STUDY OF THEOPHYLLINE PLASMA LEVELS AFTER ORAL ADMINISTRATION OF NEW THEOPHYLLINE COMPOUNDS

BY

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The pharmacological action of theophylline is well known; it relaxes the bronchi, both after histamine-induced spasm and in clinical asthma (Goodman and Gilman, 1955); it decreases the venous filling pressure and increases the cardiac output in patients in cardiac failure (Howarth *et al.*, 1948); it causes a diuresis in patients in heart failure partly by its effect on the cardiac output and also by depressing tubular reabsorption (Walker *et al.*, 1937). Theophylline with ethylenediamine (aminophylline) is highly effective in controlling Cheyne–Stokes breathing, but there is evidence that this may be due to the ethylenediamine (Marais and McMichael, 1937). These properties make theophylline a useful drug in the treatment of bronchial asthma and cardiac failure.

Parenteral administration of theophylline compounds is unsatisfactory for long-term treatment; the intravenous route is impracticable and there is some evidence that it may be dangerous (Bresnick *et al.*, 1948); intramuscular injections are often extremely painful. Oral administration of theophylline is useless because it causes severe gastric irritation and its solubility is very low (1:120). Aminophylline has a much greater solubility (1:5), but it is strongly alkaline in solution and is hydrolysed by gastric juice; free theophylline is liberated and gastric irritation occurs. Theophylline derivatives have been prepared which are readily soluble in water and are stable over a wide range of *p*H; they are stated to cause negligible gastric irritation when given in therapeutically effective doses by mouth. It is possible that the absorption of these substances from the gastro-intestinal tract may differ both in regularity and in degree, and that their therapeutic value can be related to the theophylline plasma concentrations.

In the present investigation five theophylline preparations have been compared in a small group of patients, by studying the plasma levels over a period of five hours, after a single oral dose of equivalent theophylline content. The minimal plasma concentration for relief of bronchospasm has been estimated.

Methods

Twenty-five patients were studied; 20 suffered from chronic asthma and were continuously wheezy, and five were in congestive cardiac failure (two rheumatic and three arteriosclerotic heart disease).

TABLE I

	Group 1	Group 2	Group 3	Group 4	Group 5
	Dihydroxypropyl-Theoph.	Choline Theoph.	Theoph. Ethanoate with Piperazine	Sodium Glycinate Theoph.	Aminophylline
Theophylline-content ..	79%	60%	56%	49–52%	79%
Solubility in water ..	1:6	1:1	1:5	1:5	1:5
Dosage given ..	0·6 g.	0·79 g.	0·85 g.	0·95 g.	0·6 g.

The theophylline content of each trial dose was 0·47 g. and equivalent to that contained in 0·6 g. of aminophylline.

Five groups were formed so that each contained five patients of similar age and sex distribution (four asthmatic and one cardiac failure). Each group received one of the five theophylline preparations under investigation in a single dose by mouth (Table I).

Some of the cases were studied as out-patients and therefore the test dose was given 1½ to 2 hours after breakfast (between 9.30 and 10 a.m.). Samples of venous blood were taken after one, two, three, and five hours; these were heparinized and the plasma theophylline was estimated by the method described by Schack and Waxler (1949). Plasma was used rather than whole blood because of the uneven distribution of theophylline between cells and plasma (Schack and Waxler, 1949). The double extraction process described by these authors recovers approximately 98% of the plasma theophylline.

Caffeine and barbiturates were avoided before the test because they are known to interfere with the ultra-violet spectrophotometric estimation of theophylline.

Spirographic records of each asthmatic subject were taken before the test dose and at one hour after it; for technical reasons it was not possible to repeat the test after this time. From the spirograms (drum speed 5 mm./sec.) one second vital capacities were measured. The time of onset and the duration of subjective improvement were also noted, and these were correlated with the timed vital capacities.

Results

The theophylline plasma levels of each patient are shown in Table II, in each group the mean level at one, two, three, and five hours has been calculated and these have been expressed graphically in Fig. 1.

TABLE II.—*Theophylline Blood Levels (mg. per 100 ml. of Plasma) in 25 Subjects*

Patient:	1	2	3	4	5	Mean	Standard Error of Mean
Group 1. Dihydroxypropyl-theophylline							
1 hour	0·765	0·946	0·915	0·927	0·901	0·891	±0·032
2 hours	0·935	0·990	0·805	0·765	—	0·874	±0·053
3 ,,	1·360	0·952	0·768	0·832	0·556	0·892	±0·134
5 ,,	1·185	0·475	1·074	0·697	0·385	0·763	±0·159
Group 2. Choline Theophylline							
1 hour	*1·305*	0·521	*1·263*	*0·807*	0·532	1·086	±0·184
2 hours	*1·185*	1·001	*1·032*	*1·225*	1·055	1·100	±0·044
3 ,,	*1·822*	1·070	*1·838*	*1·240*	1·138	1·422	±0·169
5 ,,	*1·442*	0·725	*0·886*	*1·172*	1·194	1·084	±0·126
Group 3. Theophylline Ethanoate of Piperazine							
1 hour	0·550	0·535	0·652	0·721	0·778	0·647	±0·047
2 hours	*0·808*	0·687	0·730	0·478	0·833	0·707	±0·063
3 ,,	*1·710*	1·190	—	0·482	0·815	1·049	±0·263
5 ,,	*0·935*	1·152	0·353	0·650	0·672	0·752	±0·136
Group 4. Theophylline Sodium Glycinate							
1 hour	1·120	0·00	*0·605*	0·994	0·652	0·674	±0·127
2 hours	1·205	0·558	*0·970*	0·436	0·553	0·744	±0·147
3 ,,	1·014	0·281	*1·490*	0·237	0·630	0·730	±0·236
5 ,,	0·932	0·092	*1·189*	0·013	—	0·557	±0·296
Group 5. Aminophylline							
1 hour	*0·700*	0·985	*0·770*	1·338	1·020	0·963	±0·112
2 hours	*1·598*	1·002	*1·545*	*1·330*	1·300	1·355	±0·106
3 ,,	*1·370*	1·090	*1·462*	*1·586*	1·148	1·331	±0·094
5 ,,	*1·030*	0·491	*1·180*	1·060	0·965	0·955	±0·108

Patient 2 in each group had congestive cardiac failure.
Italic figures indicate subjective relief from asthma.

The mean level at one hour in groups 2 and 5 rose to about 1 mg. per 100 ml. of plasma, and, except in one instance, remained over this level for the whole trial period. In contrast, in groups 1, 3, and 4 the mean levels (with one exception) did not rise above 1 mg. per 100 ml. of plasma, and were usually considerably lower.

Each compound was compared with each of the others (ten pairs) by a variance ratio test. Choline theophylline and aminophylline showed significantly higher plasma levels than the dihydroxypropyl, ethanoate, and glycinate derivatives ($P<0.001$), but no difference was shown between the choline salt and aminophylline on the one hand, or between the dihydroxypropyl, ethanoate, and sodium glycinate compounds on the other.

The theophylline plasma levels of each asthmatic patient have been plotted in Fig. 2; they have been indicated by a dot if bronchospasm was relieved and by a cross if it was not. With few exceptions relief of symptoms was noticed only when the theophylline levels exceeded 1 mg. per 100 ml. of plasma. Six patients improved within the first hour of the test, and in five of these there was slight but definite improvement in the timed and total vital capacities. In two further patients sustained relief was obtained, beginning after the first hour, but spirographic measurements were not made. When bronchospasm was relieved within the first two hours the effect was maintained until the end of the test and correlated with theophylline concentrations of over 1 mg. per 100 ml. of plasma. Subjects obtaining no relief showed no improvement of the timed or total vital capacities.

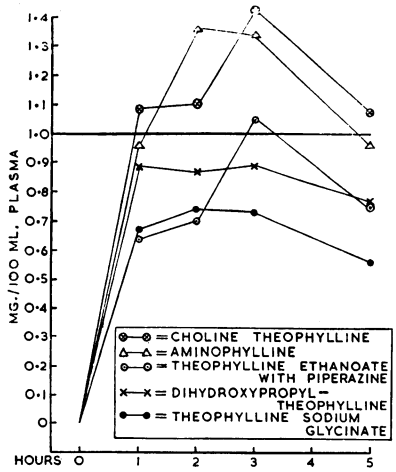

FIG. 1.—Theophylline plasma levels in a trial of five compounds.

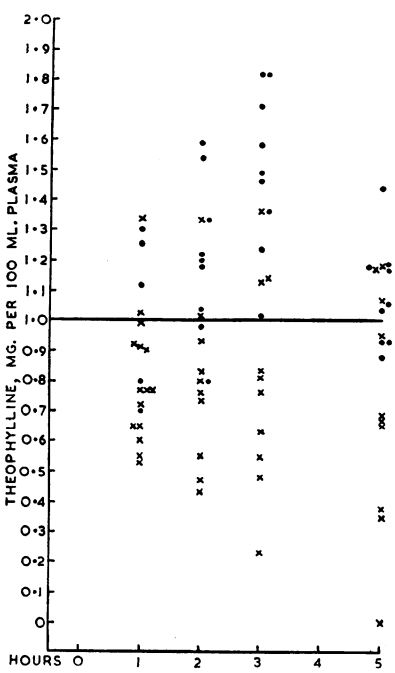

FIG. 2.—Theophylline plasma levels correlated with subjective relief from asthma. ●=Relief from bronchospasm. ×=No relief.

Five patients in congestive cardiac failure (patient 2 in each group) were studied; no gross difference in theophylline concentrations were observed between these subjects and the other members of the same group who suffered from asthma.

No patient experienced gastro-intestinal symptoms following the oral test dose.

Discussion

Comparative studies of theophylline compounds have been concerned with toxicity tests (Maney *et al.*, 1946) and with its pharmacological actions in experimental animals (Quevauviller and Morin, 1953). There are few reports upon plasma levels following equivalent theophylline dosage or upon comparatively clinical effects. Gagliani *et al.* (1954) studied the blood levels of choline theophylline and aminophylline with and without aluminium hydroxide; higher levels were found with the choline derivative. In the present study similar concentrations were found with the two drugs. Gagliani *et al.* (1954) used whole blood for their estimations, but in view of the different solubilities of the compounds it is possible that their cell-plasma partition is also different and the blood and plasma levels may not be comparable.

The results obtained from the present series of patients indicate that a theophylline level of about 1 mg. per 100 ml. of plasma is necessary to relieve bronchospasm. Truitt *et al.* (1950) correlated their studies of theophylline blood levels with a report by Segal *et al.* (1949) on the interval between theophylline administration and the relief of bronchospasm in histamine-induced asthma. They deduced indirectly that the therapeutic blood level was 0.5 mg. per 100 ml. of whole blood. Aminophylline does not appear to enter the cells (Schack and Waxler, 1949), and therefore the finding of Truitt *et al.* would correspond to a plasma level of about 1 mg. per 100 ml. and agree well with the present results.

Of the theophylline preparations studied it appears from a single-dose trial that, apart from aminophylline, choline theophylline is the drug most likely to have clinical effect. A further investigation of patients on maintenance treatment is needed to assess fully the therapeutic value of these products. In the present series only 12 subjects were studied in this way; four out of five with theophylline levels over 1 mg. per 100 ml. of plasma four hours after the morning dose were receiving choline theophylline (200 mg. q.d.s.), whereas eight out of nine patients with plasma concentrations under 1 mg. per 100 ml. were receiving one of the other preparations (dihydroxypropyl, 400 mg. q.d.s.; sodium glycinate, 640 mg. q.d.s.; ethanoate of piperazine, 500 mg. q.d.s.; these are approximately the largest recommended doses). Other workers (Truitt *et al.*, 1950) using theophylline sodium glycinate have found considerably higher blood levels ranging from 0.7 mg. at two hours to 1.2 mg. per 100 ml. of whole blood after 12 hours (dosage 650 mg. q.d.s.). In the present series two patients were treated in the first instance with theophylline ethanoate of piperazine with plasma levels of 0.4 and 0.46 mg. per 100 ml.; they were then changed to choline theophylline and the concentration was increased to 1.28 and 1.74 mg. per 100 ml. of plasma. In view of these findings the occurrence of side-effects is important. Using choline theophylline (200 mg. q.d.s.), Brown and Clancy (1955) reported gastro-intestinal symptoms in 5 out of 35 cases, and Dann *et al.* (1954) in 4 out of 18. The number of patients in the present series is too small for accuracy, but symptoms of nausea, epigastric pain, and "fullness" did occur in a few patients receiving choline theophylline but in none treated with any of the other products, although the total doses received each day were two to three times larger.

The observations reported here on theophylline plasma levels in subjects treated with theophylline compounds of dihydroxypropyl, sodium glycinate, and ethanoate of piperazine, together with those upon the absence of side-effects, suggest that the recommended dosages should be increased.

Theophylline is largely excreted in the urine. When renal flow diminishes in cardiac failure, theophylline excretion might be expected to fall and the plasma level to rise. The opportunity was taken to study five patients in cardiac failure; there was no demonstrable difference in the plasma levels in these subjects compared with asthmatics in the same group.

Summary

The theophylline plasma levels were studied in five groups of patients; each group received a single oral dose of a different theophylline compound of equivalent theophylline content (0.47 g. of theophylline).

Higher theophylline levels were found in subjects receiving choline theophylline and aminophylline (theophylline ethylenediamine) than those receiving dihydroxypropyl theophylline, theophylline ethanoate of piperazine, or theophylline sodium glycinate.

The minimal theophylline plasma level for the relief of bronchospasm appears to be about 1 mg. per 100 ml.

The importance of these findings in the maintenance treatment of patients with theophylline compounds is discussed.

Five patients with cardiac failure showed no appreciable difference in theophylline plasma levels from asthmatics receiving the same compound.

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SALM. THOMPSON GASTRO-ENTERITIS
REPORT OF TWO OUTBREAKS

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Since the end of the war *Salmonella thompson* has been one of the commoner causes of human salmonellosis in England and Wales (Wilson and Miles, 1955; Public Health Laboratory Service Reports, 1954, 1955), but until 1956 had been rarely encountered in Edinburgh. In the University Laboratory which serves the City of Edinburgh and much of the South-East of Scotland, *Salm. thompson* was not isolated from any cases in 1950–1–2, from only one in 1953, from another in 1954, and from two in 1955. In the summer of 1956, however, two apparently unrelated outbreaks of *Salm. thompson* infection came to our notice, and these seem to be of sufficient interest to be put on record.

First Outbreak

The first outbreak occurred in May, 1956, when *Salm. thompson* was isolated from faecal specimens in 10 cases of acute gastro-enteritis within a fortnight. The patients were all children (aged 10 months to 7 years); and, except for two brothers, were from different households widely distributed throughout the city. *Salm. thompson* was also isolated about this time from the faeces of three adults who frequently travelled into Edinburgh from surrounding districts; three more cases were admitted direct to the Edinburgh City Hospital for Infectious Diseases; another was found in a private nursing-home, and two more were found in the wards of a general hospital. (One of these patients had undergone an operation for hernia two days before the onset of diarrhoea.) Food-poisoning was not notifiable in Edinburgh at this time, and other cases may have occurred which did not come to our notice.

The condition varied from comparatively mild diarrhoea and vomiting, which cleared clinically and bacteriologically in a few days, to a more severe type of illness which required hospital treatment (in one case for six weeks), and in two cases with *Salm. thompson* persisting in the faeces for four to eight weeks respectively.

Owing to the unexplosive character of the outbreak and the scattered distribution of the cases, there was inevitable delay in arriving at any facts to indicate a common source of infection, for often the housewife could not recall where or what foodstuffs she had bought at the relevant time. Suspicion, however, focused on a city bakery and restaurant. One patient had eaten "trifle" from this firm 24 hours before the onset of illness; two of the patients lunched daily in this restaurant; another had had tea and cream cakes there two days before symptoms appeared, and in six instances this firm supplied goods to the household. Therefore, some two weeks after the first case was diagnosed it was decided to visit this bakery.

It was found that a good standard of general cleanliness of premises and equipment was being maintained, and all meringues, cream cakes, sponges, and similar products were being made in a separate department. From our own previous observations of samples of imported whole egg and egg albumen, and from reports from other parts of the country (Newell *et al.*, 1955), imported egg was thought to be the most likely vehicle of the infection; but at this time the bakery in question was using home-produced, hen shell-eggs instead of imported liquid egg. Egg albumen, which was being used in large quantities mainly for meringues, was, however, an imported product. Two days before this inquiry Chinese albumen crystals had been in use for meringues, but this stock of albumen had been exhausted and frozen Argentine egg albumen brought into use.

The method of handling this was to defrost a 22-lb. (10-kg.) tin and to pour the contents into a "reservoir" container from which the albumen could be ladled. The favourite container used as a "reservoir" was a round three-gallon (13.6-litre) "synthetic cream" tin, which after removal of the cream was washed in warm water and then used for the egg. It was alleged that a different tin was obtained daily for this purpose or at least every second day. However, at the time of the inspection this seemed doubtful, as deposits of dried albumen were found encrusting both the inside and the outside of the rather dented tin.

Samples for bacteriological examination were taken from (1) stock tins of frozen Argentine egg albumen; (2) tins of synthetic cream; (3) the albumen encrusted on the sides of the "reservoir" tin; and (4) fluid albumen from the ladle. *Salm. thompson* was isolated both from the dried albumen on the sides and from the fluid albumen in the centre of the container, but was not isolated from the stock tins of albumen or synthetic cream.

Immediately after obtaining the samples the reservoir container and its contents were discarded, and the firm undertook to use for this purpose special containers constructed of stouter metal which could be more effectively cleansed with boiling water. Otherwise no change was made: the stocks from which the synthetic cream and egg albumen were drawn were still the same; the shell-eggs (manipulated in another part of the building) were obtained from the same source; and no change was made in personnel. Repeated samples of cream and albumen were examined during the next few days, always with negative results; and no further clinical cases came to our notice after the infected "reservoir" tin was put out of use. No cases of *Salm. thompson* infection did, in fact, come to our notice until some ten weeks later, when this organism was isolated in the second group of cases about to be described and the infection traced to an entirely different source.