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COMBINED CHEMOTHERAPY IN BACTERIAL INFECTIONS*

BY

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The practice of combining two or even more chemotherapeutic drugs in the treatment of patients is growing commoner, and its merits demand examination. Inquiry soon reveals that the subject is one of great complexity, and that for various reasons these combinations may often be useful, sometimes may be no better than one of the pair used alone, and at least occasionally may be contraindicated because a diminished instead of an enhanced effect results. The laboratory work sometimes required for the intelligent direction of such treatment is perhaps more elaborate and time-consuming than anything previously undertaken in routine clinical bacteriology.

Some Indications for Combined Therapy

The Urgent Undiagnosed Case.—An acutely ill patient evidently suffering from some infection, in whom no bacteriological (and perhaps even no clinical) diagnosis has been made, may require treatment with one antibiotic or another, and it is perhaps tempting to give him both until the situation is clarified. This is often done, but genuine necessity for it should be very exceptional. Premature and blindly directed treatment may obscure the diagnosis, and should not be begun until any specimens have been obtained the examination of which is likely to be helpful.

Mixed and Double Infections.—There are some infections, notably those originating in the bowel, but also including some involving the lungs, the flora of which is so varied that no single drug may be expected to combat the whole of it. In peritonitis due to perforation of the lower part of the bowel treatment with both penicillin and streptomycin has been common practice and is theoretically sound, although I know of no statistical proof of its efficacy. It may well be superseded by the use of the newer antibiotics with their wider range of activity: terramycin, given at first intravenously, has in my experience been particularly effective.

A patient may, on the other hand, have two separate conditions, due to entirely different bacteria: perhaps the commonest of such pairs is a lung infection complicating one of the urinary tract or vice versa. If it should be thought necessary to treat both together no single drug may serve for both, and two may therefore be needed.

Prevention of Toxic Effects.—Another possible indication for using two drugs is the danger of toxic effects from only one. In this category comes the use of sulphonamide combinations—for example, "sulphatriad"—an advantage of which is that each component retains its individual solubility in the urine, with the result that the risk of heavy crystalline deposition and consequent tubular or ureteric blockage is minimized. Among antibiotics, streptomycin is that most liable to produce toxic effects, and any effective combination with it which enabled the dose of streptomycin to be reduced from a risky to a safe level might have this type of advantage.

These three indications are of much less significance than the prevention of acquired resistance and the achievement of synergism.

Prevention of Acquired Resistance

Acquired bacterial resistance is a change which threatens ultimately to extinguish the usefulness of all the present major antibiotics except penicillin. Its gradual development in communities where these drugs are freely available can be delayed in only two ways. One is more discriminating and restricted use, and, to judge by experience hitherto, much cannot be hoped from this direction. The second is to combine with the main drug another with an adjuvant effect. The other may not only exert its own independent effect but prevent acquisition of resistance to the first. Precisely how this is achieved we cannot pretend to say until more is known of the mode of action of antibiotics, but in general terms the mechanism is almost certainly this. Sulphonamides act by blocking a stage in an essential synthesis, and antibiotics probably act similarly. The acquisition of resistance results from a circumvention of this chemical process, another being substituted for it having the same ultimate effect, with which the antibiotic cannot interfere. If the second drug used blocks this second process the alternative metabolic route cannot be established, and the organism therefore remains sensitive.

If this explanation is true, it is perhaps remarkable that so many combinations do act in this way, and it encourages the belief that by this means itself more may be discovered about the points of attack of individual drugs. In practice it may even prove advantageous to use more than two drugs together: Carpenter *et al.* (1945) found that gonococci could be entirely prevented from acquiring resistance to sulphathiazole, rivanol, promanide, and penicillin only by exposing them to all four together, a combination of the first three not being fully effective. Similar *in vitro* studies with antibiotics have been made by Kaipainen (1951, 1952), who has shown that a mutual resistance-preventing relationship exists between streptomycin on the one hand and aureomycin, chloramphenicol, or terramycin on the other. (It does not follow that

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these actual combinations will be therapeutically effective, and indeed there is at least a theoretical objection to them on other grounds.) Streptomycin, as the antibiotic to which high resistance is most readily acquired, stands most in need of a partner to prevent this, particularly when it has to be given for long periods, as in the treatment of tuberculosis. Its combination with *p*-aminosalicylic acid, now regarded as obligatory for the treatment of this disease, has been clearly shown to have this effect, provided that the dose of P.A.S. is adequate (Report, 1949 ; Daniels and Hill, 1952), as well as securing an improved therapeutic response. The combination of streptomycin with isoniazid also militates against the acquisition of resistance to either (Report, 1953).

Synergism

Another notable advantage of combined therapy can be (but by no means always is) the achievement of a synergic effect. This is one of three possible results of combining two drugs, and the terms describing them require definition. In the simplest terms, an additive effect is the sum of those of the same dose of each drug given separately ; a synergic effect is something more than this, and an antagonistic effect something less. If these effects can be observed in the form of clinical therapeutic results they are undeniable : if, on the other hand, we are referring to something capable of assessment in the laboratory the definition must be extended by an explanation of what effect is meant. The most easily observed effect is simple inhibition of visible growth, and it is not surprising that so many workers have studied both single and combined effects by this standard only. Unfortunately it is inadequate and misleading for certain combined effects, and a true picture of them can be obtained only by studying bactericidal instead of merely bacteriostatic action, and preferably by following its progress in a series of observations. The definition of synergism proposed by Jawetz and Gunnison (1952a), whose studies of combined drug effects have contributed so largely to our present knowledge, is : " A large increase in the rate of early bactericidal action and the rate of cure of infections beyond that obtainable by simple additive effects of the agents."

An ultimately fatal case of *Str. faecalis* endocarditis was the incentive for Jawetz's (1952) work, and it is in this disease that we find the clearest example of antibiotic synergism yet known. Jawetz and his colleagues showed that when *Str. faecalis* is exposed to an optimal concentration of penicillin the initial bactericidal effect is incomplete and is followed eventually by growth ; if, on the other hand, streptomycin is added, even in a concentration quite ineffective acting alone, the initial rate of killing is accelerated and continues to the point of total sterilization (Jawetz and Gunnison, 1950). That this combination of drugs offers the only hope of cure in this infection is now fairly well recognized, and is evident from the extensive series of cases described by Robbins and Tompsett (1951). We have treated four cases of this infection at St. Bartholomew's Hospital : three were included in the four cases of penicillin-resistant bacterial endocarditis reported by Cates, Christie, and Garrod (1951), and the fourth is more recent. The antibiotic sensitivities of the organisms from these patients, stated in the Table, are strikingly uniform, and would seem to demand some other treatment than that which in fact was required. All four strains were highly sensitive to aureomycin, yet a six-weeks course of this drug was ineffective in Case 1, and an eight-weeks course, during the latter half of which the dose was 8 g. daily, was equally so in Case 2. Case 3 had a 10-weeks course of chloramphenicol, and Case 4 shorter courses of several drugs without effect (most of all this treatment was given elsewhere). The infection was controlled in all four patients by penicillin and streptomycin, although two of the patients died from heart failure, Case 1 after and Case 3 during treatment.

Antibiotic Sensitivities of Four Strains of Str. faecalis Isolated from Cases of Endocarditis; St. Bartholomew's Hospital, 1950–2

Sex	Age	Inhibitory Concentrations (μg./ml.)			
		Penicillin	Streptomycin	Aureomycin	Chloramphenicol
F	36	2·5	30	0·15	20
M	57	1·8	30	0·1	6
M	63	2·5	125	0·15	2·5
F	25	2·5	10	0·3	0·6

In relation to the task which chemotherapy has to perform, bacterial endocarditis is certainly an exceptional disease and possibly unique. It is not enough, as in most other infections, to prevent further bacterial growth and leave the disposal of survivors to the body defences ; this is a disease in which those defences are powerless. It is probably true to say that chemotherapy must eliminate the last surviving streptococcus in the vegetations if relapse is not to occur. That a bactericidal drug (penicillin) should be necessary, or in this particular infection a highly bactericidal combination, is not surprising. There are few reports of cures of this disease by any of the newer antibiotics.

In treating *Str. faecalis* endocarditis we have hitherto assumed that the higher the concentration of each drug that can be maintained in the blood the better, and our patients have accordingly been given large doses of both. The two survivors in the earlier series (Cates, Christie, and Garrod, 1951) both suffered severe vestibular damage from having received 4 g. of streptomycin daily for six and seven weeks, and in our recent case the dose was limited to 2 g. daily in the hope of obviating this effect. There is another possible reason for limiting the dose not of streptomycin but of penicillin. It has been shown by Eagle and Musselman (1948) that *Str. faecalis* is one of the species subject to the zone phenomenon in the bactericidal action of penicillin : the optimum concentration is about 6 μg. (10 units) per ml., and concentrations higher than this kill the organism more slowly. Whether this paradoxical effect operates when streptomycin is also present is of some interest: according to Gunnison, Jawetz, and Coleman (1950) it does not. Strains of *Str. faecalis* from two of our cases were tested to verify this. Both exhibited the zone phenomenon when exposed to penicillin only, a concentration of 10 μg. per ml. killing more rapidly than 500 μg. per ml. When 10 or 20 μg. per ml. of streptomycin was combined with these and intermediate concentrations of penicillin, the much accelerated death rate varied little with the latter, the higher concentrations sometimes giving a slightly faster kill and sometimes not. It appears from these results that little is either gained or lost by massive penicillin dosage in this combination, but there is some suggestion in results obtained by Martin, Chabbert, and Sureau (1953, Table I) by a rather different method that intermediate concentrations are the most effective.

Str. faecalis endocarditis is an exceptional disease, and it may well be asked whether the drug combination required for its treatment is indicated in any other condition. It may be indicated in other exceptional cases which can be identified as requiring it only by elaborate *in vitro* tests: the need for it in mixed infections such as peritonitis has to a large extent been removed by the advent of the new " broad spectrum " antibiotics. The indiscriminate use of penicillin–streptomycin combinations is certainly to be deplored, and it may be thought unfortunate that proprietary mixtures of these two antibiotics, having flourished on the American market for some time, have now been introduced in this country. That these are commercially successful is due to two ideas about them—that they are generally more potent than penicillin itself, and that they afford wider cover in the undiagnosed case. The logical outcome of the latter standpoint would be a chemotherapeutic cocktail supposedly capable of dealing with any microbic infection, and given automatically to any patient with fever. Apart from the general

undesirability of "shot-gun" therapy, the use of streptomycin in patients of whom at least nine-tenths will probably get no benefit from it is to be deprecated on three grounds—the possibility of either toxic effects or sensitization, and the likelihood of evoking bacterial resistance. These and other weighty reasons for discouraging the use of these combinations have been formulated by Jawetz and Gunnison (1952b) in a report published by the Council on Pharmacy and Chemistry of the American Medical Association.

Laboratory Tests of Combined Action

Repeated viable counts in various mixtures and the plotting of death curves constitute a major operation in any laboratory and are almost beyond the capacity of some. A simpler method of determining combined effects must consequently be found if laboratory guidance for such treatment is to be provided. Unfortunately, simple tests of growth inhibition, at least by pairs of antibiotics, seem to be worthless. A few examples from many studies made in this way will show how misleading their results can be. Richter (1952), in a long series of such tests, found that there was not even an additive effect between streptomycin and penicillin acting on *Str. faecalis*, although this is perhaps the clearest example of a synergic combination that we know. Reid, Jones, and Bryce (1952) found that most combinations of five antibiotics acting on four species of Gram-negative bacilli gave an enhanced effect, although one of their combinations, penicillin+chloramphenicol, has been proved by more appropriate methods, including therapeutic tests, to be antagonistic. In some experiments by Bigger (1950) P.A.S. and streptomycin were made under certain conditions to appear antagonistic. Price, Randall, Welch, and Chandler (1949) tested various combinations not only in this way but therapeutically in mice, and obtained agreement between the two methods in only 50% of their results, some being frankly contradictory.

These authors used liquid media; others have hoped to demonstrate synergism even more simply by an agar diffusion method. That this hope is vain may be concluded from the findings of Peyré and Velu (1952), who tested combinations both in plates and in agar columns in tubes, and found that the width or depth of the inhibition zone differed little from that given by the more rapidly diffusing and active of the pair acting alone. It has also been suggested, and found impracticable, to determine combined effect in a plate in which two antibiotics diffuse from paper strips or gutters meeting at a right angle. I have been unable to obtain true or even consistent results in trials of this method. The fact undoubtedly is that mere inhibition of growth, as judged by naked-eye appearance, affords no true reflection of what a combination of drugs does to bacteria. What matters therapeutically is whether the combination kills them, and in a culture showing no visible growth the bacteria may either have been killed within an hour or may even be slowly multiplying.

There is thus no alternative to a method involving the enumeration of survivors after a period of exposure to the drugs. That proposed by Martin, Sureau, and Chabbert (1952) is not unduly complex, and in their hands has afforded a guide to successful treatment. If four antibiotics—for example, penicillin, streptomycin, chloramphenicol, and terramycin—are to be tested singly and in every possible combination, only ten tubes of broth are required, provided that only a single concentration of each antibiotic is to be tested. After incubation for 24 hours or less a measured inoculum from each is spread over a segment of a plate: decimal dilutions of the culture used for inoculation are plated similarly, only four plates being required in all. By comparison with this control it can be seen whether the inoculum in the tubes has multiplied or diminished in numbers: if the latter, the result can be stated as a very approximate percentage surviving.

The difficulty with such a simplified method is to choose appropriate single concentrations of the antibiotics, since to vary them would immediately complicate it: even two concentrations instead of one would mean 36 tubes instead of 10. They can evidently be chosen on either of two principles: in relation to the sensitivity of the organism to each used singly or in relation to the concentrations attained in the body by the dosage it is proposed to use. Arbitrary amounts chosen on the latter basis are preferable for routine use. The test is certainly worthy of extended trial as a guide to treatment in difficult cases.

We have recently applied a simple three-tube form of this test to determining whether the penicillin–streptomycin synergy against *Str. faecalis* which can be demonstrated by this as well as by more elaborate methods is also exerted against other species. Some strains of *Bact. coli, Proteus,* and penicillin-resistant *Staph. pyogenes* reacted similarly, suggesting that this combination may have uses other than the treatment of bacterial endocarditis.

Sulphonamide–Antibiotic Combinations

I was responsible eight years ago (Garrod, 1945) for a misleading although correct observation—namely, that, as observed by viable counts over an eight-hour period, sulphathiazole reduces the rate at which penicillin kills staphylococci. An explanation of this was not far to seek: penicillin is active only against multiplying bacteria, and another agent which prevents multiplication therefore removes a condition necessary for at least its full effect. The defect of this experiment was that it was terminated too early. Others who have prolonged such experiments have shown that the initial rapid decline produced by penicillin alone may be succeeded by bacterial recovery and actual growth, whereas the slower decline caused by penicillin and a sulphonamide continues and proceeds to ultimate extinction. The explanation for this suggested by Hobby and Dawson (1946) and by Klein and Kalter (1946) is that penicillin reduces the number of living bacteria to a level at which a sulphonamide can exert its full effect: the dependence of sulphonamide activity on bacterial numbers is well known. This is therefore not so much synergy in the ordinary sense as two successive and complementary effects. It is evident from the work of the authors quoted that the result is conditioned by various factors, and, among these, time relationships between the introduction of the two drugs have been particularly studied by Gunnison, Speck, Jawetz, and Bruff (1951), who draw an interesting distinction between the apparent interfering effects of sulphonamides and the newer antibiotics with the bactericidal action of penicillin.

Simple tests of growth inhibition have consistently shown that an enhanced effect is obtained by combining penicillin and a sulphonamide: in this connexion Bigger's (1946) work on *Salm. typhi* may be recalled; Thomas and Hayes (1947) and Stewart (1947) obtained similar results with the same organism, and Bigger (1944) previously showed that they were obtainable with *Staph. pyogenes*. Most authors who have tested penicillin–sulphonamide combinations therapeutically in mice (Ungar, 1943; Soo-Hoo and Schnitzer, 1944; Nitti, Boyer, and Faguet, 1946; Kolmer, 1948; Domagk, 1952) have observed synergic effects. There have been fewer studies of the combined action of sulphonamides and streptomycin, but Klein and Kimmelman (1947) and Knapp (1950) report mutual reinforcement by such combinations, and the former emphasize particularly that the presence of a sulphonamide tends to prevent the development of bacterial resistance to streptomycin.

Clinical experience confirms the general tenor of these findings in two particulars. Waring and Smith (1944) are emphatic that sulphadiazine must be given in addition to penicillin for the treatment of pneumococcal meningitis; 11 out of 12 patients so treated recovered, whereas penicillin alone was less successful. Smith, Duthie, and Cairns (1946) base the same conclusion on a study of 38 cases. The second clear example is provided by the action of streptomycin and sulphadiazine in brucellosis, which was the only effective treatment for this disease until the discovery of aureomycin and chloramphenicol (Spink, Hall, Shaffer, and Braude, 1948; Scowen and Garrod, 1948).

Antagonistic Effects

Although interference by sulphonamides with the action of penicillin is only apparent, interference by other antibiotics due to a similar mechanism is not merely demonstrable in a similar way *in vitro,* but verifiable by therapeutic tests. That aureomycin may interfere with the action of penicillin or streptomycin was first shown by Lankford and Lacy (1949), but most of our now extensive knowledge of this subject is due to the work of Jawetz and his colleagues, which has been summarized in recent reviews (Jawetz, 1952 ; Jawetz and Gunnison, 1953). The stages in the development of this work were as follows. It was first shown that chloramphenicol interferes with the bactericidal action of penicillin *in vitro* (Jawetz, Gunnison, and Coleman, 1950), and later that aureomycin and terramycin have a similar interfering effect (Gunnison, Coleman, and Jawetz, 1950). The interfering action of chloramphenicol had meanwhile been confirmed therapeutically (Jawetz and Speck, 1950) : mice infected with *Str. pyogenes* and treated with both chloramphenicol and penicillin had a lower survival rate than those treated with the same dose of either drug alone. The objection that such acute experiments, in which only a single dose of the drug or drugs is given, are far removed from the conditions of clinical use appears to be answered by later studies (Speck and Jawetz, 1952) of a subacute streptococcal infection produced by intramuscular inoculation, of which control mice died in five to eight days. Although treatment was continued for five days, the same type of effect was observed, chloramphenicol and particularly terramycin reducing survivors to as low as 45% when combined with a dose of penicillin which alone saved 100%. In another series of experiments, both *in vitro* and *in vivo,* it was shown that the newer antibiotics also interfere with the action of streptomycin, chloramphenicol having the most pronounced effect (Jawetz, Gunnison, and Speck, 1951a).

The explanation of these findings is as follows (Jawetz and Gunnison, 1952a). Antibiotics are divisible into two classes:—Group 1 (bactericidal): penicillin, streptomycin, bacitracin, neomycin. Group 2 (bacteriostatic) : aureomycin, chloramphenicol, terramycin.

Combinations within group 1 are often synergic. Within group 2 they are no more than additive. Combinations of the two groups are apt to be antagonistic, the group 2 drug interfering with the bactericidal action of the other. Such antagonism is observed only when the organism is fully sensitive to the bactericidal component : if it is more resistant (although naturally not if completely so) the combination may actually be synergic. Instances of this paradoxical effect are cited by Jawetz, Gunnison, and Speck (1951b), and by Spies *et al.* (1951); both patients had staphylococcal endocarditis, and the successful combinations were terramycin–streptomycin and penicillin–aureomycin respectively.

These patients afford striking confirmation of one aspect of the Jawetz theory. What evidence is there of the clinical reality of antagonism ? Certainly the clearest is the experience of Lepper and Dowling (1951) in the treatment of pneumococcal meningitis. Alternate cases were treated with penicillin alone and with penicillin + aureomycin, the penicillin being given intramuscularly only in a dose of 1,000,000 units two-hourly. Of 14 patients given penicillin only 3 died (21%), and in a total of 43 patients so treated 13 died (30%), whereas only 3 out of 14 receiving combined treatment recovered, a mortality of 79%. A similar though less emphatic conclusion may be drawn from the findings of Lepper *et al.* (1952) in meningococcal meningitis : here there was almost no mortality, but the duration of fever and of "mental abnormality" seems definitely to have been prolonged by combining aureomycin with penicillin. It has been remarked by several authors that conditions in the meninges are ideal for antagonism, critical concentrations for this effect being maintained fairly constantly in an area where efficient bactericidal action is probably imperative. Whether antagonism occurs in other situations it is the task of future clinical observation to decide.

Complexity of the Problem

We have seen that there are several specific instances in which drug combinations are clearly indicated either because they delay the acquisition of bacterial resistance or because they exert a synergic effect. In pyogenic meningitis we have a disease in which certain combinations are contraindicated because their effect is antagonistic, and future clinical appraisal may possibly decide that these combinations are either contraindicated or at least without advantage in other conditions. But these conclusions refer to only a few specific and (except tuberculosis) uncommon infections, and leave the general question of the usefulness of combined therapy in everyday medicine unanswered.

It is strongly emphasized by Jawetz and Gunnison (1952b) that no general rules about synergism and antagonism can be laid down. The same pair of antibiotics may exhibit either effect against different organisms, according to their degrees of sensitivity. Other authors have found that the same pair may be either synergic or antagonistic against the same organism according to the concentrations used. The whole of this account of this subject has in fact been oversimplified, for the sake of brevity, by omitting mention of many factors which have been shown to determine the results of combined action.

In any serious case an *ad hoc* effort to provide a rational basis for treatment should be made by laboratory experiment, and the simplified test of bactericidal action proposed by Martin, Sureau, and Chabbert (1952) may afford useful indications with little delay. On the other hand, more elaborate proceedings may be very advisable, and I calculate that various studies in connexion with our last case of enterococcal endocarditis occupied well over 100 hours of laboratory time. I have contended for many years that the action of chemicals, whether simple germicides or chemotherapeutic agents, on bacteria can be determined in the laboratory in such a way as to provide a reliable guide to their use. I still adhere to this view, but I begin to doubt whether such determinations in connexion with combined antibacterial action are practicable. The feasibility of attaining a significant result from a reasonable amount of any such work depends on the number of variables involved. It might be supposed that the introduction of a second drug merely adds one more variable, and this is true in a sense, but drug interactions are so complex that it does no justice to the result. The bacteriologist who tackles individual problems of this kind seriously is facing what I believe to be the most complicated task in the whole of routine laboratory medicine.

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CLINICAL TRIAL OF A NEW ORAL DIURETIC

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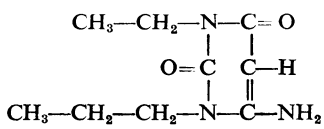
In a search for a potent non-toxic oral diuretic, Kattus, Newman, and Franklin (1951) tested for diuretic activity in dogs a series of compounds derived from the uracil nucleus. One compound, 1-propyl-3-ethyl-6-aminouracil, was found to be an effective diuretic, and produced only a minor degree of gastro-intestinal disturbance. Papesch and Schroeder (1950) had previously synthesized this substance, and for convenience it is referred to by their serial number, S.C.2614. The structural formula is:



Kattus, Arrington, and Newman (1952) subsequently reported that compound S.C.2614 was an effective diuretic when given by mouth to dogs, to normal human subjects, and to patients with oedema. No serious toxic reactions were noted in their studies.

The present report describes an investigation into the effectiveness of compound S.C.2614 as an oral diuretic when given to normal human subjects and to patients with renal or cardiac disease.

Chemical Methods.—The daily renal excretion of protein was determined by micro-Kjeldahl estimation of the protein precipitate obtained from centrifuged aliquots of the 24-hour urine. The other chemical methods have been described previously (Spencer and Franglen, 1952).

Effect on Normal Subjects

Observations on the diuretic effect of S.C.2614 were made during ten tests on nine normal males aged 21–35 years. These subjects were ambulant and took their normal diet. Urine was collected over two 24-hour periods; the first period being the control day, and the second the test day on which 1,250 mg. of S.C.2614 was taken by mouth in 125-mg. tablets over a period of six hours. The renal excretion of water, sodium, chloride, and potassium was measured on each day, and the results are summarized in Tables I and II.

TABLE I.—*Effect of Compound S.C.2614 on Normal Subjects. The 24-hour Renal Excretion of Water and Sodium on a Control Day Compared with that on a Day when 1,250 mg. of S.C.2614 was Taken by Mouth. Ten Tests on Normal Subjects*

Subject	Renal Excretion in 24 Hours			
	Water (ml.)		Sodium (mEq)	
	Control	S.C.2614	Control	S.C.2614
G.A.	800	2,320	152	260
T.R.B.	2,150	3,140	110	251
G.C.	1,210	1,430	179	320
A.M.G.	1,730	2,630	157	330
A.B.K.	2,355	3,620	167	375
M.N.	1,545	2,410	154	384
G.M.	1,390	2,230	159	210
A.G.S.	1,470	2,930	99	303
A.G.S.	1,479	2,640	158	457
L.T.	1,600	2,960	196	525

TABLE II.—*Effect of Compound S.C.2614 on Normal Subjects. The 24-hour Renal Excretion of Water and Sodium on a Control Day Compared with that on a Day when 1,250 mg. of S.C.2614 was Taken by Mouth. Means and Standard Deviations of the Data from Ten Tests on Nine Normal Subjects*

	Water (ml.)	Sodium (mEq)	Chloride (mEq)	Potassium (mEq)
Control day	1,573 S.D.=460	153 S.D.=29	166 S.D.=32	71 S.D=9
S.C.2614 day	2,631 S.D.=530	341 S.D.=28	378 S.D.=66	97 S.D.=28
Mean difference	+1,058	+188	+212	+26
Mean diuresis as % of control	161	222	227	136
Level of significance	P=0·01	P=0·01	P=0·01	P=0·02

In nine of the ten tests there was a considerable increase in the urine volume and excretion of sodium and chloride on the test day. One subject did not show a significant water diuresis, although his excretion of sodium on the test day was 178% of that on the control day. For all the ten tests the mean excretions on the test day compared with the control day were: water, 161%; sodium, 222%; chloride, 227%; potassium 136%. In these tests the water diuresis in response to oral S.C.2614 was equivalent to that reported by Blumgart *et al.* (1934) for mercurial diuretics when given by intramuscular injection to normal subjects. The effect of oral S.C.2614 on sodium excretion also compared favourably with that reported by Kattus *et al.* (1952) for a 2-ml. injection of a mercurial diuretic in a comparable series of normal subjects, and was much greater than that which was produced by 1.2 g. of aminophylline by mouth.

Toxic Reactions.—Two subjects complained of epigastric pain and abdominal discomfort, one of anorexia, and two of tinnitus. During the administration of S.C.2614 there was no significant change in the low normal rate of daily excretion of protein in the urine, and in no case did urine microscopy show any abnormality.

Effect on Patients: Method of Investigation

The patients were not deliberately selected, and included those with every degree of fluid retention, from little or no pitting oedema to gross anasarca. Fifty tests were completed on 30 patients, 22 with cardiovascular and 8 with renal disease. All patients were confined to bed and, except when otherwise stated, were given a standard hospital low-salt diet (10–25 mEq) of sodium and a measured fluid intake of 30–50 oz. (850–1,420 ml.). There was a preliminary period of observation of at least six days in which to