

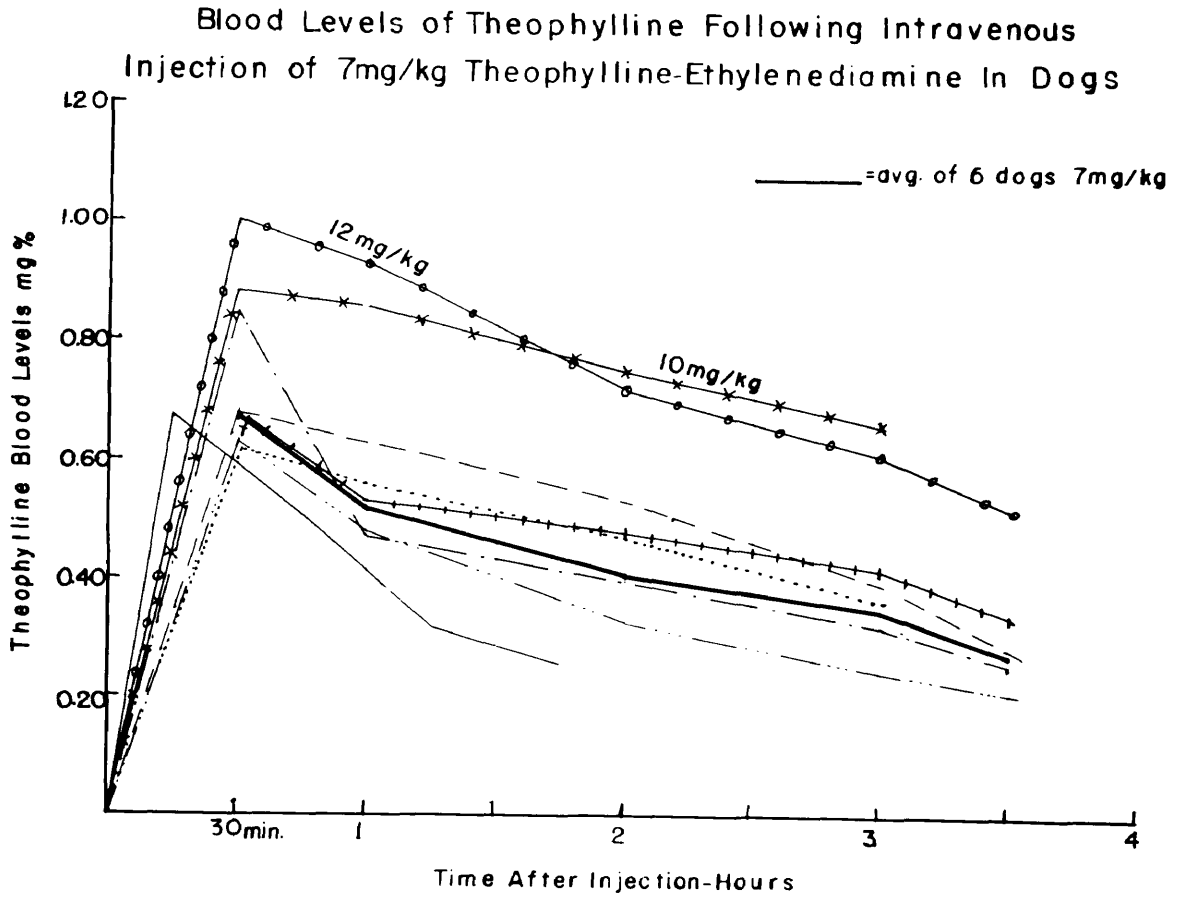
a. Results Obtained in Dogs

Intravenous doses of 7 mg./kg. of theophylline-ethylene-diamine were made in 6 dogs and doses of 10 and 12 mg./kg. in one dog each. Blood samples were drawn at half-hour intervals up to 3½ hours. Fig. 3 shows the plot of the blood levels found against time. The average rate of fall in blood level in dogs was calculated from the average of 6 dogs to be 0.13 mg.-%/hr. The intravenous administration of 7 mg./kg. is roughly equivalent to a dose of 0.5 Gm. to a 70 kg. man.

b. Results Obtained in Man

A study was made of the blood levels following the slow intravenous administration of 0.5 Gm. of theophylline-ethylenediamine in 11

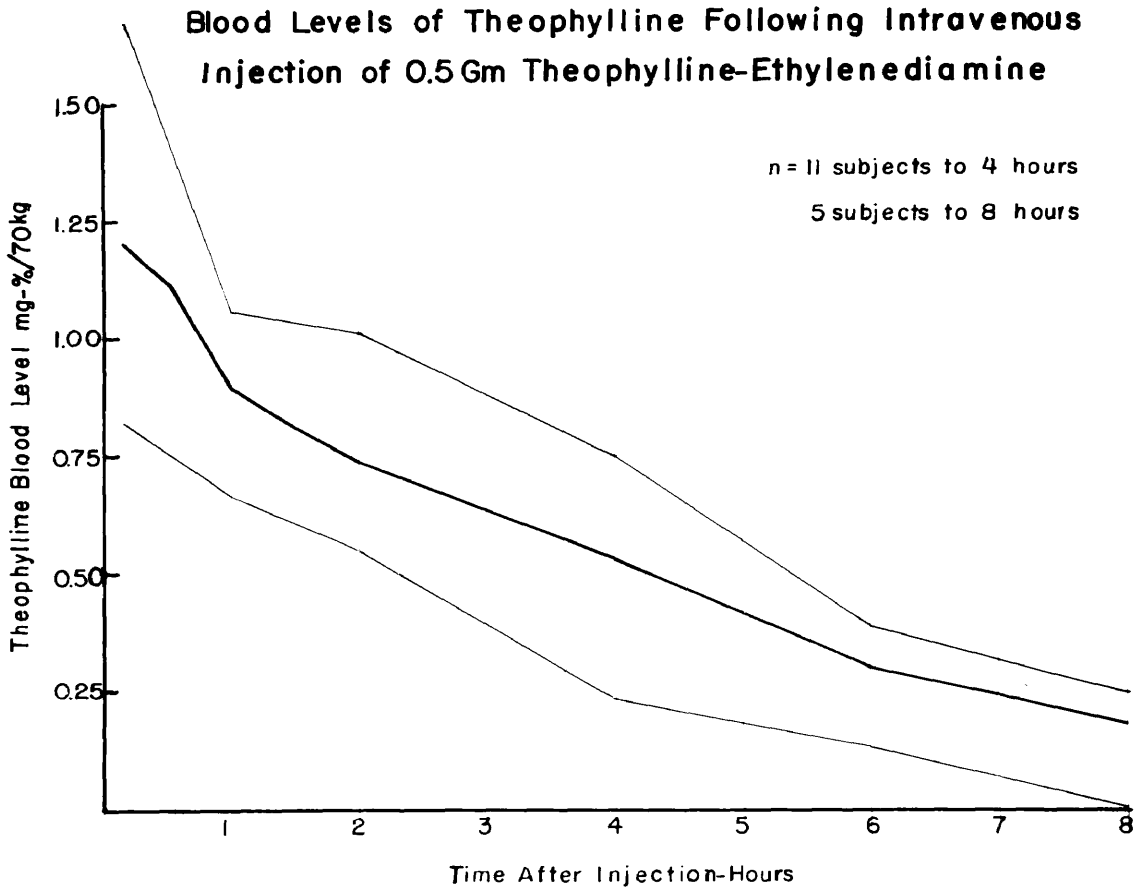
Fig. 3



subjects. Blood samples were drawn at various intervals up to 4 hours in all but one subject and up to 8 hours in five subjects. Because of the necessity of slow injection to avoid side effects with so large a dose of the drug, the first samples drawn at 10 or 15 minutes showed wide variation in blood concentration, depending on the completeness of distribution of the drug from the blood stream into the extracellular body

water. The 30-minute samples more closely reflect the effective concentration, and after this time disappearance follows a more regular pattern. Table I (see Appendix) shows the blood levels obtained expressed on a mg. per 100 cc. of blood or mg.-% basis. For better comparison of blood concentrations following the same 0.5 Gm. dose, these results were converted to a mg.-%/70 kg. basis by inverse proportion to the body weight. This is shown in Table No. II (see Appendix). Fig. No. 4 shows the relation of the average blood concentrations of these 11 subjects, calculated to a 70 kg. body weight basis, to time after injection. The upper and lower lighter lines show the maximum variation for the points plotted.

Fig. 4

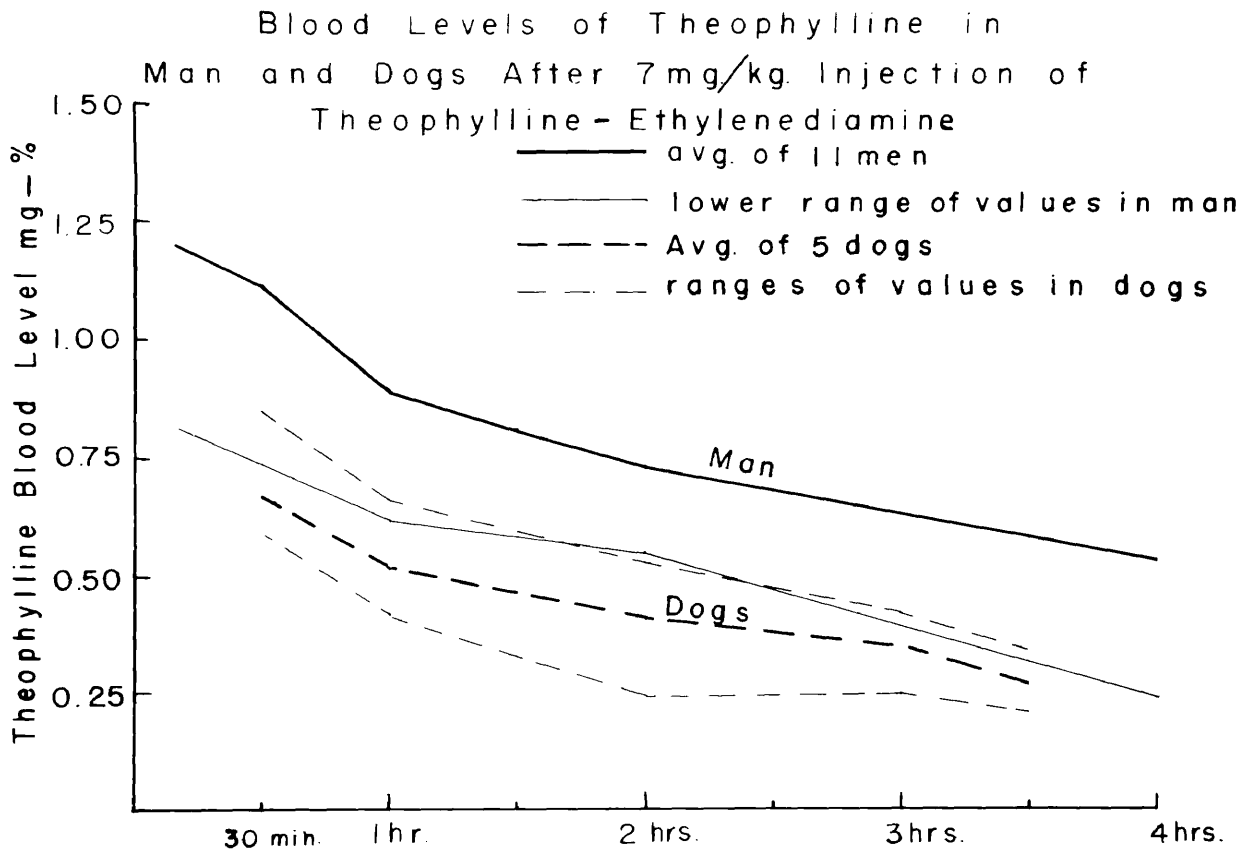


From the average blood concentrations at these intervals the rate of fall in blood level was calculated to be 0.13 mg.-% hr.

c. Comparison of Blood Levels in Men and Dogs

In view of the number of experimental studies on the action of theophylline in dogs it was considered advisable to compare the blood concentration following comparable doses by intravenous injection. Fig. 5 shows the graphic comparison of the average human blood levels for 11 subjects adjusted to a 70 kg. weight basis following 0.5 Gm. doses (approximately 7 mg./kg.) with the average results of 6 dogs for a 7 mg./kg. dose. The finer lines show the upper and lower limits of individual variation for each group.

Fig. 5

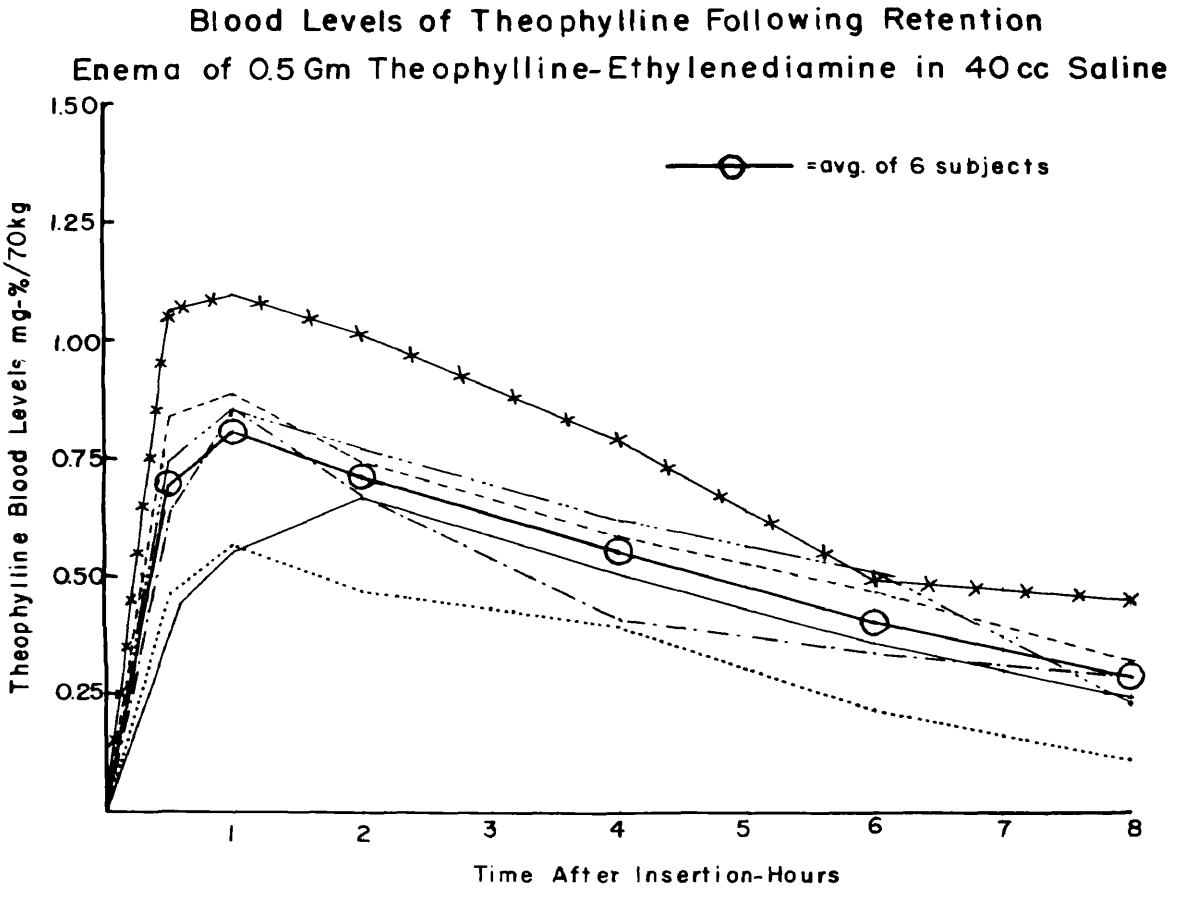


Although the blood concentrations in man are significantly higher, this discrepancy does not seem to be due to more rapid metabolism in dogs. The metabolic pattern of theophylline is similar, but slightly different in the dog (see Chapter II, Section V). The variation may be due to a higher percentage of adipose tissue in man.

The Rectal Route of Administration

Recent attention has been directed to the rectal route of administration of theophylline, particularly in the treatment of asthma (Dees (58), 1945; Barach (57), 1944; and Hartman (60), 1949). Rectal absorption was studied by two routes in human subjects. Rectal enemas containing 0.5 Gm. of theophylline-ethylenediamine were dissolved in 40 cc. of saline and instilled into the rectum. In order to duplicate the variations to be expected with patients using this route of self-medication, no effort was made to evacuate the rectum prior to administration. No rectal irritation or problems in retention occurred with this method. The individual results with this route of administration are summarized in Table No. III (see Appendix). The graphic relation of these blood levels to time is shown in Fig. No. 6. It is apparent from the graph that absorption is rapid and maximum blood levels are reached within 30 minutes to 1 hour. It is believed that these data indicate the efficacy of retention enemas as a method of administration of theophylline. On the other hand, absorption from rectal suppositories was not so good. Blood levels of theophylline were measured at intervals for 8 hours following the rectal insertion of a suppository containing 0.5 Gm. of theophylline-ethylenediamine. As can be seen in Fig. 7, the absorption is slow, and blood levels never reach much more than 20% of the corresponding level produced by intravenous administration of the same dose. It is to be noted that the ordinate scale

Fig. 6

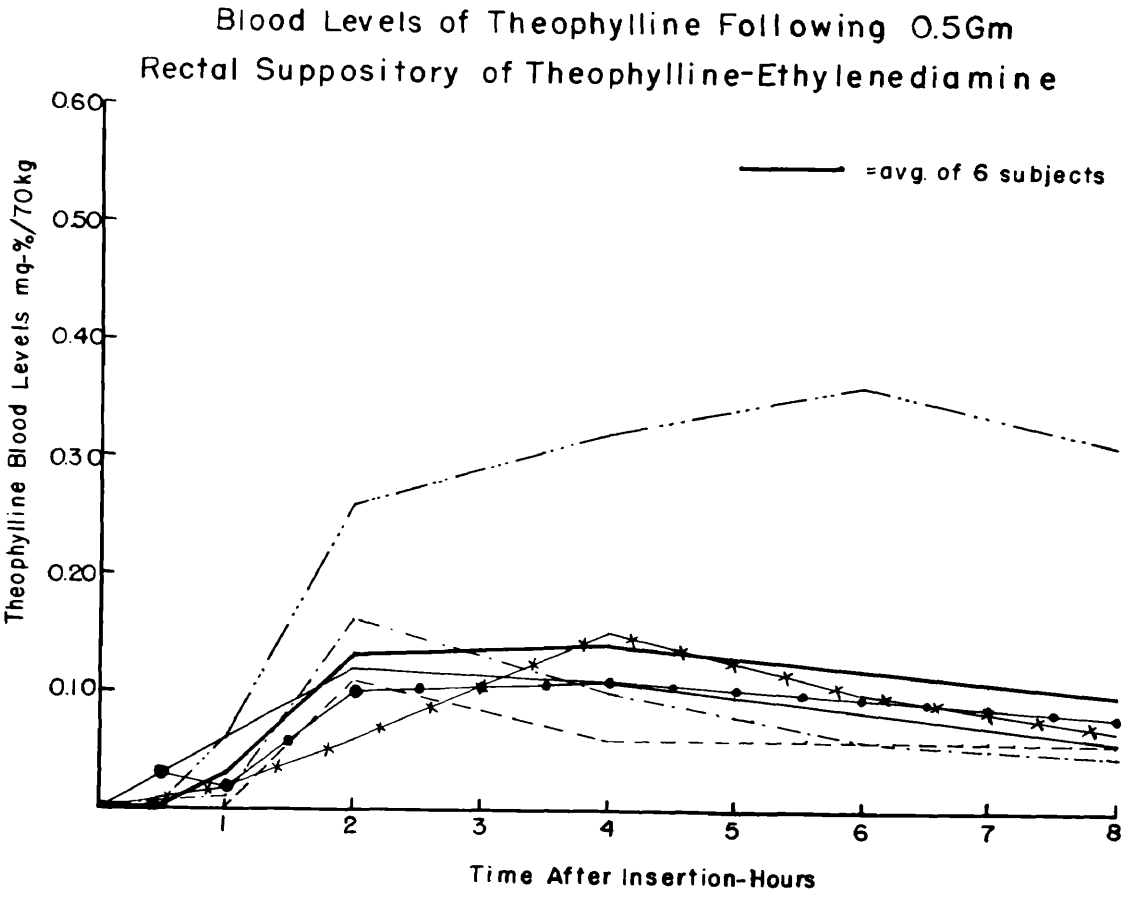


is magnified 2.5 times that for intravenous and rectal enema data. Complete data for these patients are given in Table IV (see Appendix).

The Oral Route of Absorption

Studies of theophylline blood concentrations were made following two oral doses of theophylline-ethylenediamine. Since the usual dose for repeated oral administration is 0.3 Gm., this dose was first chosen.

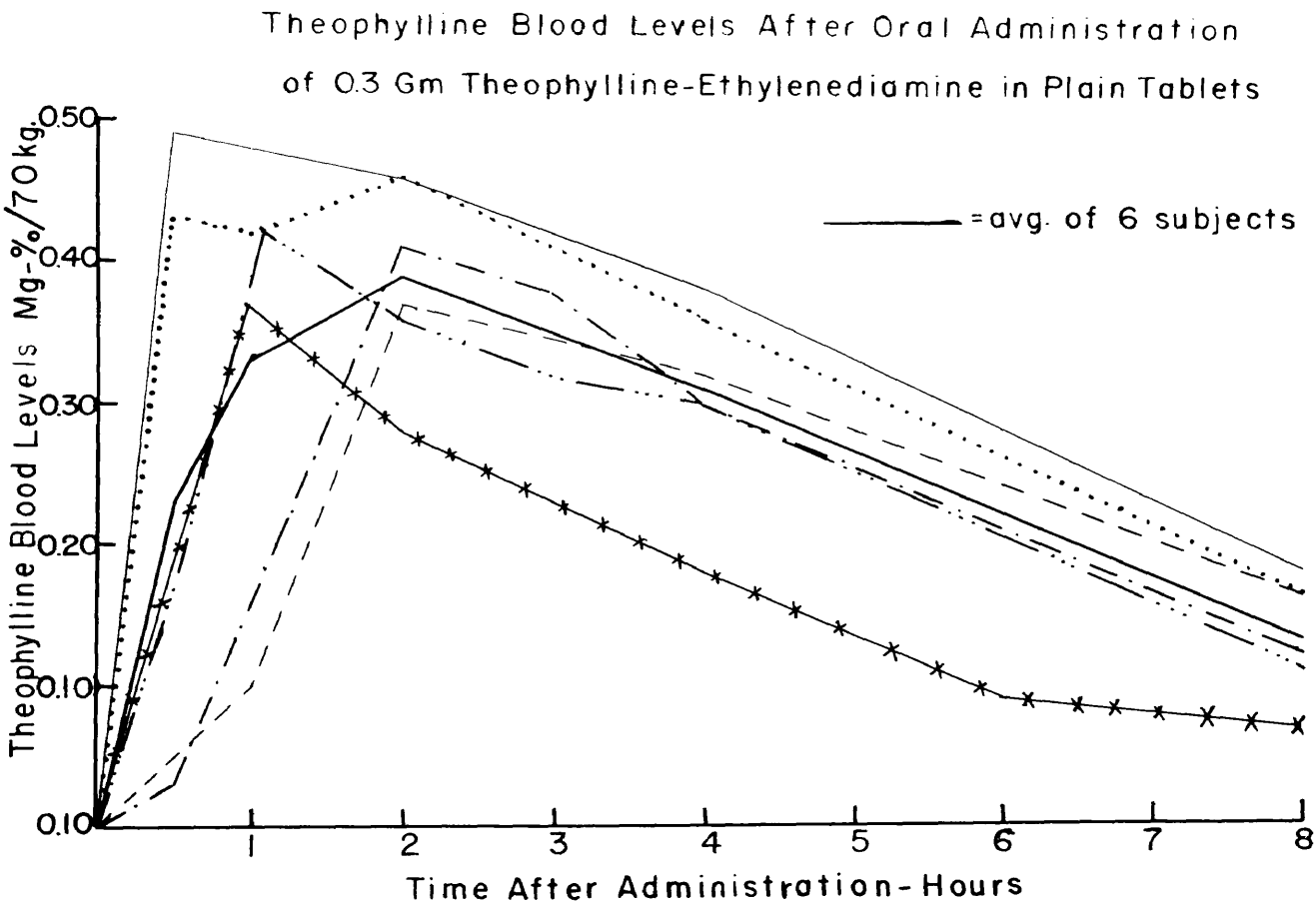
Fig. 7



An additional series of patients was studied with a dose of 0.5 Gm. for comparison with other routes. No gastric distress occurred in any patients with either dose. These doses were administered approximately one-half hour following a typical hospital breakfast. While this may, to some extent, affect the rate of absorption, it is to be remembered that most clinicians advise taking the drug with or after a meal to avoid gastric

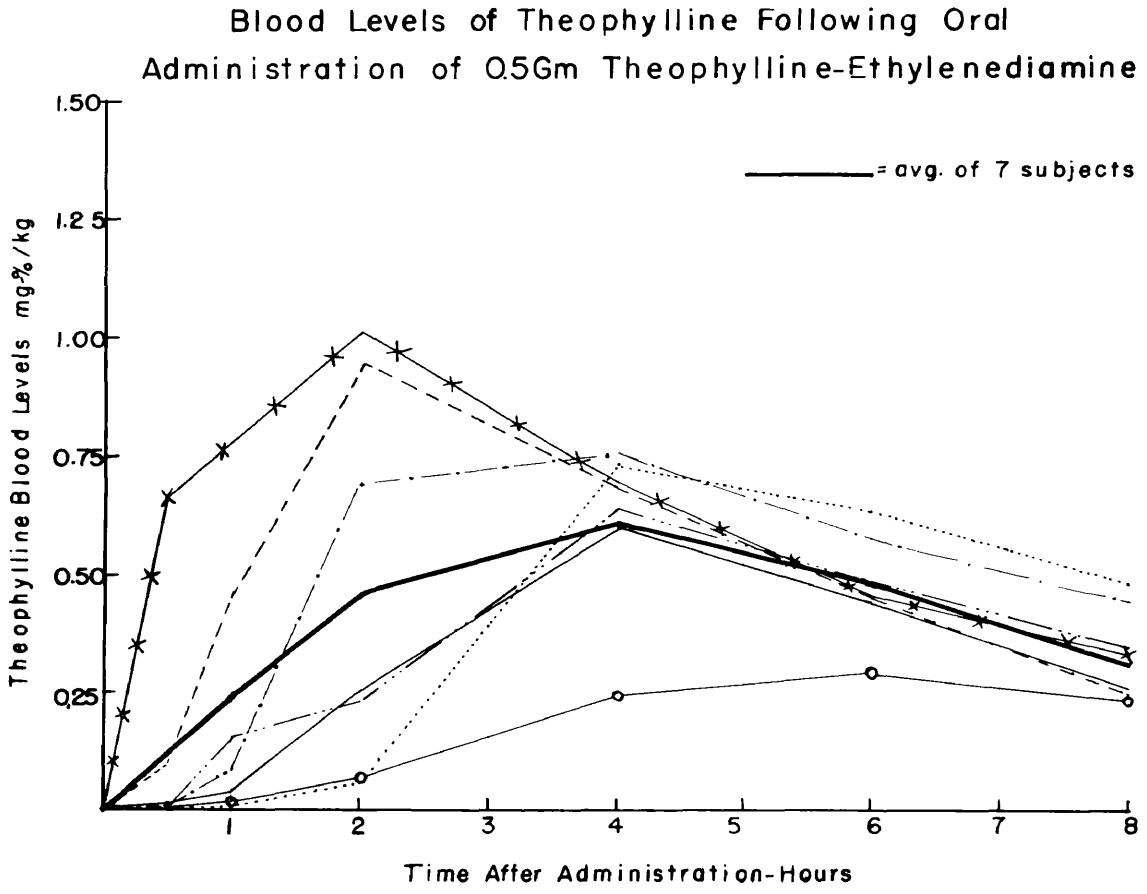
distress. Figs. 8 and 9 show the graphic representation of the individual absorption, as well as the average curve. Complete summaries of these data are given in the Appendix in Tables No. V and VI. Maximal

Fig. 8



absorption with the lower dose was reached between 1 and 2 hours. With 0.5 Gm. doses this peak of absorption varied from 1½ to as long as 6 hours, the average peaks being at about 4 hours. These curves show the wide

Fig. 9



variation possible in oral absorption, particularly with large doses.

A further study was made of the blood levels following repeated oral dosage of theophylline. Theophylline and sodium aminoacetate (Theoglycinate) is reported to have a better than average gastric tolerance because of the buffering action of the amino group in the glycine portion of the molecule (Krantz et al. (122), 1947; and Paul and

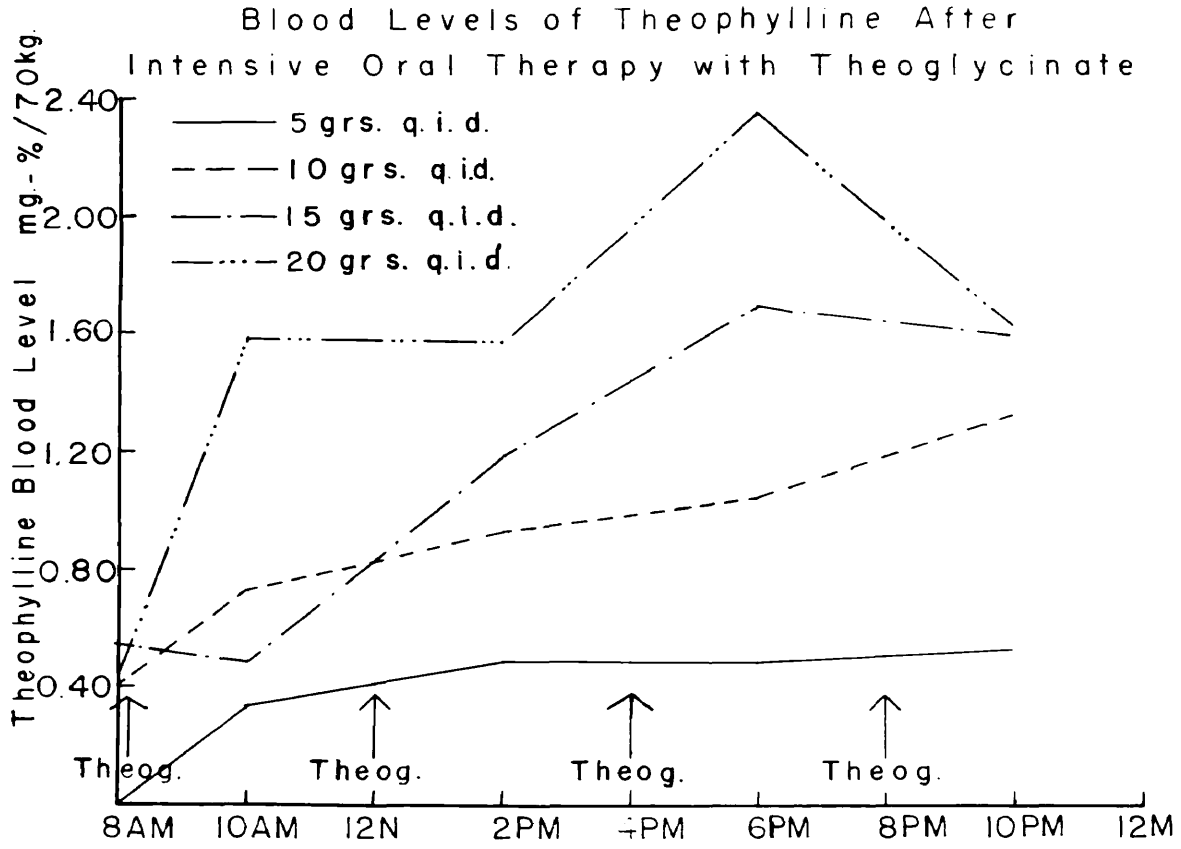
Montgomery (15), 1948). Theophylline-sodium amino-acetate contains 50% of theophylline. A study was made in 4 patients of the blood levels obtainable after dosage with this form of theophylline 4 times a day. Blood samples were drawn 2 hours following each dose, as well as before the first morning dose. In Fig. 10 the lower solid line represents the average blood levels of the four subjects after 5 gr. doses of Theoglycinate at the times indicated. After 3 days the dose was raised to 10 grs. The broken line shows the average results of the four, plus a repeat analysis for three of them two days later at the same dose for a total of 7 analyses. In these three patients the dose was not further raised because of mild gastric distress. They were all elderly cardiac patients. The upper two lines show the extremely high blood levels obtainable by repeated dosage of 15 and 20 grs. per dose in the one remaining subject. However, with both of these doses he incurred nervousness and with the higher dose, severe headache. A summary of the individual blood levels is presented in Table VII in the Appendix.

Comparison of Various Routes of Absorption

In Fig. 11 a comparison of the average results of routes and doses is presented with all blood concentrations adjusted to a 70 kg. basis.

Here the efficacy of the blood levels following retention enemas is shown to be comparable to the intravenous blood levels, providing the need for action is not more urgent than a half hour. The poor comparison of rectal absorption from suppositories is also evident. The slow rate of absorption for the higher dose of Aminophylline gives levels comparable to the intravenous route only after 4 hours. However, individual variation was great.

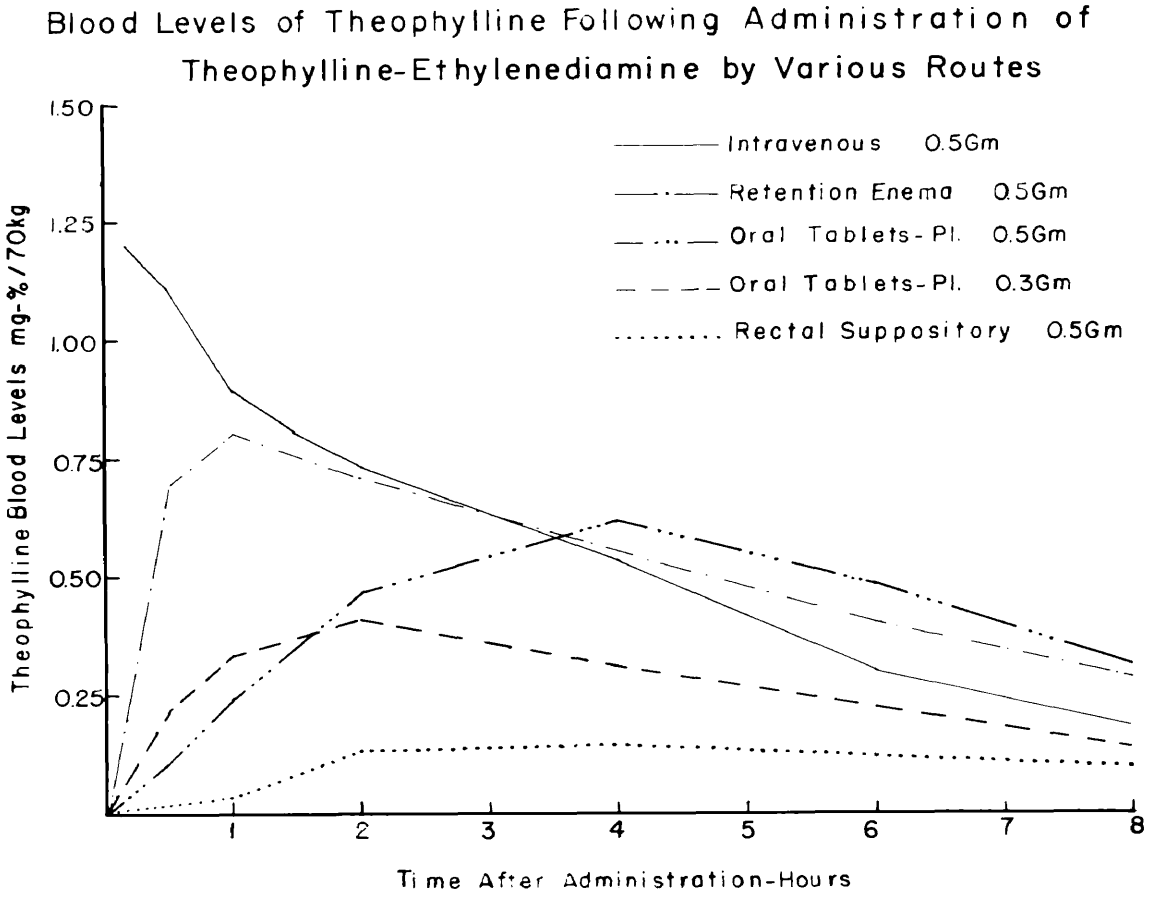
Fig. 10



Discussion:

It appears from the foregoing data that, although intravenous administration gives immediately elevated blood levels, the level rapidly falls during the first hour. Schack and Waxler (269), 1949, have shown that theophylline is confined to the extracellular body fluids. Diffusion of the drug from the blood stream into the body tissues may account for

Fig. 11



this initial rapid fall in blood level. The increases in renal function which have been shown to occur (see p. 42) could also cause increased excretion of the unmetabolized drug. Plummer (268), 1948, showed increased excretion of theophylline during the first 30 minutes in dogs. Thus, unless the action of theophylline is urgently required, retention enemas of 0.6 Gm. or more will provide adequate blood levels within one hour.

The delayed peak of absorption and the variability between patients with the oral route does not indicate this method to be a reliable route of administration. This delay could be due to the preceding breakfast. However, the drug is very difficult to take on an empty stomach. Because of the slow rate of absorption from suppositories, this method is not efficacious in producing adequate blood levels.

CHAPTER IV

BLOOD LEVELS OF THEOPHYLLINE AS RELATED TO PHARMACOLOGIC ACTION

In order to correlate theophylline blood levels with the pharmacologic action of the drug, the diuretic effect was selected as being easily measured and therapeutically substantiated. Since the major portion of the analyses in Chapter II was made in man, this response to theophylline is also desirable because the effect on the kindey can be conveniently measured in man. The diuretic action of theophylline accomplishes two therapeutic effects. Although it increases the urine volume, this action varies considerably with the degree of hydration of the subject. A more constant response is the increased electrolyte excretion which can be conveniently measured by chloride, sodium, and potassium ion analyses of the urine.

Davis and Shock (37, 206), 1949, studied the effects of an intravenous dose of 0.5 Gm. of theophylline-ethylenediamine on renal function in an extensive series of control subjects. The most outstanding effect of the drug was the approximately threefold increase in sodium clearance, which lasted for 55 to 60 minutes after the intravenous injection. The concentration of sodium did not rise until the urine volume decreased. The effect on urine volume was a similar threefold increase, but the values returned to normal more quickly.

In order to assay the diuretic action of theophylline, it was decided to measure the changes in urine volume and electrolyte excretion. However, the effect of the drug on the latter was taken as the better criterion.

Method

The subjects used were young male patients from the United States Marine Hospital. All subjects had no history or evidence of cardiac or renal disfunction. They were well hydrated before and during the test to assure a measurable flow or urine. Urine samples were removed by catheterization at the end of 30-minute periods. Control samples were removed at the end of two 30-minute periods. Theophylline-ethylenediamine, 0.5 Gm., was injected intravenously at the start of period 3. Urine collections were taken at 30-minute intervals for 2 to 2½ hours. Samples were analyzed for chloride content by the Van Slyke and Hiller modification of the Sendroy method (Van Slyke and Hiller (285), 1947). Sodium and potassium determinations were made, using the flame photometer. Theophylline blood levels were determined by the modified method in Chapter III, Section I.

Five subjects were studied with the intravenous dose. In addition, one subject each was done with 0.3 Gm. and 0.5 Gm. oral doses, and a 0.5 Gm. rectal suppository.

Results

The results obtained are tabulated in Table No. 2.

Discussion

Because of the variability of the action of theophylline with the degree of hydration of the subject, no comparison of these doses and routes of administration can be made from the minute volume of urine flow. When the patient is dehydrated, even large doses of theophylline are ineffective in producing increases in urine. However, repeated toxic doses of the xanthines eventually cause death because of continued decreased

Table No. 2

The Effect of Theophylline-Ethylenediamine (TED) on Water and Electrolyte Excretion with Intravenous, Oral, and Rectal Administration

Averages of 5 Subjects	Period No.							
30-Min. Period	1	2		3	4	5	6	7
Urine Flow-cc/min.	1.93	5.87	T.E.D. 0.5 Gm. I.V.	12.90	7.98	12.50	10.20	
Chloride Content--mEq.	3.41	5.94		11.14	11.62	12.35	8.48	
Sodium Content--mEq.	8.26	4.71		17.53	19.84	16.34	12.11	
Potassium Content--mEq.	3.42	2.92		4.47	5.31	5.56	3.58	
Theophylline Level--Mg.-%	----	----		1.17	0.98	0.87	0.81	
1 - Subject			T.E.D. 0.5 Gm. Oral					
Urine Flow--cc./min.	1.08	1.66		6.68	18.14	9.0	1.6	
Chloride Content--mEq.	2.22	1.74		4.03	12.9	9.18	1.44	
Sodium Content--mEq.	3.93	3.38		3.41	14.2	9.45	4.52	
Potassium Content--mEq.	0.50	0.55		3.45	8.2	5.24	0.29	
Theophylline Level--Mg.-%				0.49	0.66	0.82	0.75	
1 - Subject			T.E.D. 0.5 Gm. Oral					
Urine Flow--cc./min.	2.0	5.7		6.77	15.8	15.4	13.6	15.5
Chloride Content--mEq.	4.95	8.10		12.7	----	9.83	6.29	6.95
Sodium Content--mEq.	9.2	9.4		16.3	----	12.0	7.73	7.88
Potassium Content--mEq.	0.32	3.19		3.62	----	5.72	1.29	1.45
Theophylline Level--Mg.-%				0.49	0.48	0.51	0.52	0.53
1 - Subject			T.E.D. 0.5 Gm. Suppository					
Urine Flow--cc./min.	1.86	7.86		8.06	14.0	11.5	10.9	13.2
Chloride Content--mEq.	1.69	11.05		11.6	18.3	14.4	13.3	17.5
Sodium Content--mEq.	2.80	9.91		9.44	16.8	11.7	13.1	16.6
Potassium Content--mEq.	1.14	4.06		3.82	6.04	3.83	3.53	3.94
Theophylline Level--Mg.-%				0	0.05	0.10	0.28	0.30

reabsorption of electrolytes, potassium in particular (Grunwald (207), 1908, and Kerpel-Fronius and Butler (208), 1935).

The most significant change following the intravenous dose of 0.5 Gm. theophylline-ethylenediamine was an approximately twofold increase in chloride and potassium excretion. This effect took place with blood levels varying from 1.17 mg.-% down to 0.81 mg.-%. Even at the end of two hours substantial increases in sodium and chloride excretion were evident. A comparison with the blood level-effect relationships following oral and rectal administration gives an estimation of the theophylline blood levels necessary to produce moderate and slight diuretic effects. Following oral dosage of 0.5 Gm. very little effect was noted in the first period after the drug with a blood level of 0.49 mg.-%. A moderate effect was caused by theophylline blood levels of 0.66 and 0.82 mg.-% in the next two periods. However, no explanation can be made for the lack of effect with blood levels of 0.75 mg.-% in the last period. With an oral dose of 0.3 Gm. scattered, but inconsistent, increases in excretion were noted in the electrolyte content, although urine volume was increased throughout $2\frac{1}{2}$ hours following the drug. The blood levels produced were approximately three-fifths of those produced by the higher dose. With the rectal route some increases were noted in various periods following the drug. However, considering the control fluctuations of this subject, it is doubtful that these are very significant changes.

Because of the great variability between subjects during the control periods, even in the same individual, no attempt has been made to treat each set of results statistically. From the variations observed in eight subjects in the two control periods, the following coefficients of variation were calculated from the standard deviations and the means:

chloride content, 88%; sodium content, 84%; and potassium content, 81%.

Admittedly, more rigid control of water intake, environmental temperature, salt intake, food intake, as well as calculation of the results on the basis of body area and control of other variables would produce more consistent results. However, these data are more of an exploratory nature to point out one method of correlating theophylline activity with blood concentrations. An exact determination of the minimum effective blood level for diuretic activity will have to wait for more extensive quantitative studies.

In searching the literature for other quantitative data on the effects of theophylline in human subjects, a study of the protective action against experimentally induced bronchospasm (Segal et al. (286), 1949) was found. Those authors measured the effectiveness of theophylline-ethylenediamine in protecting against reductions in vital capacity induced by histamine and mecholyl. The similarity in shape of their curves with intravenous, rectal, and oral doses to those for blood levels of theophylline in Chapter III was striking. Segal and co-workers measured the effect of intravenous doses of 0.5 Gm., retention enemas of 0.5 Gm., and oral doses of 0.4 Gm. In evaluating their data they only considered protection of more than 40% against control reductions in vital capacity as significant. The activity of theophylline against mecholyl was, in most cases, less than that against histamine. If it can be assumed that the blood levels produced in their patients are roughly similar to those found in Chapter II, the following table gives a general idea of the blood levels associated with the broncho-spasmolytic action of theophylline against histamine:

Table 3

Estimated Blood Levels Corresponding to Significant Protection (40% or more) Against Histamine-induced Bronchospasm in Man

Dose and Route	Latent Period of Development of 40% Protection*	Duration of Significant Protection*	Estimated Blood Levels
0.5 Gm. -- I. V.	0	130 min.	1.20 to 0.70 mg.-%
0.5 Gm. -- Retention Enema	45 min.	150 min.	0.70 to 0.65 mg.-% (0.80 mg.-% max.)
0.4 Gm. -- Oral	105 min.	50 min.	0.50 to 0.50 mg.-% (0.60 mg.-% max.)

* From data of Segal et al. (286), 1949.

Although this is an estimated correlation between blood levels and antispasmodic activity, it seems that blood levels of the order of 0.50 mg.-% or more are required for effective action. This study by Segal and associates also points to the efficacy of rectal absorption from retention enemas. Inhalation absorption from aerosol produced very poor protection. Intramuscular absorption was also poor.

No other data were found in the literature which showed the duration and effectiveness of theophylline compounds in man that permitted correlation with the blood levels obtained. A review of the quantitative data from experimental preparations revealed very few actions of theophylline that employed any concentration close to the therapeutic levels reported herein. Most studies in excised and perfused organs found effective concentrations to be on the order of 1: 50,000 (2 mg.-%) or more. One exception was the action of theophylline in potentiating the action of

potassium on the contraction of striated muscle. Torda and Wolff (252, 1945, reported concentrations of 0.02 mg. per 100 cc. to increase contractions of the rectus abdominis muscle of the frog. - Thus it seems that until increases are made in the sensitivity of experimental methods to estimate the effectiveness of xanthine action, no estimation can be made of the therapeutic concentrations required in man from these preparations.

CHAPTER V

INVESTIGATIONS ON THE CIRCULATORY ACTION OF THEOPHYLLINE

Direct Measurement of Coronary Inflow

A number of trials were made to find a quantitative method to establish the relationship between a coronary vasodilating action of theophylline and its blood level. It was first attempted to measure this action by recording the changes in coronary inflow to a cannulated branch of the left coronary. The changes in flow were recorded by means of the bubble flow meter method of Eckenhoff et al. (287, 288), 1947. Blood was supplied from the carotid artery and the animal was heparinized to prevent coagulation.

The difficulties involved in this method did not recommend it as a suitable procedure for correlating the coronary action of theophylline with blood levels. The reasons for this conclusion are first, the nature of the method itself and second, the technical difficulties involved. Furthermore it was difficult at the time to obtain large, healthy dogs (13 to 16 kg.). The method is beset with many difficulties, such as: maintenance of a constant level in oxygenation by artificial respiration to maintain a constant oxygen supply to the heart, tendency of the blood pressure to gradually fall making it difficult to get comparable readings in the same animal, and the gradual blood platelet deposition in the instrument.

Although these difficulties could have been overcome in time, it became apparent through trial that the method had other shortcomings. Some of these are: (1) the trauma involved in the exposure and ligation

of the coronary arteries which Bayley (130), 1943, has shown to be sufficient to depress or invert the T-wave in the portion of the myocardium supplied by the vessel; (2) the inaccuracies involved in determining the amount of myocardium supplied by the vessel; (3) the long delay to the blood and the pressure loss involved in the instrument; and (4) the inability of the method to determine whether the increase in flow is primarily an action on the coronary vessels or secondary to myocardial stimulation. For these reasons this study was not extended further.

Indirect Measurement of Coronary Action

If theophylline does have a beneficial action on the coronary vessels, it may only be manifested in cases in which the coronaries are spasmodically constricted. Thus, it may have only an antispasmodic action on actively constricted coronary smooth musculature similar to its action against bronchospasm in asthma and biliary colic. Of the several known coronary vasoconstrictors, posterior pituitary was selected, since Hanzlik and Moy (134), 1945, have reported that Aminophylline antagonized the adverse effects of posterior pituitary on the blood pressure and heart rate. These adverse effects are believed to be due directly to the coronary constriction produced.

To avoid, if possible, the trauma involved in coronary operations in order to measure the flow changes, it was decided to interpret the adverse or favorable effects of the drugs by changes in the electrocardiograms taken from a lead in a precordial position. The effects of cardiac hypoxia in depressing or inverting the pattern of the T-wave as well as displacements of the RS-T segment are well known. The method used was similar to that used by Melville (289), 1938, for ephedrine and posterior pituitary except for the use of the precordial lead. It was found that

upon repeated administration the circulatory response to posterior pituitary diminished rapidly. On the other hand, if the animal was allowed to rest for several days, the response to the same dose was sometimes increased. In addition to this, the day-to-day variations in the electrocardiogram of dogs made it impossible to obtain a quantitative basis for comparison of various doses of theophylline. Furthermore, in some anesthetized, atropinized animals intravenous doses of 10 mg./kg. of Theoglycinate seemed to have an adverse effect on the heart, as interpreted by the electrocardiographic record. This method was also abandoned, as was that using the bubble flow meter, for lack of specificity and quantitative reproducibility.

Isolated Mammalian Heart Experiments

Although the limitations of the use of the isolated saline perfused mammalian heart discussed in Chapter II are many, the method, if carefully controlled, gives a comparison of the relative action of the drug on the myocardium and the coronary vessels at the same time. Furthermore, it was decided to make a closer study of the time relationships between the increases in coronary flow and the action on the myocardium, caused by theophylline, to see if the former preceded, coincided with, or occurred later than the increased vigor of contraction.

Method

The isolated rabbit heart was used for perfusion according to a very modified Langendorff preparation (Langendorff (98), 1895). The apparatus used was that prepared by Anderson and Craver (290), 1948, following the requisites of the method as reemphasized by Chenoweth and Koelle (291), 1946. The apparatus was slightly modified with a flowmeter

described by Anderson and Cameron (292), 1950. In addition to this, electrograms of the isolated preparation were taken by means of fine platinum hooks placed at the apex and in the connective tissue around the auricle. These were connected to fine platinum wires to avoid interference with movements of the heart. Recordings were made, using the Sanborn Electrocardiette.

Results

It was early noted with the above apparatus, before the addition of the electrocardiogram, that the increases in coronary flow occurred after the increases in amplitude of contraction, as recorded on the drum simultaneously by the flowmeter and the lever arm attached to the ventricle. With higher doses of theophylline compounds, there appeared an interval immediately after the onset of the vigorous contractions during which the coronary flow decreased or ceased entirely if the contraction was vigorous enough. Then followed the usually-observed increase in flow. The increased flow was also observed to frequently outlast the effect on contraction.

Several attempts were made to prevent the coronary-constricting action of posterior pituitary injections by the simultaneous injection of theophylline compounds. As in the case of the whole animal, repeated injections of posterior pituitary had less effect and no quantitative relation could be developed. However, qualitatively theophylline appeared to prevent the decreases in rate of coronary perfusion caused by doses of posterior pituitary alone.

Discussion

Although this method does vary from the conditions in situ

in many respects, it does provide an experimental preparation in which the drug is delivered to the heart via the coronary arteries. By means of changes in the amplitude of contraction and in the T-wave of the electrocardiogram, the onset of the myocardial stimulation of theophylline could be determined. The increase in coronary flow was found to follow these changes with respect to time. This lends credence to the theory that the increase in coronary perfusion rate is secondary to the myocardial stimulation and is the result of an accumulation of metabolites. The evidence obtained that theophylline antagonized coronary constriction was only qualitative.

Action of Theophylline on Smooth Muscle

A study was made to determine whether theophylline was capable of depressing the oxygen consumption of smooth muscle obtained from arterial tissue. It was desired to know if the mechanism of action of theophylline in producing vasodilatation might be indicated by a decrease in the oxygen utilization of the smooth muscle in the walls of the vessels. Although such a method might not indicate the exact site of action, it would give a hint as to the possible mechanisms involved.

Method

The oxygen consumption of rat arterial tissue was measured after the method of Briggs, Chernick and Chaikoff (293), 1949, using the direct method of Warburg (294), 1945.

Results

Because of the inconstancy of the method (mean Q_{O_2} - 1.12 $\pm$ - 0.18, C. V. - 16%), no significant depression of oxygen consumption was observed. Theophylline was added during the second hour in concentrations

varying from 0.2 mg.-% up to 20.0 mg.-%. The variability of this method does not recommend it for the study of the action of drugs on arterial tissue.

Discussion

Since this and most of the previous experiments in this chapter have supplied more or less negative answers, one wonders whether theophylline is an active vasodilator affecting the vascular mechanism directly. It leads one to believe that perhaps the effects observed clinically (peripheral vasodilatation, etc.) are secondary to the effects on the myocardium or due to other causes.

CHAPTER VI

Discussion and Conclusions

A complete and exact correlation of the pharmacologic actions of theophylline with blood levels has not been made, as might be indicated from the title of this thesis. However, it is believed that this study will indicate some of the problems involved in such an undertaking. Many of these are problems in basic physiology and pharmacology which must be solved before the complete quantitation of the action of theophylline and related compounds on the body can be made. It is felt that a contribution has been made herein toward that end.

Because a discussion section was included following the experimental sections, no lengthy discussion will be made here. The methods for the measurement of theophylline blood concentrations described in Chapter III should provide a reference standard for the comparison of activities of the drug in experimental animals and in clinic patients. The modified and simplified version of the method approaches some clinically used methods in applicability. The use of a colorimetric reaction, which avoids the use of an ultraviolet spectrophotometer, is an advantage. The method could be further increased in accuracy by further steps to eliminate more of the extractables from blood, but it was felt that the method was suitable for application to blood because of high comparative sensitivity to therapeutic levels of theophylline.

Conclusions

1. The problems associated with the therapeutic uses of theophylline have been reviewed and discussed.

2. The experimental investigations leading to the theories of the mechanisms of action of the methyl-xanthines have been reviewed and discussed.
3. A method for the quantitative estimation of theophylline has been developed. Its application to the measurement of blood levels has been described. A modification and simplification of this method has been made to decrease the time involved and to increase its accuracy.
4. A comparison of the blood levels obtained after intravenous, oral, and rectal absorption in man and intravenous administration in dogs have been measured.
5. A comparison of the effective blood levels of theophylline producing diuresis in man has been reported. Also a comparison has been made with the blood level expected and the activity of the drug on experimentally induced bronchospasm and other actions reported in the literature.
6. Several investigations have been made to determine the nature of the action of the drug on the circulation.

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APPENDIX

I. Assay of Specificity of Method for Theophylline in Blood

Brodie et al. (284), 1947, have set forth the principles involved in ascertaining the specificity of a reaction or procedure when applied to biological fluids. Briefly, the assay consists of the application of the colorimetric reaction or other procedure developed to the measure of the distribution ratio between an organic solvent and buffers of various pH of the drug and of the substance obtained from the animal after administration of the drug. If the distribution ratio of the true drug is the same as that for the apparent drug extracted from biological fluid by the extraction procedure used in the method, then the determination may be considered specific. If the reaction is also measuring a metabolite of the drug, it is very doubtful that the metabolite would have the same distribution ratio as the original drug. In general, most metabolites produced in the body tend to be more water-soluble than the parent drug.

An assay of specificity of the method described in Chapter III, Section I, was carried out to determine whether a part of the theophylline blood levels found could be attributed to a metabolite, possibly 1 methyl xanthine or 1,3 dimethyluric acid.

Method

The distribution ratio was measured between equal quantities of chloroform containing 5% isopropanol and buffers of pH 4.0, 8.0, 9.0, and 10.0 prepared from Coleman buffer tablets. Samples of appropriate size were evaporated to dryness and analyzed by the modified diazo

coupling method described on page 69. The per cent of theophylline or apparent theophylline was calculated for each phase.

Large amounts of plasma, taken 1½ hours after the administration of a very large dose of Aminophylline, were extracted with the chloroform-isopropanol extraction mixture after addition of 1.0 N HCl to pH 4.5. The extraction solvent was evaporated and the residue dissolved in a small quantity of 0.1 N NaOH. This was concentrated to about 5 ml., and 1.0 ml. was added to each separatory funnel. Anhydrous theophylline, 1.0 mg. in 1.0 cc. H_2O, was added to separatory funnels containing the same combinations of buffers and organic solvent.

The mixtures were shaken at intervals for 30 minutes, and samples of appropriate size were taken for analysis. The results are shown in Table 4.

Results

Table 4

Distribution of Theophylline and Apparent Theophylline Between Chloroform-Isopropanol (20:1) and Various Buffers

pH	Per Cent Theophylline in Organic Phase	
	True Theophylline	Apparent Theophylline
4.0	40	39
8.0	35	35
9.0	12	10
10.0	1	2

Discussion

From the results in Table 4 it is apparent that the agreement

is good and that after 1½ hours not more than 1% of a possible metabolite could be included in the apparent theophylline measured by the method.

Table No. I

Blood Concentrations of Theophylline Following
Intravenous Injection of 0.5 Gm. Theophylline-Ethylenediamine

Patient No.	Body Wgt.	Time After Injection								
		10 min.	15 min.	30 min.	1 hr.	1½ hrs.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
	Kg.	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%
1	54.6	2.15			0.86		0.70	0.55		
2	49.5	1.58		1.35	1.23		1.05			
3	84.0	0.68			0.56		0.38	0.19		
4	75.0	1.09		1.10	0.99		0.95	0.70		
5	70.6	1.36		1.09	1.01		0.91	0.58		
6	68.0	1.06		1.06	0.88		0.67	0.47		
7	71.4		0.98	1.18	1.00	0.88	0.83	0.58	0.38	0.24
8	68.3		1.06	1.13	0.85	0.75	0.65	0.57	0.13	0
9	56.8			1.43	1.21	1.05	0.93	0.71	0.42	0.28
10	72.4			0.93	0.88	0.78	0.72	0.58	0.38	0.20
11	64.1			1.18	0.98	0.83	0.75	0.49	0.29	0.22

Table No. II

Blood Concentrations of Theophylline Following Intravenous Injection of 0.5 Gm. Theophylline-Ethylenediamine Adjusted to 70 Kg. Body Weight by Proportion

Patient No.	Body Wgt.	Time After Injection								
		10 min.	15 min.	30 min.	1 hr.	1½ hrs.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
	Kg.	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%	Mg.-%
1	54.5	1.67			0.67		0.55	0.43		
2	49.5	1.12		0.94	0.87		0.73			
3	84.0	0.82			0.67		0.46	0.23		
4	75.0	1.17		1.18	1.06		1.02	0.75		
5	70.6	1.37		1.09	1.01		0.91	0.58		
6	68.0	1.04		1.04	0.88		0.66	0.47		
7	71.4		1.00	1.20	1.02	0.90	0.85	0.59	0.39	0.25
8	68.3		1.03	1.10	0.85	0.73	0.63	0.57	0.13	0
9	56.8			1.16	0.98	0.85	0.75	0.58	0.34	0.23
10	72.4			0.96	0.91	0.80	0.74	0.60	0.39	0.21
11	64.1			1.08	0.90	0.76	0.69	0.45	0.27	0.20
	Avg.:	1.20		1.12	0.89	0.81	0.73	0.53	0.30	0.18

Table No. III

Theophylline Blood Levels Following Intravenous Enema of 0.5 Gm. Theophylline-Ethylenediamine in 40 cc. Saline

Upper fig. -- Mg. of Theophylline/100 cc. of Blood (Mg.-%)
Lower fig. -- Mg.-%/70 Kg. (Mg.-% adjusted to 70 Kg. basis by inverse proportion of body weight)

Patient No.	Body Wgt. Kg.	Time After Administration					
		30 min.	1 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
		(+ 5 min.)					
1	72.7	0.42	0.53	0.60	0.48	0.35	0.23
		0.44	0.55	0.62	0.50	0.36	0.24
2	57.5	0.56	0.68	0.57	0.47	0.27	0.14
		0.46	0.56	0.47	0.39	0.22	0.11
3	57.0	1.02	1.08	0.91	0.71	0.58	0.45
		0.83	0.88	0.74	0.58	0.47	0.37
4	79.5	0.56	0.75	0.58	0.36	0.30	0.25
		0.64	0.86	0.66	0.41	0.34	0.28
5	67.7	0.76	0.88	0.80	0.64	0.53	0.24
		0.74	0.85	0.77	0.62	0.51	0.23
6	74.4	1.00	1.03	0.96	0.74	0.46	0.42
		1.06	1.09	1.01	0.78	0.49	0.45
Avg. Mg.-%/70 Kg.		0.69	0.80	0.71	0.55	0.40	0.28

Table No. IV

Theophylline Blood Levels Following Rectal Insertion of Suppository containing 0.5 Gm. Theophylline-Ethylenediamine

Upper Fig. -- Mg.-% Theophylline
Lower Fig. -- Mg.-%/70 Kg. (See Table No. III)

Patient No.	Body Wgt. Kg.	Time After Administration					
		30 min.	1 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
		----	0.06	0.12	0.11	----	0.06
1	72.7	----	0.06	0.12	0.11	----	0.06
		0	----	0.28	0.34	0.38	0.33
2	66.0	0	----	0.26	0.32	0.36	0.31
		0.04	0.03	0.14	0.16	----	0.12
3	49.5	0.03	0.02	0.10	0.11	----	0.08
		0	0	0.09	0.05	----	0.05
4	83.3	0	0	0.11	0.06	----	0.06
		----	0.01	0.14	0.09	0.05	0.04
5	79.5	----	0.01	0.16	0.10	0.06	0.05
		----	0.03	0.09	0.21	0.14	0.10
6	50.0	----	0.02	0.06	0.15	0.10	0.07
Avg. Mg.-%/70 Kg.		----	0.03	0.13	0.14	----	0.10

Table No. V

Blood Levels of Theophylline After Oral Administration of 0.5 Gm. Theophylline-Ethylenediamine in Uncoated Tablets

Mg.-% -- upper figure; mg.-%/70 Kg.--lower figure

Patient No.	Body Wgt. Kg.	Time After Administration					
		30 min.	1 hr.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
1	66.0	0.02 0.02	0.04 0.04	0.27 0.25	0.64 0.60	0.48 0.45	0.28 0.26
2	57.4	0.12 0.10	0.56 0.46	1.16 0.95	0.84 0.69	0.56 0.46	0.30 0.25
3	68.3	0 0	0.01 0.01	0.06 0.06	0.75 0.73	0.66 0.64	0.50 0.49
4	45.5	0.02 0.01	0.13 0.08	1.06 0.69	1.17 0.76	0.89 0.58	0.70 0.45
5	95.5	0.49 0.67	0.66 0.90	0.75 1.02	0.51 0.70	0.34 0.46	0.26 0.38
6	71.0	0 0	0.15 0.15	0.23 0.23	0.63 0.64	0.48 0.49	0.29 0.29
7	75.4	0 0	0.02 0.02	0.06 0.06	0.22 0.24	0.27 0.29	0.22 0.24
Avg.	Mg.-%/70 Kg.	0.11	0.24	0.47	0.62	0.48	0.32

Table No. VI

Blood Levels of Theophylline After Oral Administration of 0.3 Gm. Theophylline-Ethylenediamine in Uncoated Tablets

Upper figure -- Mg.-%; lower figure -- Mg.-%/70 Kg.

Patient No.	Body Wgt. Kg.	Time After Administration: 30 min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	8 hrs.
1	73.6	0.47	0.46	0.44		0.36		0.17
		0.49	0.48	0.46		0.38		0.18
2	54.6	0.06	0.13	0.47	----	0.42		0.20
		0.05	0.10	0.37	----	0.32		0.16
3	61.4	0.49	0.48	0.52		0.41	0.27	0.18
		0.43	0.42	0.46		0.36	0.24	0.16
4	60.0	0.03	(+ 10 min.)	0.48	0.44	0.35	----	0.14
		0.03	0.23	0.41	0.38	0.30	----	0.12
			0.20					
5	79.5	0.15	(+ 5 min.	0.32	0.28	0.26		0.10
		0.17	0.35	0.36	0.32	0.30		0.11
			0.40					
6	66.0	----	0.39	0.30	----	0.19	0.10	0.07
		----	0.37	0.28	----	0.18	0.09	0.07
Avg. Mg.-%/70 Kg.		0.23	0.33	0.39		0.31		0.13
*	73.0	0.14	0.30	0.40		0.43	----	0.30
		0.15	0.31	0.42		0.44	----	0.31

* Patient with carbon tetrachloride nephrosis.

Table No. VII

Blood Levels of Theophylline After Repeated Oral Therapy With Theophylline-Sodium Amino Acetate at 8 a.m., 12 M., 4 p.m., and 8 p.m.

Upper figure -- Mg.-% Lower figure -- Mg.-%/70 Kg.

Patient No.	Body Wgt. Kg.	Dose Given at Above Time	Time of Analysis				
			8 a.m.	10 a.m.	2 p.m.	6 p.m.	10 p.m.
1	52.3	5 grs.	Control	0.45	0.58	0.57	0.78
			Blank	0.34	0.43	0.43	0.58
		10 grs.	0.56	0.63	1.05	1.32	1.47
			0.42	0.47	0.78	0.99	1.10
		10 grs.	0.44	1.00	1.24	1.32	1.53
			0.33	0.75	0.93	0.99	1.14
2	83.8	5 grs.	Control	0.45	0.41	0.46	0.37
			Blank	0.54	0.49	0.55	0.44
		10 grs.	0.12	0.60	0.28	0.68	0.95
			0.14	0.72	0.34	0.81	1.14
		10 grs.	0.16	0.80	1.06	1.05	1.32
			0.19	0.96	1.27	1.26	1.59
3	69.1	5 grs.	Control	0.24	0.70	0.51	0.64
			Blank	0.24	0.69	0.50	0.63
		10 grs.	0.93	0.89	1.37	1.57	2.00
			0.92	0.88	1.35	1.55	1.97
		10 grs.	0.85	0.78	1.52	0.69	1.42
			0.85	0.77	1.50	0.68	1.40
4	71.4	5 grs.	Control	0.18	0.32	0.43	0.43
			Blank	0.18	0.33	0.44	0.44
		10 grs.	0.48	0.48	0.28	1.00	0.93
			0.49	0.49	0.29	1.02	0.95
		15 grs.	0.53	0.47	1.15	1.66	1.57
			0.54	0.48	1.17	1.69	1.60
		20 grs.	0.24	1.54	1.53	2.32	1.60
			0.45	1.57	1.56	2.36	1.63
Avg. Mg.-%/70 Kg.		5 grs.	0	0.33	0.48	0.48	0.52
		10 grs.	0.41	0.72	0.92	1.04	1.33