the test duration, subcultures from all remaining sera were made at the 24- and 40-hour periods from tubes showing no visible growth or hemolysis.

RESULTS AND CONCLUSIONS

The results of this investigation, including the data presented in Tables 1 and 2, demonstrate that:

(1) Inhibitory substances to the streptococcal strain C203 and *B. subtilis* exist in human sera.

(2) The effect of these substances may be very easily misinterpreted as penicillin activity, especially in concentrations of the order of 0.02 to 0.05 units/ml of serum.

(3) The inhibitory activity of normal adult sera was much more pronounced against *B. subtilis* than against the streptococcus. In the former the effect was bactericidal as well as bacteriostatic; in the latter the effect was only bacteriostatic, which activity could be nullified by subculture after 24 hours' incubation of the tubes.

(4) The contrast between the streptococcus and *B. subtilis* cultures carried over to sera from children. Of twelve sera only one, and even then only in the 1 ml serum volume, showed inhibitory activity against the streptococcus, whereas bactericidal activity was demonstrated in one third of the sera against *B. subtilis* at 24 hours.

(5) Sera from ailing adults showed pronounced bacteriostatic and bactericidal activity against both microorganisms. This agrees with the observations of Tillet and is probably due to the greater concentration of inhibitory substances whose effect can not be eliminated by subculture.

TABLE 2

THE EFFECT OF HEAT (56° C. WATER BATH FOR 5 MINUTES) ON THE INHIBITORY AND BACTERICIDAL ACTIVITY OF SERA FROM 47 NORMAL ADULTS*

Tube inhibition at incubation period of	Strept. C203		*B. subtilis*	
16 hrs.	27.7 per cent.		55.3 per cent.	
24 "	8.5 " "		48.9 " "	
40 "	4.3 " "		14.9 " "	
Bactericidal activity as demonstrated by absence of growth on subculture at	*24 hrs.*	*40 hrs.*	*24 hrs.*	*40 hrs.*
	0 per cent.	0 per cent.	12.8 per cent.	8.5 per cent.

* 1 ml volumes of sera were contained in 2 ml final volumes with broth as diluent. Results are expressed in per cent. of cases showing inhibitory activity as in Table 1

(6) Heating normal adult sera at 56° C. for five minutes lowered the incidence of bacteriostatic activity against the streptococcus and *B. subtilis* but did not eliminate the bactericidal effect against the latter culture.

(7) In the comparison of penicillin dosage forms the streptococcal method, with subculture, has proven of value when sera from children or normal adults are used in such tests.

(8) Neither streptococcal nor *B. subtilis* method are absolutely reliable in the determination of low concentrations of penicillin in ill adults, since neither method can distinguish between penicillin activity and other bacterial inhibitory substances in sera from such individuals.

(9) The sensitivity of a strain of *Staphylococcus aureus*[12] to inhibitory substances in human sera was determined. Although this organism was not inhibited by 1 ml volumes of serum in 50 cases of normal adult serum, because of its insensitivity to penicillin it is not applicable to the determination of low concentrations of penicillin in serum. This organism required 0.08 unit of penicillin per tube for inhibition as compared to 0.02 unit for the streptococcal strain C203.

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CALCIUM CARBONATE AS AN ANTACID FOR ORAL PENICILLIN

EARLY work by Abraham, Florey and associates[1] and Rammelkamp and Keefer[2] indicated penicillin to be ineffective by the oral route because of destruction by stomach acidity. Later, Free *et al.*,[3] using larger doses and more purified penicillin, showed that some penicillin escapes destruction by the stomach acid and is absorbed, thereby renewing interest in the oral route as a possible mode of administration. More recently, somewhat greater absorption has been reported to take place when the penicillin is administered in combination with agents to protect it from destruction by stomach acidity. Libby[4] suspended penicillin in fixed oils and reported a protective effect, but McDermott[5] was not able to show a significant protection by the use of oils. Reports on the administration with certain gastric antacids appear the most favorable. Sodium bicarbonate has been found unsuited for this purpose because of its alkalinity.[1,2,3] Charney *et al.*[6] and Gyorgy *et al.*[7] reported increased absorption by the simultaneous administration of sodium citrate. Sodium phosphate was also

1 E. P. Abraham, H. W. Florey *et al.*, *Lancet*, 2: 177, 1941.
2 C. H. Rammelkamp and C. S. Keefer, *Jour. Clin. Invest.*, 22: 425, 1943.
3 A. H. Free *et al.*, SCIENCE, 100: 431, 1944.
4 R. L. Libby, SCIENCE, 101: 178, 1945.
5 W. McDermott *et al.*, SCIENCE, 101: 228, 1945.
6 J. Charney *et al.*, SCIENCE, 101: 251, 1945.
7 P. Gyorgy *et al.*, *Jour. Am. Med. Asn.*, 127: 639, 1945.

found to be effective by Charney's group, but aluminum hydroxide or calcium carbonate in milk was not found to be particularly effective. Enteric coating has not given a satisfactory answer to the problem[1, 4, 8] probably due to the variability in the time and location at which the enteric coating disintegrated in the gastro-intestinal tract. Burke *et al.*[9] reported increased absorption by the use of both an enteric coating and aluminum hydroxide as an antacid. It was not determined which of the two factors contributed most to the increase in absorption.

In preliminary experiments with artificial gastric juice *in vitro* and in trials of oral penicillin medication on humans, we also found gastric antacids to be the most promising aid to oral penicillin administration reported up to the present time. It occurred to us that calcium carbonate might be an effective antacid for this purpose because of its very weak alkaline reaction on one hand and its ability to quickly neutralize comparatively large amounts of hydrochloride acid on the other. We reasoned that the milk administered simultaneously with the calcium carbonate and penicillin by Charney's group[6] may have induced a delaying effect on stomach emptying, thereby allowing more time for penicillin destruction in the stomach before its passage into the duodenum for absorption. Therefore, calcium carbonate and five other antacids, including those reported on by other investigators, were studied for comparative effectiveness.

TABLE 1

BLOOD SERUM CONCENTRATIONS OF PENICILLIN FOLLOWING ORAL ADMINISTRATION OF 100,000 UNITS OF CALCIUM SALT WITH VARIOUS ANTACIDS

Antacid	Subject	Serum concentrations of penicillin in units per cc. ½ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Calcium carbonate	1	.312	.624	.312	.156	.078
	2	.312	.039	.019	.019	Trace
	3	.039	.078	.312	.312	.156
	4	.156	.156	.078	.039	.019
	5	...	.312	.078	.039	.039
Average serum concentration		.202	.242	.160	.113	.058
Sodium citrate	6	.039	...	.078	.039	.019
	7	.312	.312	.078	.019	.019
	8	.156	.156	.078	.039	.039
	9	.156	.039	.019	.019	Trace
	10	.156	.156	.039	Trace	Trace
Average serum concentration		.164	.166	.058	.023	.015
Sodium phosphate buffer 12.5% NAH_2PO_4 87.5% Na_2HPO_4 (pH 7.5)	11	.039	.039	.039	.039	Neg.
	12	.039	.039	.039	.039	.039
	13	.039	.039	.078	.039	.019
	14	.039	.039	.039	Trace	Neg.
	15	.156	.078	.039	.039	Trace
Average serum concentration		.062	.047	.047	.031	.012
Aluminum hydroxide	16	.019	.019	.019	.039	.039
	17	.019	.019	.039	.019	.019
	18	.039	Trace	Neg.	Neg.	Neg.
	19	.156	.078	.078	.019	.019
	20	.078	.078	.039	Trace	Trace
Average serum concentration		.062	.039	.035	.015	.015
Magnesium trisilicate	21	.078	.016	Trace	Trace	Trace
	22	Trace	Trace	Trace	Neg.	Neg.
	23	.156	Trace	.019	Trace	Neg.
	24	Trace	.019	.039	.019	Trace
	25	.312	.156	.019	Neg.	Neg.
Average serum concentration		.109	.038	.015	.004	...
Magnesium hydroxide	26	.019	Trace	Trace	Trace	Trace
	27	Trace	Trace	Trace	Neg.	Neg.
	28	Trace	Trace	Trace	Trace	Trace
	29	.019	Trace	Trace	Trace	Trace
	30	.039	Trace	Trace	Trace	Neg.
Average serum concentration		.015	...	...	...	...
No antacid (Penicillin only)	31	.078	.078	.019	.019	.019
	32	.039	.019	Trace	Trace	Trace
	33	.156	.156	.078	.019	Trace
	34	.039	.156	.156	.078	.078
	35	.156	.156	.039	Trace	Trace
Average serum concentration		.094	.113	.058	.023	.019

All penicillin-antacid combinations were mixed in the dry state and administered in gelatin capsules with a glassful of water to fasting human subjects. The dose of penicillin used was 100,000 units calcium penicillin combined with 2 grams of the antacid being studied. This dose of antacid was arbitrarily selected as an average therapeutic dose and one which could be conveniently administered in tablet or capsule form. Each penicillin antacid combination was administered to a group of five subjects. The same dose of penicillin with no antacid was also administered to a control group. Blood samples were drawn at intervals up to four hours after administration and the serum was assayed for penicillin by the method of Rammelkamp.[10]

COMMENTS

The average serum levels following the administration of 100,000 units calcium penicillin with 2 grams of calcium carbonate are approximately twice as high at all test periods as the average serum levels following the administration of 100,000 units of calcium penicillin alone. The average serum levels obtained with the penicillin-sodium citrate combination are also generally higher than those obtained by the administration of penicillin alone, which is in accord with the work of Gyorgy.[7] The average serum levels obtained following the administration of penicillin with dry sodium phosphate buffer, aluminum hydroxide, magnesium trisilicate or magnesium hydroxide are all inferior to those obtained by the administration of penicillin alone. Therefore, the latter compounds in the dry form do not appear to be indicated as an aid to the oral administration of penicillin.

Calcium carbonate is classified as a non-systemic

[8] M. E. Florey and H. W. Florey, *Lancet*, 1: 387, 1943.

[9] F. G. Burke *et al.*, *Jour. Am. Med. Asn.*, 128: 83, 1945.

[10] C. H. Rammelkamp, *Proc. Soc. Exp. Biol. and Med.*, 51: 95, 1942.

antacid because of the insoluble and non-absorbed calcium compounds reformed in the intestinal tract after the passage of the chloride from the stomach.[11] It may therefore prove to be a safer antacid to administer repeatedly with penicillin than a soluble buffer salt such as sodium citrate, which is a systemic antacid and can lead to alkalosis. Dosage schedules for the maintenance of therapeutic blood levels by the oral administration of penicillin with calcium carbonate are being investigated.

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SYNTHESIS OF A COMPOUND IDENTICAL WITH THE L. CASEI FACTOR ISOLATED FROM LIVER[1]

PREVIOUS work has indicated the existence of a new growth factor(s) essential for the growth of *Lactobacillus casei* and *S. faecalis* R and necessary for growth and hemoglobin formation in the chick. These fractions or compounds have been variously designated as the norite eluate factor,[2,3] folic acid,[4] vitamin Bc,[5] *L. casei* factor from liver[6] and *L. casei* factor from a fermentation residue.[7] We wish to report the synthesis of a compound which is identical with the *L. casei* factor isolated from liver. The synthetic compound is active for *L. casei*, *S. faecalis* R and is effective in promoting growth and hemoglobin formation in the chick.

The identity of the synthetic compound and the *L. casei* factor isolated from liver is based on the following observations. The ultraviolet absorption spectra of the synthetic and natural compounds are identical. The $E^{1\%}_{1\text{ cm}}$ values for the two compounds are shown in Table 1.

The infra-red spectra of the synthetic and natural compound were determined and compared by Dr. R. C. Gore, of the Stamford Research Laboratories, American Cyanamid Company. The per cent. transmission of the two compounds is given in Fig. 1. Dr. Gore states, "With correspondence in absorption exhibited at so many frequencies the probability is extremely high that the two molecules are identical."

TABLE 1

ULTRAVIOLET ABSORPTION SPECTRA OF NATURAL AND SYNTHETIC L. CASEI FACTOR

Solvent		mμ	*L. casei* factor from liver $E^{1\%}_{1\text{ cm}}$	Synthetic *L. casei* factor $E^{1\%}_{1\text{ cm}}$
0.1 N NaOH	Minima	235	287	290
	Maxima	256	565	570
	Minima	268	485	495
	Maxima	283	550	560
	Minima	332	133	135
	Maxima	365	195	199
0.1 N HCl	Minima	262	253	265
	Maxima	296	440	445

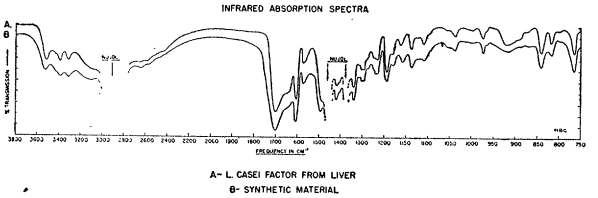

FIG. 1. Infrared absorption spectra of natural and synthetic *L. casei* factors.

Microscopical examination of the natural and synthetic compound was performed by Dr. A. F. Kirkpatrick, of the Stamford Research Laboratories, American Cyanamid Company. Dr. Kirkpatrick reported:

> The compounds which were crystallized as the free acids formed thin lenticular crystals, exhibiting birefringence and parallel extinction. The refractive index for light vibrating parallel to the length of the crystals was 1.559 ± 0.003 for the natural compound and 1.559 ± 0.003 for the synthetic compound; the refractive index for light vibrating parallel to the width was 1.744 ± 0.003 for the natural product and 1.744 ± 0.003 for the synthetic compound.

The natural and synthetic compounds were equally active when assayed by *L. casei* or *S. faecalis* R. The amount required per ml of medium for half-maximum growth of *L. casei* was 0.00007 micrograms for the natural and 0.00007 micrograms for the synthetic compound. For *S. faecalis* R the amount required per ml for half-maximum growth was 0.0003 micrograms for the natural and 0.0003 micrograms for the synthetic compound. The amounts required for half-maximum growth are slightly greater than previously reported, but the amount required to produce half-maximum growth varies somewhat with different experiments.

[11] L. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," The Macmillan Company, 1941.

[1] The announcement of the synthesis of this compound and its availability for experimental use was made at Gibson Island, Maryland, July 18, 1945.

[2] E. E. Snell and W. H. Peterson, *Jour. Bact.*, 39: 273, 1940.

[3] B. L. Hutchings, N. Bohonos and W. H. Peterson, *Jour. Biol. Chem.*, 141: 521, 1941.

[4] H. K. Mitchell, E. E. Snell and R. J. Williams, *Jour. Am. Chem. Soc.*, 63: 2284, 1941.

[5] J. J. Pfiffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan and B. L. O'Dell, SCIENCE, 97: 404, 1943.

[6] E. L. R. Stokstad, *Jour. Biol. Chem.*, 149: 573, 1943.

[7] B. L. Hutchings, E. L. R. Stokstad, N. Bohonos and N. H. Slobodkin, SCIENCE, 99: 371, 1944.