TABLE I.
Growth Response of Rats to Riboflavin and Iso-riboflavin (Both compounds were dosed as a suspension in 0.5 ml gum acacia).

No.	Group	Gain in wt 28 days (Avg initial wt, 46 g) Period I g	Gain in wt Succeeding 21 days Period II* g
1.	Riboflavin deficient 0.5 ml gum acacia	15	
2.	2 mg Isoriboflavin	5	
3.	10 μg riboflavin	69	94
4.	10 μg riboflavin + 2 mg isoriboflavin	29	118
5.	40 μg riboflavin	96	
6.	40 μg riboflavin + 2 mg isoriboflavin	92	

* Riboflavin increased to 80 μg daily between periods I and II.

mg of nicotinamide, and 100 mg of choline chloride per 100 g of diet.

The riboflavin and isoriboflavin were fed by stomach tube as a suspension in gum acacia.

Isoriboflavin inhibited the growth of rats in the absence of riboflavin (Group 2, Table I, Period I) although the manifestations of deficiency (greasiness of the fur and alopecia) and the survival time were not significantly different between the animals in groups 1 and 2. The analog, in the dosage employed, markedly depressed the growth of the rats receiving a sub-optimal intake of the vitamin (Groups 3 and 4). The growth-depressing effect of isoriboflavin was almost completely overcome by the feeding of 40 μg of riboflavin (Groups 5 and 6).

Thus, it would appear that the effect of isoriboflavin was that of an inhibitor of riboflavin.

The counteraction of large doses of the vitamin upon the inhibiting effect of the analog was further shown in the animals of Groups 3 and 4. After 28 days on test the level of riboflavin was increased from 10 μg to 80 μg for these groups: the feeding of isoriboflavin to the rats in group 4 was continued at the level of 2 mg. As evidenced by the growth response (Table I, Period II), as well as by the general improvement in the condition of the animals, the inhibiting effects of isoriboflavin were overcome by the curative feeding of 80 μg of riboflavin.

Summary. The feeding of isoriboflavin to rats receiving a sub-optimal intake of riboflavin inhibited the growth-promoting action of the vitamin. The antagonistic effect of the isomer was prevented or overcome by the feeding of an adequate level of riboflavin.

14512

Influence of Bacteriologic Peptones on Hydrogen Sulfide, Indol and Acetyl Methyl Carbinol Production by Enterobacteriaceæ.

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The production of hydrogen sulfide (H_2S) by members of the family Enterobacteriaceæ is regarded by microbiologists as an important differential diagnostic criterion. In addition, the production of indol and acetyl methyl carbinol (A.M.C.) is used as a means of separating two significant genera, namely, the Aerobacter from the Escherichia. Bacteriologic peptones serve as sources of (a) sulfur compounds for H_2S formation, (b) tryptophane for indol formation, and (c) compounds possessing the guanidine nucleus necessary for the detection of A.M.C. That peptones vary between manufacturers is almost axiomatic.

These differences may be due to the variations in chemical composition which in turn are determined by (a) the substrate from which the peptones are prepared and (b) methods of preparation. It occurred to the writers that the brand and kind of peptone might, therefore, materially influence any or all of the above three diagnostic reactions.

Peptones recommended as good sulfur sources for the H_2S test seem to be a matter of choice. Levine[1] *et al.*, Hunter and Crecelius,[2] Utermohlen and Georgi[3] recommend Bacto proteose-peptone while ZoBell and Feltham[4] found Bacto tryptone to be quite satisfactory; the Society of American Bacteriologists' "Manual of Methods"[5] suggests any peptone containing available sulfur as being satisfactory. As for sources of tryptophane, Tilley[6] found that peptones varied considerably in their content of this amino acid; he studied relatively few brands. Recently, however, Hook and Fabian,[7] employing the Rosenheim test for tryptophane, obtained strong positive tests with all the commercial peptones investigated by them. The "Manual"[5] recommends casein digests such as Bacto tryptone. As for the presence of compounds possessing the guanidine structure, Barritt[8] found Bacto peptone to be satisfactory while the "Standard Methods"[9] and the S.A.B. "Manual"[5] demand the use of either Witte or Difco proteose peptone for the Voges-Proskauer test. It is apparent, therefore, that certain commercial peptones are superior to others.

Not only has the sulfur source been an important factor in the production of H_2S, but the selection of a suitable detector ion is likewise essential. A number of compounds have been reported to yield satisfactory results. Lead, in the form of the acetate, is the detector ion used almost exclusively and is the accepted one in the technic outlined in the S.A.B. "Manual."[5] Levine[1] successfully employed iron (ferric citrate) while ZoBell and Feltham[4] and others[2] have found bismuth (citrate) to be suitable detectors of sulfide production. The latter two elements have been reported to be superior to lead because of their lower toxicity toward bacteria. What effect different peptones may have on either the toxicity of Pb^{++}, Fe^{+++} or Bi^{+++} or upon detection of sulfides by the latter, has never been extensively determined. That differences do exist were noted[3] but were not thoroughly investigated. It is the purpose of this paper to determine those differences.

Materials. Twenty commercial bacteriologic peptones were employed. The names of the peptones and the manufacturers appear in Table I. Bacterial cultures employed for the H_2S tests were as follows (number in parentheses indicates number of strains used): *Aerobacter aerogenes* (2), *Aerobacter oxytocum* (1), *Eberthella typhosa* (2), *Proteus vulgaris* (2), *Salmonella typhimurium* (2), *Salmonella schottmuelleri* (2), *Salmonella pullorum* (2), *Shigella gallinarum* (2), *Shigella sonnei* (1), *Shigella dysenteriæ* (2), *Escherichia freundii* (1), *Streptococcus fæcalis* (1). For the indole and Voges-Proskauer tests, the following were used: *A. aerogenes* (7), *A. cloacæ* (1), *A. oxytocum* (1), *E. coli* (7). The Escherichia and Aerobacter were found to be "typical" in their biochemical reactions.

Methods. Media used for the detection of H_2S was prepared in accordance with the procedure recommended by (a) the S.A.B. "Manual"[5] with lead acetate, (b) Levine[1] with Fe as the indicator, and (c) Hunter *et al.*[2] with Bi as the detector. The only variable in each of these media was the pep-

[1] Levine, M., Vaughn, R., Epstein, S. S., and Anderson, D. O., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1022.

[2] Hunter, C. A., and Crecelius, H. G., *J. Bact.*, 1938, **35**, 185.

[3] Utermohlen, W. P., and Georgi, C. E., *J. Bact.*, 1940, **40**, 449.

[4] ZoBell, C. E., and Feltham, C. B., *J. Bact.*, 1934, **28**, 169.

[5] *Manual of Methods for Pure Culture Study of Bacteria*, Leaflet V, 1942, Society of American Bacteriologists, Geneva, N.Y.

[6] Tilley, F. W., *Am. J. Pub. Health*, 1921, **11**, 834.

[7] Hook, A. E., and Fabian, F. W., *Tech. Bull. No.* 185, 1943, Mich. Agr. Exp. Sta.

[8] Barritt, M. M., *J. Path. Bact.*, 1936, **42**, 441.

[9] *Standard Methods for the Examination of Water and Sewage*, 8th ed., 1936, Am. Pub. Health Assn., New York, N.Y.

TABLE I.
Comparison of Pb, Fe, and Bi as Indicators of H_2S Using 20 Peptones as Sulfur Sources and 20 Cultures Representative of the Enterobacteriaceæ.

Peptones			No. cultures yielding vigorous positive H_2S tests			No. cultures growing but failing to produce H_2S			No. cultures failing to grow in presence of following		
Name	Manufacturer	Code	Pb	Fe	Bi	Pb	Fe	Bi	Pb	Fe	Bi
Peptone	Pfanstiehl	P	9	6	6	10	20	4	0	2	9
Nutri Peptone	Wilson	W	12	0	16	1	17	0	0	0	0
Bacteriologic Peptone	Parke-Davis	PD	14	9	7	0	6	1	2	0	6
Peptone Siccum	Stearns	S	8	0	11	6	20	1	0	0	4
" "	Armour	AS	7	0	6	5	20	3	0	0	6
Peptic Digest Pork Muscle*	"	AP	9	3	16	8	9	0	0	0	4
Pancreatic Digest Beef Muscle*	"	AB	6	0	15	8	17	1	0	0	2
Pancreatic Digest Casein	"	AC	9	0	15	2	20	0	1	0	1
Infusion Peptone	"	AI	0	0	18	20	19	0	0	0	0
Bacteriological Peptone	Paul-Lewis	PL	19	6	11	0	10	1	0	0	5
Hog Protein Peptone	Cudahy	CHP	0	0	3	3	20	5	14	0	11
Hog Peptone	"	CH	8	3	9	6	12	2	0	0	3
Beef Peptone	"	CB	12	5	14	3	6	4	2	0	1
Bacto Tryptose	Difco	DT	11	5	16	6	11	0	2	0	1
Proteose Peptone	"	DI	10	3	16	4	6	0	1	0	2
Bacto Peptone	"	DP	11	0	9	2	16	3	5	1	5
Bacto Tryptone	"	DY	2	0	17	15	20	0	0	0	0
Neopeptone	"	DN	1	0	18	0	17	1	19	0	0
Proteose Peptone No. 3 + $NaHSO_3$	"	DPP	8	8	11	9	11	7	2	0	0
Witte's Peptone	Fr. Witte	WT	0	3		0	16		20	0	

* Experimental peptones.

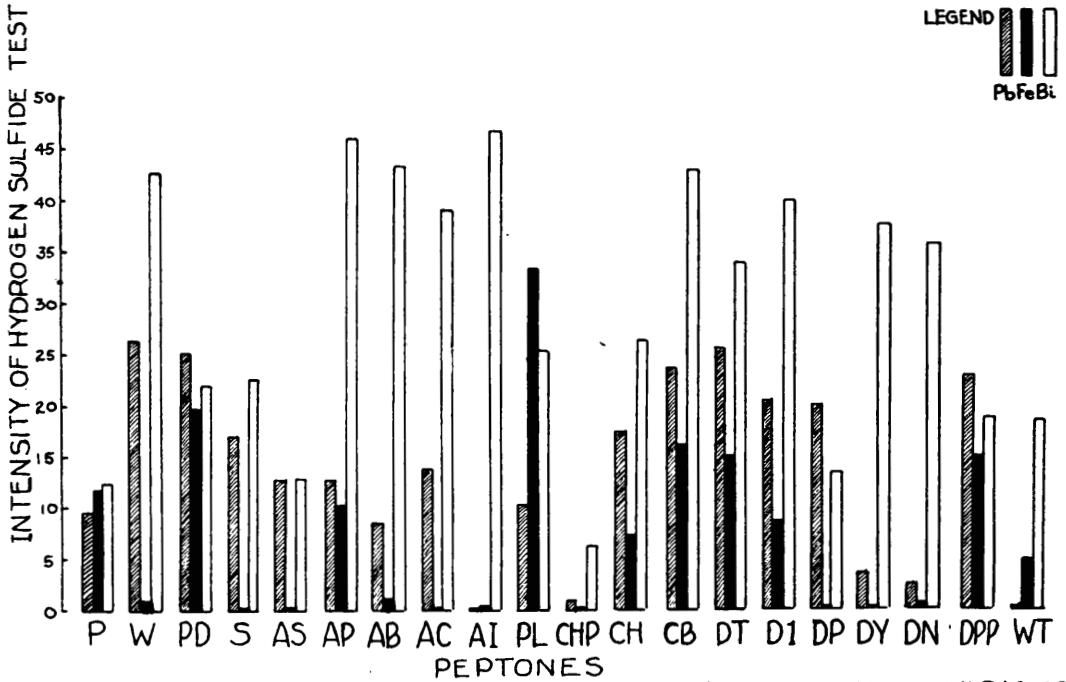
tone as the sulfur source.

The media used for the detection of indol and acetyl methyl carbinol production were both prepared as prescribed in the "Standard Methods."[9] The only variable in each medium again was the peptone which in the first test served as a source of tryptophane and in the second as a source of a guanidine-containing compound. The test for indol was Kovac's reagent[9] but A.M.C. was detected by the sensitive procedure outlined by Barritt.[8] Tests for the latter were conducted at 24- and 72-hour intervals.

Results. A. Hydrogen sulfide. From the data in Table I, it is apparent that many more positive responses to H_2S production were obtained when Bi was employed as the indicator ion. Ten peptones in particular were found to possess components which, when converted to sulfides by bacterial reduction, produced darkening along the line of inoculation. The best were W, AP, AB, AC, AI, DT, DI, DY, and DN. The intensity of the test for peptone CB was high (Fig. 1) but only 14 of the 20 cultures gave satisfactory results. There were some cultures which grew in the presence of Bi but failed to produce H_2S; these were considerably fewer than when either Fe or Pb were used as the detector ion (Column 3, Table I). Peptone WT formed a dark precipitate with Bi on autoclaving, making that combination useless. In general, Bi seemed to produce an unmistakable positive test.

Hydrogen sulfide detection by ferric iron was generally poor. A great number of cultures grew in the presence of Fe but failed to produce H_2S. Best results were obtained with peptones PD and DPP. In the presence of peptone DI, 3 cultures were good sulfide producers while 11 failed to yield this gas. Peptones PL, CB, and DT showed tests of good intensity, in terms of iron (Fig. 1), however only 25% of the cultures responded vigorously. In general, the iron sulfides were the weakest in intensity.

With 7 peptones (W, PD, PL, CB, DT, DI, and DP), 50% or more of the cultures yielded strongly positive tests when Pb was the detector. About 3 times as many cultures grew in the presence of Pb and did not produce H_2S than in the case of Bi. The intensity



HYDROGEN SULFIDE PRODUCTION BY BACTERIA GROWN ON VARIOUS PEPTONES EMPLOYING THREE DIFFERENT INDICATOR IONS

FIGURE 1

of the tests was not of the order of Bi but was superior to Fe. Peptones AI, DY, and DN are unusual in that very few positive tests were reported with Pb but many with Bi.

B. Growth. The growth of some bacterial cultures was completely inhibited by two of the elements depending upon the peptone used. Iron was toxic only 3 times in 400 trials. Lead and bismuth were about equal, *i.e.*, 68 per 400 and 60 per 380 trials respectively. Peptones CHP, DN, and WT accounted for 53 of the 68 lead inhibitions whereas with bismuth the distribution was quite even amongst the 20 sulfur sources.

C. Indol production. The results indicate that two peptones, S and CHP, failed completely to yield indol whereas one (P), is a questionable source of available tryptophane. Fourteen others were satisfactory and of these the most clear cut positive tests were produced in peptones DI, AS, AC, and WT, while W, PL, DT, and DY gave very good results based upon the intensity of the test and color of the reaction mixture.

Several of the peptones produced water-white aqueous solutions which made the cherry-red color reaction appear more pronounced than where it was partially masked by a yellow tinge frequently encountered in solutions of peptones.

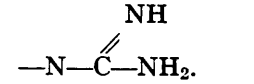
The Hopkins-Cole test for the detection of tryptophane was *positive* for 2 peptones (S, CHP) which failed to yield indol biologically. Obviously, the tryptophane is present but in combination with other constituents of the peptone which render it unavailable to the bacteria.

There is still another possible explanation as to the variation in the indol response, *i.e.*, the pH of the medium. Tilley[6] found a direct relation between hydrogen ion concentration and indol production; peptones whose aqueous solutions were near neutrality yielded positive results. Of the 4 peptones which gave the most striking positive results, solutions of 3 gave pH's near neutrality and one (AS) was acid. Peptones DI, AC, and WT were then acidified to pH 5.4 and AS brought to neutrality; following inoculation, tests were again made for indol, the results being in exact

agreement with those obtained on the same peptones before neutralization or acidification. Of 4 other peptones, which also gave good tests, 3 were nearly neutral and the fourth was acid. Hydrogen ion concentrations of aqueous solutions of the peptones yielding either variable results or wholly negative tests, were between pH 5.2 and 5.9. Neutralization, before inoculation, failed to improve the indol test. Of the remaining 7 peptones which gave moderately good indol tests, 4 were nearly neutral and 3 were acid.

It appears that there is a slight correlation between the indol response and the "natural" pH of the aqueous solution of a peptone. However, alteration in hydrogen ion concentration of peptones normally yielding good indol tests or peptones yielding poor tests failed to bring about a reversal in response to Kovac's reagent.

D. Acetyl methyl carbinol production. In the Voges-Proskauer test, the A.M.C. condenses with constituents of the peptone containing the guanidine nucleus to give a red color in the presence of alkali. The peptone is of value then, in this test, because it supplies compounds having the configuration



From Barritt's[10] investigations, it appears that the brand of peptone materially influences the *speed* with which the Voges-Proskauer test becomes readable. Some peptones permit the test to be detected in 24 to 48 hours while others can be read only at 72 hours. In our studies, tests which were positive or negative at 24 hours were the same at the end of 72 hours.

Seven peptones were found to be slightly superior to the remaining peptones used while 3 strains of *A. aerogenes* were variable in their activity. The 7 peptones are S, AS, AP, AB, AC, CB, and DT. Peptone PL was unsatisfactory. Barritt[10] found Witte's peptone (WT) to be wholly unsatisfactory.

No strains of *E. coli* were V-P positive. Hydrogen ion concentrations of the glucose-peptone waters after autoclaving ranged from pH 7.0 to 7.3.

Discussion. From the experimental data on hydrogen sulfide production, it becomes apparent that the ability of a peptone to render sulfur available to bacteria and to support their growth is correlated with the character of the indicator ion incorporated in the medium. The sensitivity of the detector ions to the H_2S test differs, viz., Bi>Pb>Fe. However, the order of toxicity is Pb>Bi>Fe. Perhaps the microbial enzyme which brings about the reduction of sulfur is inactivated in some bacteria to a greater extent by certain metallic ions than by others or possibly the indicator combines with the sulfur compound forming a complex rendering it unavailable.

The hydrogen ion concentration of aqueous solutions of peptones apparently influences the availability of tryptophane for indol production. Where the pH is favorable and indol is not produced by bacterial action, it is possible that the tryptophane may be chemically bound so as to be biologically unavailable although the Hopkins-Cole test was positive.

Compounds containing the guanidine nucleus, possibly arginine, must be abundantly distributed in commercial peptones for in no instance did this appear to be a limiting factor.

Summary. Hydrogen sulfide production by bacteria varied greatly with the indicator ion employed; bismuth was superior, as a detector, to iron and lead. Iron was the least toxic. Occasionally the tryptophane content but rarely the guanidine configuration serve as limiting factors in peptones used for differential bacterial tests.

[10] Barritt, M. M., *J. Hyg.*, 1943, **43**, 214.

14513 P

Use of B-Proteus OX19 Agglutination in Diagnosis of Cancer.

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In a recent study the sera of pregnant women uniformly were found to contain a high titer of agglutinins for B-proteus OX19. In the course of that study[1] it appeared that the sera of women with malignant neoplastic disorders likewise strongly agglutinated proteus organisms. Even though the reaction was non-specific, yet it was believed that the agglutination test often might provide evidence for the existence of cancer.

For this reason the titer of B-proteus OX19 agglutinins* was measured[2,3] in the sera of 30 normal women, 27 with mammary carcinoma, and 3 with carcinoma of the cervix. The diagnosis of carcinoma in each instance was confirmed by microscopic examination of biopsied sections.

The results of this study failed to indicate any difference between the sera of the two groups (Table I). Of the 30 sera taken from persons having cancer, 14 gave positive agglutinations with B-proteus OX19, the titer ranging from 1:20 to 1:160 (only one serum agglutinated as high as 1:160). Of the control group, 21 sera showed positive reactions, the titers ranging from 1:20 to 1:80.

Conclusion. By the methods used, there was no evidence that the agglutination of B-proteus OX19 antigen would be of value in determining the presence of cancer.

TABLE I.
The Agglutination Titer of the Sera Studied for B-Proteus OX19.

	Titers				
	0	1:20	1:40	1:80	1:160
Control individuals	9	7	9	5	0
Patients with mammary or cervical carcinoma	16	6	5	2	1

1 Gratch, I., *Am. J. Surg.*, 1943, **60**, 411.
* Lederle Laboratory antigen.
2 Welch, H., *Am. J. Pub. Health*, 1937, **27**, 141 (Yearbook).
3 Welch, H., and Mickle, F. L., *Am. J. Pub. Health*, 1936, **26**, 248.

14514

Effect of Cooling Isolated Parts upon the Comfort of Man Resting in Hot Humid Environment.*

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Hill[1] and Kuno and Ikeuchi[2] inhibited sweating in human subjects by cooling isolated portions of the body. They made no attempt to correlate the temperature of the bath cooling the part, the temperature and humidity of the environment, and the size of the area cooled, with the quantity of sweat and the comfort of the subjects. Nor were attempts made to evaluate the efficiency of

* Aided by a grant in aid from the Rockefeller Foundation.

1 Hill, L., *J. Physiol.*, 1921, **54**, 137.
2 Kuno, G., and Ikeuchi, K., *J. Orient. Med.*, 1928, **9**, 53.