obtained with the HI and the neutralization tests with dengue type I virus was remarkably good.

As mentioned above, the HI studies had indicated the possibility that one of the donors had undergone an infection with RSSE virus. In a neutralization test with RSSE virus, this serum neutralized 300 LD_{50} of the virus, whereas 3 HI negative sera showed no neutralizing action. The donor of this serum had lived all his life in Greece.

These results indicate clearly that the epidemic of dengue in Athens in 1927-28 was, in all probability, due to a dengue type I virus. There is no evidence that any West Nile infections occurred. The high titers obtained in HI tests with a West Nile antigen are possibly the result of the high reactivity of the antigen used. Antigens prepared with the IG 2266 strain of West Nile virus used in these studies have consistently reacted to a higher titer with antisera than antigens prepared from other strains of West Nile.

Discussion. These studies have provided a unique opportunity for acquiring information about the persistence of HI antibodies following a group B arthropod-borne virus infection. In most regions where these agents occur, the infections are endemic, and individuals are exposed to repeated infections by one or more group B agents. In the present instance, however, the complete absence of any group B antibodies in the sera of the younger age group shows that no group B arthropod-borne virus infection has occurred in Athens since the epidemic of 1927-28. Under these circumstances, the close correspondence between the results obtained in the HI and neutralization tests is good evidence that HI antibodies persist for at least 30 years after a dengue type I infection.

Summary. The results of serological investigations on sera obtained from residents of Athens, Greece, indicate clearly that the epidemic of dengue which occurred in that country in 1927-28 was, in all probability, due to type I dengue virus. Antibodies were demonstrated in approximately 70% of sera obtained from persons living at the time of the epidemic. Sera from individuals born subsequent to the epidemic were negative.

1. Sabin, A. B., in Rivers, T. M., Horsfall, F. L., Ed., *Viral and Rickettsial Infections of Man,* 3rd ed., J. B. Lippincott, 1959, 361.

2. Sabin, A. B., Schlesinger, R. W., *Science,* 1945, v101, 640.

3. Schlesinger, R. W., Frankel, J. W., *Am. J. Trop. Med. and Hyg.,* 1952, v1, 66.

4. Bernkopf, H., Levine, S., Nerson, R., *J. Infect. Dis.,* 1953, v93, 207.

5. Goldblum, N., Sterk, V. V., Paderski, B., *Am. J. Hyg.,* 1954, v59, 89.

6. Marberg, R., Goldblum, N., Sterk, V. V., Jasinska-Klingberg, W., Klingberg, M. A., *ibid.,* 1956, v64, 259.

7. Taylor, R. M., Work, T. H., Hurlbut, H. S., Rizk, F., *Am. J. Trop. Med. and Hyg.,* 1956, v5, 579.

8. Robinson, M. C., *Tr. Roy. Soc. Trop. Med. & Hyg.,* 1955, v49, 528.

9. Ross, R. W., *J. Hyg.,* 1956, v54, 177.

10. Clarke, D. H., Casals, J., *Am. J. Trop. Med., & Hyg.,* 1958, v7, 561.

11. Casals, J., *N. Y. Acad. Sci.,* Ser. 2, 1957, v19, 219.

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Absorption and Urinary Excretion of Chloramphenicol and 2 Analogues, Thiocymetin and U-15,442 in Normal Men.* (25475)

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Following the discovery of chloramphenicol and elucidation of its chemical structure, numerous derivatives were prepared in different laboratories. However, in the various test systems employed, none of them have shown

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antimicrobial activity as great as that of the parent compound(1), nor have any thus far proved to be as useful therapeutically. The possibility remains that certain of the chloramphenicol derivatives, by virtue of some special pharmacologic property, may be more desirable than chloramphenicol as a therapeutic agent in man. Chloramphenicol has been shown to be rapidly inactivated *in vivo* by conjugation with glucuronic acid, and this largely accounts for the recovery in urine of man of only 5-15% of microbially active, unconjugated drug(2,3,4). Conceivably a weaker derivative which is inactivated much more slowly than the parent compound *in vivo* may produce an equivalent or even greater amount of antimicrobial activity in blood, tissue and urine. With this in view, an investigation of absorption and urinary excretion of 2 chloramphenicol derivatives that had good activity *in vitro* was undertaken in man. Both of these compounds differ from the parent drug only in substitutions for the nitrophenyl group, one derivative, Thiocymetin® (Win 5063-2), substituting 4-methylsulfonylphenyl, the other U-15,442 (Win 5094-2), substituting 4-methylmercaptophenyl.

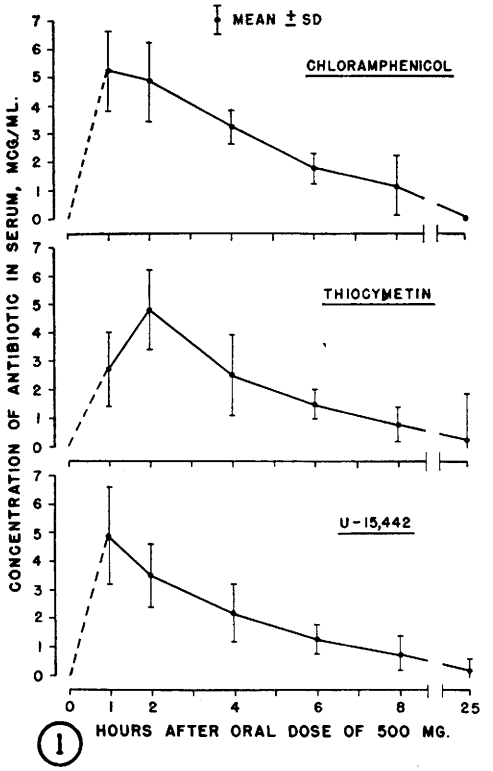
Materials and methods. An oral dose of 500 mg of chloramphenicol, Thiocymetin and U-15,442§ was given, according to a Latin square design, in rotation to 6 healthy young men. Doses were separated by at least one week. On each test day the subjects were in the fasting state except for liberal water intake. Venous blood samples were obtained prior to and at 1, 2, 4, 6, 8 and 25 hours after the dose. The first meal was taken after the 4-hr specimen. Serums were separated as soon as possible and stored at –20°C until time of assay. All urine passed during 48 hours following the dose was collected in sterile containers and immediately refrigerated; aliquots from pools obtained at 0-4, 4-8, 8-12, 12-24 and 24-48 hours were kept frozen at –20°C until time of assay. Specimens of serum and urine were assayed by a modified cup-plate method employing *Pasteurella bovisepticus* as the test organism and levels were expressed in μg/ml based on comparison with standard drug corresponding to the one administered. These tests were done in the Research Laboratories, Upjohn Co. (to be referred to as Laboratory A) to which the specimens were delivered in the frozen state. Urines were also assayed in this laboratory (hereafter referred to as Laboratory B) using the standard *Sarcina lutea* cup-plate method described for chloramphenicol(5), and employing standards of all 3 drugs simultaneously with each test so that results could be expressed in terms of activity equivalent of each drug. The assay strain of *P. bovisepticus* was inhibited by 0.19, 0.09 and 0.09 μg/ml of chloramphenicol, Thiocymetin and U-15,442, respectively. The strain of *S. lutea* was less sensitive but otherwise satisfactory; it was inhibited by 3, 10, and 15 μg/ml of chloramphenicol, Thiocymetin and U-15,442, respectively.

Results. Serum levels. Average concentrations of the 3 analogues in serum of 6 subjects are shown in Fig. 1. Average peak levels were nearly the same for the 3 analogues but were achieved later with Thiocymetin than with the other 2. At 6 hours, average concentrations of all 3 drugs were again about the same. Slight activity of Thiocymetin and U-15,442 was still measurable 25 hours after these doses, but none was detectable after the dose of chloramphenicol.

Urinary excretion. Average amounts of the 3 drugs recovered in urine, as determined in each laboratory, are given in Table I. Values obtained in Lab. A were slightly lower than those obtained in Lab. B for chloramphenicol and U-15,442, but for Thiocymetin they were about one-third lower. Despite these differences the patterns of the 48 hour cumulative excretion of the 3 drugs as determined in the 2 laboratories were quite similar (Fig. 2). In both studies considerably more thiocymetin was recovered than U-15,442; recoveries of chloramphenicol in urine were lowest of the 3.

The data presented in Fig. 3 permit comparison of relative antimicrobial activity of each analogue in urine, expressed in chloramphenicol "equivalents" as determined in the *S. lutea* method. Thiocymetin, in each sequential time period, and over the total pe-

§ Kindly supplied in coded capsules by Upjohn Co. from bulk material obtained from Winthrop Laboratories; the generic name of Thiocymetin is dextrosulphenidal.



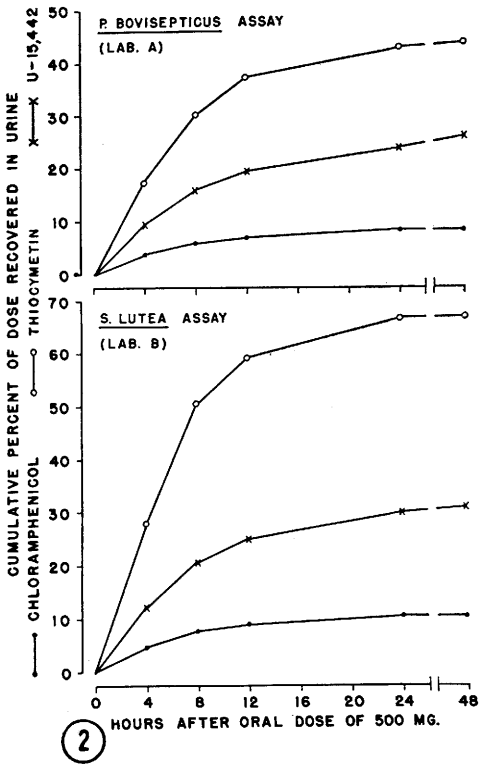
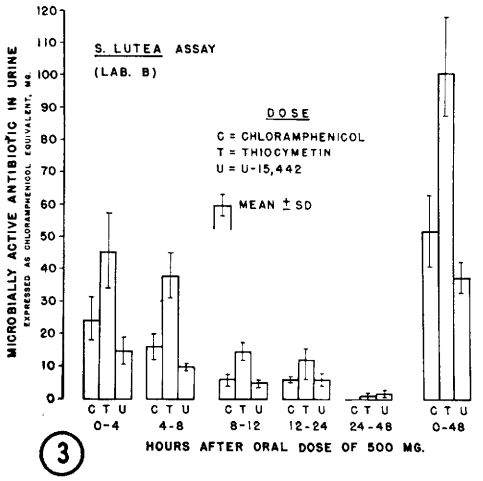


FIG. 1. Avg concentrations of chloramphenicol and 2 analogues in serum of same 6 subjects after single oral doses of 500 mg.

FIG. 2. Avg excretion of microbially active chloramphenicol and 2 analogues in urine of 6 subjects after single oral doses of 500 mg as determined in 2 laboratories.

FIG. 3. Avg excretion of active chloramphenicol and 2 analogues, each calculated as chloramphenicol equivalents, after single oral doses of 500 mg.



riod of 48 hours, produced significantly more antimicrobial activity in urine than did either chloramphenicol or U-15,442. The same relationships hold for results expressed in terms of the other 2 drugs; only the heights of all the bars are different.

Discussion. These data indicate that levels of microbially active thiocymetin and U-15,442 produced in serum during the first few hours after an oral dose are quite comparable to those obtained with chloramphenicol; however, peak levls were achieved somewhat later with Thiocymetin and activity was no longer detectable in serum 25 hours after the dose of chloramphenicol, but was still measurable in some subjects after doses of the other 2 ana-

TABLE I. Recovery of Chloramphenicol and 2 Analogues in Urine of 6 Healthy Young Men after a Single Oral Dose of 500 mg.

Hr after dose	Urinary excretion: Chloramphenicol mg ± S.D.	% *	Thiocymetin mg ± S.D.	%	U-15,442 mg ± S.D.	%
	Laboratory A, *P. bovisepticus* assay					
0- 4	18.0 ± 6.4	3.6	87.1 ± 22.6	17.4	47.5 ± 12.8	9.5
4- 8	12.3 ± 2.9	2.4	65.0 ± 10.6	13.0	31.6 ± 3.7	6.3
8-12	5.3 ± 1.6	1.0	33.9 ± 7.3	6.7	18.1 ± 8.2	3.6
12-24	5.5 ± 1.8	1.1	28.4 ± 8.9	5.6	23.3 ± 4.4	4.6
24-48	.8 ± .5	.1	5.6 ± 5.4	1.1	8.6 ± 5.5	1.7
Total	41.7 ± 9.2	8.2	220.0 ± 28.5	43.8	129.1 ± 24.9	25.7
	Laboratory B, *S. lutea* assay					
0- 4	23.8 ± 6.8	4.7	140.3 ± 35.5	28.0	61.3 ± 17.1	12.2
4- 8	16.2 ± 3.7	3.2	112.5 ± 26.4	22.5	41.6 ± 4.1	8.3
8-12	5.9 ± 1.5	1.1	44.4 ± 7.7	8.8	21.6 ± 3.9	4.3
12-24	5.9 ± 3.2	1.1	35.5 ± 11.4	7.1	25.8 ± 8.0	5.1
24-48	†	—	2.7 ± 5.2	.5	5.4 ± 4.2	1.0
Total	51.7 ±11.0	10.1	335.7 ± 41.5	66.9	155.5 ± 21.0	30.9

Each value shown is the mean for the same 6 subjects, expressed as activity of drug ingested.
* % of administered oral dose. † Undetectable.

logues. Recovery of the active drug in urine was several times as great after Thiocymetin and significantly greater after U-15,442 than after chloramphenicol, when expressed as percent of dose ingested. However, when expressed in the equivalent of chloramphenicol activity, the dose of Thiocymetin still yielded significantly more activity in urine than a similar dose of chloramphenicol, but the advantage of U-15,442 was lost due to the lesser activity of the latter against the assay organism.

From the therapeutic point of view at least 2 factors other than absorption, excretion and inactivation must be considered in comparing these 3 drugs, namely their relative *in vitro* activity against human pathogenic bacteria and their relative toxicity. Since chloramphenicol has been widely used recently in treatment of infections with staphylococci that are resistant to other antibiotics, a number of recently isolated strains were tested for their susceptibility to these 3 agents by a 2-fold dilution method on agar plates. The results (Table II), indicate that chloramphenicol is the more active agent, on the average at least twice as active as the other two. Since blood levels over an 8-hour period after a dose in normal individuals were at least as high with chloramphenicol as with the other derivatives, the systemic antistaphylococcal effect may be expected to be greater. The activity recoverable in urine, on the other hand, was greater with Thiocymetin even when expressed as active chloramphenicol equivalents, so that the former might be considered advantageous in treatment of urinary tract infections. The results do indicate, however, that Thiocymetin and U-15,442, particularly the former, are inactivated more slowly or by different mechanisms than chloramphenicol. The overriding consideration from the therapeutic point of view is the much greater toxicity of Thiocymetin as revealed both in laboratory animals and in preliminary clinical trials which preclude its use (personal communication). Nevertheless, these findings indicate that further search for chloramphenicol derivatives that are less toxic and also inactivated more slowly or to a lesser degree than chloramphenicol, may be worthwhile.

TABLE II. Susceptibility of 178 Recently Isolated Strains of *Staphylococcus aureus* to Chloramphenicol and 2 of Its Analogues.

Minimum inhibiting conc., μg/ml	Chloramphenicol (% of strains)	Thiocymetin (% of strains)	U-15,442 (% of strains)
6.3	12.9		
12.5	55.1	4.5	5.1
25	23.6	47.8	83.1
50	1.7	37.6	6.7
100	5.1	1.1	2.8
>100	1.7	9.0	2.2

Summary. Comparison of absorption and urinary recovery of chloramphenicol and 2 of its derivatives, Thiocymetin and U-15,442, has been carried out following administration of single oral doses of 500 mg each in 6 healthy human volunteers. Similar peak concentrations of these drugs were noted in serum but they were achieved later after the dose of Thiocymetin. At 25 hours slight antibiotic activity was still discernible with the 2 derivatives, but not with chloramphenicol. In view of the superior antimicrobial activity of chloramphenicol the 2 derivatives are considered to be inferior to the parent compound in producing antimicrobial activity in serum. In contrast, much higher urinary recoveries were achieved with the 2 derivatives than with chloramphenicol. In the case of Thiocymetin urinary recovery was so great as to more than offset its inferior antimicrobial activity; this was not the case with U-15,442. The high recovery of active Thiomycetin in urine suggests that it is inactivated more slowly or by a different mechanism than is chloramphenicol.

1. Hahn, F. E., Hayes, J. E., Wisseman, C. L., Jr., Hopps, H. E., Smadel, J. E., *Antibiot. and Chemother.*, 1956, v6, 531.

2. Glazko, A. J., Wolf, L. M., Dill, W. A., Bratton, A. C., *J. Pharm. and Exp. Ther.*, 1949, v96, 445.

3. Chittenden, G. E., Sharp, E. A., Vonder Heide, E. C., Bratton, A. C., Glazko, A. J., Stimpert, F. D., *J. Urol.*, 1949, v62, 771.

4. Kunin, C. M., Glazko, A. J., Finland, M., *J. Clin. Invest.*, 1959, v38, 1498.

5. Grove, D. C., Randall, W. A., *Assay Methods of Antibiotics. A Laboratory Manual,* 238 pages, Medical Encyclopedia, Inc., N. Y., 1955.

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A Simple Chromatographic Method for Preparation of Gamma Globulin.* (25476)

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Many serological procedures might be improved by use of gamma globulin rather than whole serum as the source of antibody. For example, non-specific inhibitors of viruses might be removed in some cases where antiviral sera are being studied. Furthermore, in work with fluorescent antibody, non-specific staining might be reduced if gamma globulin fractions were used instead of serum. Most methods for preparation of gamma globulin are not convenient for use by the usual virology or immunology laboratory. The high capacity of the cellulose ion exchange adsorbents(1), and the relatively mild conditions of salt and pH required for adsorption and elution have suggested their use in such preparation(2). A slight modification of the chromatographic procedure described by Sober and Peterson(3) is presented here which allows preparation of gamma globulin in a stepwise procedure, free from macroglobulins, high molecular weight serum protein with gamma globulin mobility, and non gamma globulin serum proteins.

Methods. Ion exchange adsorbents. The anion exchanger, diethylamino-ethylcellulose (DEAE), (40-100 mesh, 0.96 meq/g) was prepared by the method of Peterson and Sober(1), or purchased. It was washed with 0.5 N NaOH 3 times to remove color and to regenerate the column, then suspended in water and washed to neutrality. The slurry was adjusted to pH 6.3 by adding 0.2 M NaH_2PO_4, and washed several times with the starting buffer (0.0175 M phosphate (Na^+), pH 6.3). A large batch of the adsorbent may be so prepared and stored in the refrigerator.

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