at the ankle, and after the first injection there was very little change in the degree of spasticity at the knee. This patient had acquired a pronounced scoliosis as a result of his neurological lesion, which has been developing over a period of 10 years, and it seems that, despite efforts to prevent the downward movement of the phenol solution, roots as low as S1 were affected. Later injections were carried out on this patient at a higher space with greater elevation of the buttocks and successful relief of the extreme spasticity in the leg.

Duration of Benefit

It is not yet possible to be definite about the expected duration of the reduction in spasticity, for the effects vary greatly from patient to patient. It is now over three years, however, since an intrathecal injection was carried out in an elderly patient who had developed a spastic flexion paralysis of the left leg as a result of a cerebrovascular (thrombotic) accident. Phenolization was carried out by one of us (R. M. M.) in April, 1956, using 1 ml. of phenol 5% in glycerin at a level of L 2/3. The pain and flexor spasms ceased immediately, and by the aid of physiotherapy the leg was gradually brought into extension. This patient is still ambulant three years later. Another female patient suffering from multiple sclerosis, which resulted in very frequent painful flexor spasms and made sleep almost impossible for her, received two injections, each of 1 ml. of intrathecal 5% phenol, in November, 1957. For six months pain and spasm were completely abolished, but then the spasms began to recur, though, curiously enough, without any associated pain. Two further injections at L 2/3 were carried out in January, 1959, with success, and when last seen (September, 1959), she was still completely relieved, though of course quite immobile.

At the other end of the time scale we have one patient in whom flexor spasms, seemingly abolished by intrathecal injection, returned within one week of treatment though again the pain element had been removed. We feel that in this particular patient the pathological process (multiple sclerosis) is still active and that this may be responsible for the return of spasticity on each occasion. In the main, however, it seems that appreciable benefit can be expected for an average of at least six to 12 months, though further observation may cause us to modify this opinion. Two patients (each suffering from active multiple sclerosis) have died within three months of the treatment being carried out, though in neither case did it appear that the procedure had accelerated the underlying disorder, and in both cases severe pain and flexor spasms were relieved. We have concluded, however, that it is probably inadvisable to administer this form of treatment to patients in whom the demyelinating process appears to be active.

Summary

The technique of intrathecal injection of the solution of phenol in glycerin is described, and the results of its administration to 31 patients are discussed. The manoeuvre is thought to be of circumscribed value in the relief of painful spasms in cases of spasticity. Careful positioning of the patient is required, and the possibility of resultant retention is emphasized. Care should be exercised in administering the treatment to patients who are still ambulant.

A number of the patients discussed in this paper were under the care of Dr. Fergus R. Ferguson and Dr. G. E.

Smyth, to whom we are indebted for interest and advice. It is with pleasure and gratitude that we acknowledge a substantial grant from the Multiple Sclerosis Society in connexion with this work.

ADDENDUM.—Since this article was submitted for publication two others have appeared on this subject, both in the *Lancet*, December 19, 1959. The first, by P. W. Nathan, deals with the use of 20% phenol solution in cases of spastic paraplegia in flexion, and the second, by R. E. Kelly and P. C. Gautier-Smith, with problems very similar to those discussed in the article.

REFERENCES

- Brown, A. S. (1958). *Lancet*, 2, 975.
 Maher, R. M. (1955). *Ibid.*, 1, 18.
 — (1957). *Ibid.*, 1, 16.
 Nathan, P. W., and Scott, T. G. (1958). *Ibid.*, 1, 76.

BIOASSAY OF THEOPHYLLINE PREPARATIONS USING HUMAN SUBJECTS

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The two chief disadvantages ascribed to theophylline are well known; it is poorly absorbed, probably because of low solubility, and it produces gastric irritation. To overcome these disadvantages, numerous theophylline salts and complexes have been introduced. In this investigation, using pure theophylline as a control, we have attempted to establish a hierarchy of clinical effectiveness among the various compounds by comparing the height and duration of blood levels which they produce in normal subjects and patients.

Method

Normal Subjects.—A group of 12 normal subjects, 21 to 35 years of age, selected at random, were used to study the blood levels of the preparations. Except for one subject (N. E. G.) all were males. At intervals of seven days, each subject received a single oral dose of each preparation listed in Table I in a dose equivalent to 400 mg. of theophylline.

Patients.—A group of 10 patients, 45 to 72 years of age, suffering from bronchial asthma, coronary artery disease, or primary hypertension each received a single

TABLE I.—Preparations Used to Obtain Comparative Blood Levels in Normal Subjects

Preparation	Theophylline Content (%)	Dosage Form
Aminophylline (theophylline ethylenediamine)	75-82	Capsule
Oxtriphylline (choline theophyllinate)	64.4	Coated tablet*
Theophylline	100	Capsule
Theophylline-choline	50	Coated tablet*
7-Hydroxyethyltheophylline	89	Capsule

* Commercial tablets were used.

dose of theophylline-choline* and their blood levels were compared with those of the normal subjects.

Both subjects and patients were in a 10- to 12-hour fasting state, and were told not to take barbiturates, coffee, tea, chocolates, or cola drinks during the testing periods, which occurred at seven-day intervals. Blood samples were drawn and heparinized before the administration of each drug for the "blank" and then at 15, 30, 60, 120, and 240 minutes afterwards.

Blood theophylline levels were determined by the double extraction method of Schack and Waxler (1949).

Results

Theophylline levels were measured in micrograms per millilitre of whole blood. As can be seen from Figs. 1 and 2 and Table II, theophylline rose to a peak of 4.5 ± 1.7 in 60 minutes and remained elevated for another 60 minutes. Aminophylline showed a peak of 4.2 ± 2.3 in 15 minutes and remained statistically constant for 240 minutes. Choline theophyllinate showed low initial levels that reached a peak of 5.5 ± 0.6 in 240 minutes. The 7-hydroxyethyltheophylline showed a level of 1.0 ± 0.5 in 15 minutes and remain statistically constant for 240 minutes.

Subject A.G.G. received a single dose of 1,200 mg. of choline theophyllinate, and a peak blood level concentration of 9.0 occurred in 120 minutes.

In an additional study on a subject, B.G., the single dose of pure theophylline produced a detectable blood level after 24 hours.

Discussion

The blood levels obtained on the normal subjects acting as their own controls revealed that aminophylline produced the highest levels in the first 15 minutes. Between 120 and 240 minutes no statistically significant difference was found in the theophylline blood levels when theophylline, aminophylline, or choline theophyllinate was administered. The low initial levels of choline theophyllinate and theophylline-choline are probably due to the tablet coating. Since there was no statistically significant difference in blood levels between the normal subjects and patients (Fig. 3), it may be assumed that theophylline-choline was absorbed equally well in both groups.

*These tablets are a simple mixture containing 100 mg. of theophylline (anhydrous), 0.05 mg. of reserpine alkaloid, and 209 mg. of choline bitartrate (equivalent to 100 mg. of choline), and supplied as "theracor" by Carroll Dunham Smith Pharmacal Co., New Brunswick, New Jersey.

Fig. 1.—Blood theophylline levels after oral administration of a single dose of equivalent amounts of this drug to normal subjects.

Fig. 2.—Blood theophylline levels after oral administration to normal subjects.

These findings confirm those of Waxler and Schack (1950) as well as those of Schluger, McGinn and Hennessy (1957), who also reported the rapid appearance of theophylline in blood after the ingestion of uncoated aminophylline tablets.

Though pure theophylline is less soluble in water at room temperature (1:120) than the other two prepara-

TABLE II.—Whole-blood Levels ($\mu\text{g./ml.}$) Obtained in Normal Subjects

Name	15 Minutes					30 Minutes					60 Minutes					120 Minutes					240 Minutes				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
V.R.A.	13.3	2.0	1.2	0.0	1.0	10.0	1.1	4.1	0.0	1.0	12.0	1.0	4.4	0.0	1.0	3.1	4.1	3.5	0.0	1.0	8.0	9.0	4.6	0.0	1.0
P.B.B.	3.0	0.0	3.0	0.0	2.0	2.5	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.8	0.5	0.0	0.0	2.1	4.8	4.0	0.0
H.R.C.	—	0.0	1.7	0.0	—	—	0.0	2.5	0.0	—	—	—	0.1	—	—	3.4	3.1	0.0	—	—	—	2.0	8.9	1.1	—
R.R.F.	—	0.3	2.0	0.0	0.0	—	—	0.2	0.0	2.0	—	5.3	12.9	0.0	2.0	—	6.0	16.3	1.43	0.0	—	2.1	1.3	2.0	4.2
A.G.G.	0.0	0.0†	0.0	0.0	4.2	0.2	1.2†	0.0	0.0	8.0	3.5	7.0†	5.1	0.0	0.9	3.0	9.0†	0.5	0.0	0.0	2.4	7.3†	0.8	4.0	—
N.E.G.	0.0	0.0	2.7	1.9	0.0	5.5	0.0	0.5	0.0	0.0	3.0	0.0	0.0	0.0	0.0	4.0	3.0	0.0	0.0	0.3	3.2	3.1	0.0	0.0	3.3
R.L.G.	0.0	0.0	3.7	0.0	0.3	0.4	0.0	1.5	0.2	1.0	1.4	3.2	1.7	0.0	0.2	2.6	7.0	1.3	0.1	0.0	4.8	2.3	0.8	0.1	0.0
I.I.I.	—	0.0	—	0.0	—	—	0.0	—	0.0	—	—	0.1	—	0.8	—	—	2.2	—	0.0	—	—	4.2	—	0.0	—
G.G.K.	0.1	3.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	3.0	1.0	—	0.0	2.0	4.0	3.8	3.4	0.0	0.0	4.0	9.0	0.0	4.0	0.0
C.J.P.	13.0	0.0	1.2	5.0	0.2	10.0	0.0	2.2	2.0	0.3	12.1	0.0	2.6	2.0	0.1	3.0	0.0	2.2	2.0	0.1	11.3	2.0	2.0	1.3	0.0
B.J.S.	—	2.4	—	0.0	—	—	0.3	—	0.0	—	—	0.7	—	0.0	—	—	3.7	—	0.0	—	—	8.0	—	2.0	—
N.D.W.	—	1.0	—	0.0	—	—	0.0	—	0.0	—	—	3.0	—	0.0	—	—	1.0	—	0.0	—	—	5.0	—	0.0	—
Mean average	4.2	0.8	1.7	0.5	1.0	4.1	0.1	1.4	0.2	1.5	5.0	1.4	4.5	0.3	0.8	3.1	3.4	4.0	1.0	1.2	4.8	4.4	2.6	2.0	1.2
S.E. . .	2.3	0.5	0.4	0.4	0.5	1.7	0.1	0.4	0.1	0.3	1.8	0.5	1.7	0.1	0.3	0.4	0.8	1.2	0.3	1.0	1.4	0.9	0.9	0.6	0.0

† Dose of 1,200 mg. of choline theophyllinate (not included in statistical analysis). A=Aminophylline, 500 mg. B=Choline theophyllinate, 600 mg. C=Theophylline, 400 mg. D=Theophylline-choline, 800 mg. E=7-Hydroxyethyltheophylline, 500 mg.

FIG. 3.—Comparative blood levels after oral administration of theophylline-choline to normal subjects and patients.

tions, it has been pointed out by Nelson (1957) that it is the solution rate rather than the solubility in a medium which determines blood levels. In order to determine whether there was any correlation between these findings and the clinical effectiveness of these various preparations, we used as a test subject a patient who had been under our continuous observation for the past three years.

Bioassay

A. H., a 69-year-old man, was under medical treatment for chronic bronchiectasis complicated by bronchial asthma. The number of asthmatic attacks he had experienced had been decreased by daily injections of 0.25 ml. of adrenaline 1:1,000 as the sole drug. Various oral theophylline preparations were administered to determine their effect on the number of injections required for the relief of his attacks as well as his vital capacity.

The drugs were administered by a modified double-blind technique. A plain gelatin capsule was used as the placebo for pure theophylline, aminophylline, and 7-hydroxyethyl-theophylline. Because choline theophyllinate and the theophylline-choline preparation were in the form of yellow coated tablets, similar-appearing placebo tablets were used as controls for them.

TABLE III.—Preparations and Dosages Used in Bioassay of Theophylline in Human Subject

Preparation	Dosage Form	Initial Daily Dosage (mg.)	Maximum Daily Dosage (mg.)
Aminophylline ..	100-mg. plain tablet	200	1,200
Choline theophyllinate ..	200-mg. coated "	400	2,400
Theophylline ..	100-mg. capsule	200	1,600
Theophylline-choline ..	200-mg. coated tablet	400	1,600

TABLE IV.—Results Obtained by Oral Administration of Various Theophylline Preparations

Drug	Total Daily Dosage	Theophylline Equivalent (mg.)	Daily Doses of Adrenaline (0.25 ml., 1:1,000) Required	A.I.*	Vital Capacity (L.)
Placebo ..	—	—	Mean ± S.E.	100	1.7
Adrenaline ..	0.25 ml. (1:1,000)	—	5.2 1.2	—	2.5
Aminophylline ..	800 mg.	640	3.0 1.0	58	2.1
" ..	1,200 "	960	2.0 0.2	38	2.4
Choline theophyllinate ..	1,600 "	1,040	2.6 1.1	50	2.0
Theophylline ..	2,200 "	2,200	2.0 1.1	38	2.3
Theophylline-choline ..	2,400 "	1,200	2.3 1.0	44	2.1
Theophylline ..	1,600 "	1,600	1.7 1.7	33	2.0
Theophylline-choline ..	3,200 "	1,600	1.8 0.9	35	2.0

* See text.

During the test period the patient alternately received placebo for three-day periods and a theophylline preparation for three-day periods. Each preparation was given by the step-dosage methodology and each dosage level was administered for a three-day period. The preparations used and the dosage ranges are shown in Table III. The maximum daily dose was limited by the number of tablets or capsules which could be administered.

The results obtained are shown in Table IV. The various preparations are listed in ascending order of theophylline content, which ranged from 640 to 1,600 mg.

In order to amplify the changes produced in the daily adrenaline requirements, the following formulation was devised:

$$\text{adrenaline index (A.I.)} = 100 \times N_1/N_2,$$

where N_1 represents the number of adrenaline injections when the test theophylline preparation was administered orally and N_2 denotes the total number of adrenaline injections when no theophylline preparation was used.

In general, the A.I. quantitated the therapeutic effectiveness of various theophylline preparations in bronchial asthma, since there was a gradual reduction of the A.I. with increased theophylline dosage. This indicates that as the dosage of theophylline is increased the duration of action is prolonged, resulting in reduced requirement for adrenaline injections. On the other hand, the change in vital capacity did not vary in the same direction. The vital capacity increased by approximately 24% regardless of the dosage of theophylline administered and by 48% after the administration of adrenaline.

These data suggest that blood concentrations and clinical effectiveness of the various theophylline preparations depend on the dosage of pure theophylline present after the various agents undergo hydrolysis in the gastric juice. As a matter of fact, various workers (Truitt, McKusick, and Krantz, 1950; Turner-Warwick, 1957; Seppälä and Kärkelä, 1959) have shown that the minimum blood level of theophylline which is essential for diuresis or the relief of bronchospasm is 5 $\mu\text{g./ml.}$

In clinical practice, therefore, "the problem of reducing side-effects while retaining therapeutic effectiveness is virtually insoluble, for the active principle of all these xanthine derivatives is, in fact, theophylline" (British Medical Journal, 1957).

Summary

When single doses of four different theophylline preparations were administered to normal individuals, no statistically significant difference could be detected in blood concentrations from 120 to 240 minutes.

The adrenaline index revealed that the clinical effectiveness of various theophylline preparations depends on the absolute amount of theophylline present. Large doses produce an increase in duration of action rather than in intensity of action.

REFERENCES

- Brit. med. J., 1957, 2, 689.
 Nelson, E. (1957). *J. Amer. pharm. Ass., sci. Ed.*, 46, 607.
 Schack, J. A., and Waxler, S. H. (1949). *J. Pharm. exp. Ther.*, 97, 283.
 Schluger, J., McGinn, J. T., and Hennessy, D. J. (1957). *Amer. J. med. Sci.*, 233, 296.
 Seppälä, T., and Kärkelä, A. (1959). *Ann. Med. exp. Fenn.*, 37, 59.
 Truitt, E. B., McKusick, V. A., and Krantz, J. C. (1950). *J. Pharm. exp. Ther.*, 100, 309.
 Turner-Warwick, M. (1957). *Brit. med. J.*, 2, 67.
 Waxler, S. H., and Schack, J. A. (1950). *J. Amer. med. Ass.*, 143, 736.