THE REDUCING PROPERTIES OF MICROÖRGANISMS WITH SPECIAL REFERENCE TO SELENIUM COMPOUNDS[1]

VICTOR E. LEVINE

Department of Biological Chemistry and Nutrition, School of Medicine, Creighton University, Omaha, Nebraska

Received for publication July 23, 1924

The reducing power possessed by bacteria was a characteristic early recognized. In 1843 Helmholtz observed in putrefactive mixtures the reduction of litmus to its leuco base. The first observers to make direct researches in reference to the reducing power of bacteria were, however, Cahen, Spina, and Rozahegyi. Later came the work of Petruscky, Würtz, Behring, Smith, Müller, Uffelmann, Grasser, Marpmann and Rothberger, all of whom used dyes as indicators. Besides dyes other organic compounds may undergo reduction. Amino acids are reduced by anaerobes with the formation of saturated fatty acid and ammonia. Gayon and Dubourge observed the conversion of fructose into mannitol, Labbé, of oxyhemoglobin into hemoglobin, and Alsberg, of oxyhemocyanin into hemocyanin. The formation in human feces of coprosterol from cholesterol and of stercorcin from bilirubin is believed to be the result of bacterial reduction.

Inorganic compounds also suffer reduction. The reduction of ammonium molybdate has been reported by Capaldi and Proskauer and also by Levine and Jahr, and ferric compounds have been transformed into ferrous compounds by Poehl. That nitrates in the soil are capable of being converted to nitrites was first observed in 1862 by Goppelsröder. Meusel attributed this

[1] This work was partly done in the biochemical laboratory of the College of Physicians and Surgeons, Columbia University, and partly at the Museum of Natural History, New York City.

phenomenon to bacterial action. Sulfates are reduced to hydrogen sulfid, according to Stockvis and Saltet, Kochmann, Van Delden and others. Not only sulfates but also sulfites, tetrathionates, pentathionates, sulfur in organic complexes such as peptone, cystine and thiourea yield hydrogen sulfid. Free sulfur is also converted into hydrogen sulfid, but selenium, tellurium, arsenic and antimony do not yield the corresponding hydrogen compounds.

Bacteria are also capable of carrying on oxidations. The existence of autotrophic microörganisms, capable of deriving energy through the oxidation of purely inorganic substances has become an established fact. The nitrate and nitrite bacteria, the sulfur bacteria and the iron bacteria are examples of such types. Recently Lipman and Waksman have added another group of organisms to the already known autotrophic forms. These organisms secure energy from the oxidation of selenium. Lipman and Waksman mixed a small quantity of selenium with the soil and observed an increase in the acidity of the latter. They transferred some of this soil to a culture medium consisting of inorganic materials with elemental selenium as the only source of energy. The medium became cloudy within a few days and there appeared in it a minute rod-shaped bacterium capable of oxidizing selenium to selenic acid, just as the sulfur bacterium is capable of oxidizing elemental sulfur to sulfuric acid.

Whether selenium or tellurium could replace sulfur in the metabolism of sulfur bacteria was the object of an investigation of W. Brenner. One species, which he isolated from marine mud, *Micrococcus selenicus*, made good growth in sodium selenid without sodium selenite. The latter could be replaced by other substances such as sodium selenate, sodium thiosulfate, indigocarmin and litmus but not by potassium tellurite, potassium sulfate, potassium nitrate or atmospheric oxygen. Another species isolated from marine mud grew well on a substance containing sodium selenite, but a third species, *Thiobacillus thioparus*, gave entirely negative results. Free selenium was found in the bacterial cell when grown on selenite medium. An odor

similar to that emanating from molds, when grown with sodium selenite was observed in the presence of sodium selenid, but not in the presence of sodium selenite alone.

That bacteria reduced certain compounds of selenium was first mentioned by Japha in 1842. To quote from his dissertation, "*Bacillus ferreus* urinae immissus post aliquod tempus colorae fusco rubro tinctus est, paulatimque selenium purum floccis rubiis in illo praecipitatum." He utilized the reducing power of *Bacillus ferreus* to determine the presence of selenium dioxid. Not finding any reduced selenium by this biochemical method as well as by several purely chemical procedures, he concluded that the selenium was not eliminated in the urine.

In 1890 Chabrié and Lapicque performed a single experiment which showed that a 0.2 per cent solution of selenious acid prevented the putrefaction of bouillon. In smaller concentration, however, putrefaction did occur. At the same time the selenious acid was reduced and colonies, which alone were colored red, were observed in the liquid. These authors attributed the reduction of the selenium compound to the activity of the microorganisms in the bouillon.

In 1900 Scheuerlen grew cultures of *Bacillus anthracis* on a medium containing either sodium selenite or sodium tellurite. These he added to the medium because of a desire to grow the bacillus in pure culture and in the absence of atmospheric oxygen, which might bring with it contaminating organisms. He had hopes that this sodium or tellurium salt, which is easily reduced, would act similarly to the loosely bound oxygen in oxyhemoglobin and would be almost as favorable to the growth of the anthrax bacillus as blood itself. He found, however, that although these salts were reduced to red selenium or dark gray tellurium, growth nevertheless suffered retardation. Continuing his observations, he found that not only *Bacillus anthracis* but also all of the growing bacteria he examined were colored in the presence of sodium selenite or tellurite. Pigmentation occurred in the bacterial cell, but the nutrient medium itself was free from color.

The results of Scheuerlen led directly to the more extensive

work of his pupil, Klett, who made a study of twenty-seven species of bacteria and molds under the influence of sodium tellurite, sodium selenite and sodium selenate. He also reported that the tellurite and the selenite were reduced by living organisms. On agar streak cultures, colonies grew in gray-black streaks in the presence of the tellurium compound. Reduced selenium accumulated for the most part in the water of condensation.

In general Klett found that sodium selenite or tellurite did not favor the growth of bacteria and that the intensity of the reduction was proportional to the intensity of growth. These two salts had no effect on the virulence of bacteria. Aerobic bacteria grown under anaerobic conditions could not thrive on the oxygen set free in the course of the reduction of sodium selenite. Klett also observed that, when the nutrient medium contained glucose, reduction took place at 37°C. even in the control tubes. He was obliged therefore to allow growth in such media to proceed at room temperature.

Klett believed that reduction took place within but not without the bacterial cell. He observed that reduction followed the line of growth, the red or gray color not being diffused throughout the medium. A microscopic examination of bacterial bodies revealed the presence of yellowish or grayish particles within the cell. Sodium selenate, like sodium sulfate or sodium phosphate showed no inhibition of growth, nor was there any evidence of reduction of the selenate to brick red selenium.

Gosio in 1904 took up the problem of the bacterial reduction of selenium and tellurium salts in the hope of obtaining a method for determining the sterility of sera, culture media, body fluids, foods and other biological substances. With this end in view he examined 181 organisms grown upon selenite or tellurite media. He came to the conclusion that sodium selenite was more easily reduced than the tellurite by substances other than bacteria. He therefore preferred the tellurite and employed this salt for most of his experiments. King and Davis have recently confirmed Gosio in proving the value of tellurite medium as an indicator of microbial life.

According to Gosio tellurite reduction was accelerated in a milk medium or in a medium containing glucose, sucrose or lactose. Glucose seemed to be the best accelerator. He attempted to explain these findings on the basis of the supposition that lactic acid was produced, which, interacting with the potassium tellurite gave rise to the more easily decomposed tellurous acid. The limit of sensitiveness in a sugar-free medium was 1:20,000, whereas in a sugar medium, reduction was noticeable when the latter contained one part of the tellurite in 75,000, or even 100,000 parts of the nutrient substance. Levine has found that at 30°C. to 37.5°C. or over and under sterile conditions, glucose, lactose, and lactic acid themselves cause reduction of sodium selenite. The accelerated reduction, noted by Gosio, was really due to action of these reducing substances in the nutrient medium.

Gloger in 1906 repeated some of the work of Gosio. He agreed with the latter that the presence of glucose accelerated reduction and stated that the kind of medium used was a very important factor in determining bacterial reduction. He, however, did not make any effort to determine what chemical substances present in the nutrient medium would interfere. He formulated a relationship between the production of hydrogen sulfid and the reduction of potassium tellurite. Finding that *B. tuberculosis-hominis, B. pseudotuberculosis* Pfeiffer, *B. acidi-lactici, Spirillum rubrum, B. diphtheriae* and *B. pseudodiphtheriae* did not reduce, he put forth the proposition that these organisms were not active because they evolved only traces of hydrogen sulfid or none at all, the hydrogen sulfid itself having the power of causing the reduction of selenite or tellurite. W. Kruse in his "Allegemeine Mikrobiologie" published in 1910 stated that anaerobes did not reduce, and that small amounts of selenite or tellurite impeded their growth, while small quantities of sulfite acted favorably. He also mentioned the fact that *B. tuberculosis-hominis, B. pseudotuberculosis, B. diphtheriae, B. pseudodiphtheriae,* and *B. acidi-lactici* did not reduce. He, too, gave hydrogen sulfid as the cause of reduction.

In the work here reported the effect of various selenium com-

pounds upon the growth and upon the reducing power of some of the important groups of bacteria was studied. Experiments were also undertaken to prove or disprove the statement that anaerobes did not reduce, and to ascertain the validity of Gloger's conclusion that hydrogen sulfid was the cause of reduction. Still other experiments were carried out to determine the nature of bacterial reductase. The organisms studied were obtained from Prof. C.-E. A. Winslow's valuable collection in the American Museum of Natural History, New York City.

EFFECT OF SELENIUM COMPOUNDS UPON THE GROWTH OF BACTERIA

The effect on growth was studied with a view towards discovering any relation between growth and reduction. Selenium dioxid, selenic acid, sodium selenite, sodium selenate, and potassium selenocyanid were the compounds employed. Colloidal selenium was not included, but it has recently been shown by Henry Crooke that it has no germicidal action.

The organisms tested were *Bacillus coli-communis* and *Streptococcus pyogenes-aureus*. The medium used was liquid broth, acid in reaction (pH 6.8). The nutrient media were made up with increasing amounts of the selenium salts or acids. The concentrations employed for sodium selenate were 0.15, 0.28, 0.40, 0.50, 0.60, 0.68, 0.77, 0.83, 0.90, 0.96 per cent. These figures are on the basis of crystalline sodium selenate ($Na_2SeO_4 \cdot 10H_2O$). For selenium dioxid (SeO_2), selenic acid (H_2SeO_4), sodium selenite (Na_2SeO_3) and potassium selenocyanid (KCNSe) lower dilutions were used: 0.06, 0.11, 0.16, 0.20, 0.24, 0.27, 0.30, 0.33, 0.36, and 0.38 per cent. The tubes were examined after twenty-four, forty-eight and seventy-two hours.

Briefly stated, the results were as follows: At the end of twenty-four hours only the tubes of the lowest concentration showed growth but after seventy-two hours all of the tubes gave evidence of growth. *Streptococcus pyogenes-aureus* proved more sensitive to the selenium compounds tested than did *Bacillus coli*. Thus at the end of the first day *B. coli* showed growth in the tubes containing 0.15 to 0.95 per cent sodium selenate, while

the streptococcus gave growth in the 0.15, 0.28 and 0.40 per cent tubes only. In degree of toxicity the compounds may be arranged in the following series of diminishing effect: selenium dioxid (selenious acid), selenic acid, sodium selenite, sodium selenate, potassium selenocyanid. This order agrees well with that found by Levine in a study of the toxic effect of selenium compounds upon yeast, plants and animals.

Selenium dioxid and selenic acid in concentrations as low as 0.06 per cent exercised a marked inhibitory effect. Sodium selenite did not retard growth as much as selenious acid. Sodium selenate and potassium selenocyanid in the very low concentrations had slight effect on the growth of the organisms tested. It is interesting to mention that Němec and Kás found that extremely small amounts of sodium selenate increase the development of certain types of molds, even in the presence in the culture medium of zinc and manganese compounds.

A very interesting feature of the experiments was the formation in cultures containing selenious acid, sodium selenite, and selenic acid of free brick-red selenium, which in part precipitated and in part suffered colloidal dispersion in the liquid medium. Reduction took place only where growth occurred and the profuseness of reduction paralleled that of growth. Selenic acid was reduced more slowly than selenious acid. This fact seems to indicate that reduction takes place in two stages. At first the selenic acid is reduced to selenious acid and this is further reduced to free selenium.

Sodium selenate was not reduced to free selenium. The formation from potassium selenocyanid of free selenium only occurred as a result of the production by the organism of acid metabolites. The formation of free selenium from this compound cannot be looked upon as a vital phenomenon. Levine and Ahana have shown that acid solutions have the power to decompose it with the formation of free selenium. The reaction depends upon the hydrogen ion concentration. They have proposed the use of potassium selenocyanid as a qualitative test for free acidity in the gastric contents and have found it as efficient as Töpfer's reagent. While growth after

TABLE 1

	SELENIUM COMPOUNDS	STRENGTH OF SOLUTION	ORGANISM	REMARKS
1	Selenium dioxid.....	1:4,000	*B. coli*	At the end of twenty-four hours, colonies only pinkish. The rest of medium unchanged in color. Number of colonies not so many as in the control plates. Control containing selenium dioxid, but no bacteria, showed no evidence of reduction. When again examined at the end of six days the pink-red spots were larger in diameter and brick-red in tint
2	Selenium dioxid.....	1:2,000	*B. coli*	Slight growth, and slight reduction. When examined again at the end of six days profuse pigmentation of the colonies noticed. There seemed to be greater reduction than in plate 1 and also better growth
3	Selenium dioxid.....	1:4,000	*Strep. pyog.*	No microscopic evidence of bacteria at end of first day. Slight pigmentation of the colonies observed after six days
4	Selenium dioxid.....	1:2,000	*Strep. pyog.*	No growth and no reduction. At the end of six days fewer colonies and slighter reduction than in plate 3
5	Selenic acid.....	1:4,000	*B. coli*	Pink colonies at the end of first day. Colonies more in number than in plate 7. At the end of six days localized reduction profused. Good growth
6	Selenic acid.....	1:2,000	*B. coli*	Few pink colonies. At the end of six days reduction profuse and growth good
7	Selenic acid.....	1:4,000	*Strep. pyog.*	Few pink colonies at end of first day. At end of a few days number of colonies slight and reduction therefore not profuse
8	Selenic acid.....	1:2,000	*Strep. pyog.*	At the end of first day no growth and no reduction. At end of six days very few slightly pigmented colonies

9	Sodium selenite.....	1:2,000	*B. coli*	Growth less than in control. Few pink colonies. At end of six days profuse pigmentation of colonies
10	Sodium selenite.....	1:1,000	*B. coli*	Growth less than in plate 9. Reduction also less marked. At the end of six days more marked reduction.
11	Sodium selenite.....	1:2,000	*Strep. pyog.*	Growth slight. Small pink-red colonies noticeable. At the end of six days very marked reduction
12	Sodium selenite.....	1:1,000	*Strep. pyog.*	Growth very slight. Very few pink-red colonies. Profuse localized reduction at end of six days
13	Sodium selenate.....	1:1,000	*B. coli*	Growth as good as in control. No reduction observed even at the end of six days
14	Sodium selenate.....	1:500	*B. coli*	Growth as good as in control. No reduction to be found after six days
15	Sodium selenate.....	1:1,000	*Strep. pyog.*	Growth as good as in control. No reduction
16	Sodium selenate.....	1:500	*Strep. pyog.*	Growth as good as in control. No reduction
17	Potassium selenocyanid.....	1:2,000	*B. coli*	Excellent growth as good as in control but no pigmentation. At end of six days colonies pigmented
18	Potassium selenocyanid.....	1:1,000	*B. coli*	Growth but no reduction. When examined at end of six days pinkish-red colonies observed
19	Potassium selenocyanid.....	1:2,000	*Strep. pyog.*	Growth but no reduction. At the end of six days colonies reduced
20	Potassium selenocyanid.....	1:1,000	*Strep. pyog.*	Growth not as good as in control. Colonies pigmented. More abundant pigmentation at the end of six days

the first day occurred in all the *B. coli* and *streptococcus* tubes, none showed reddening. On the second day as acidity developed some tubes showed reddening and the number of tubes giving free selenium increased on the third day.

LOCALIZATION OF SELENIUM IN THE CULTURE MEDIUM

In order to gain a better idea as to the localization of the red deposits it was thought advisable to employ a solid nutrient medium such as peptone agar. All the solid media used in this series of experiments and in experiments following were rendered sugar-free by inoculation with *B. coli* and subsequent sterilization. To each petri dish were added peptone agar, 0.05 cc. of broth containing a heavy growth of *Bacillus coli* or *Streptococcus pyogenes*, and also a definite amount of the selenium compound. The total volume was made up to ten cubic centimeters. The culture plates were then kept in the incubator at 37.5°C.

The results are tabulated in table 1.

It is seen from these experiments that reduction followed the path of growth. Colonies alone were colored brick-red. The barren regions of the medium remained unchanged in color.

Tellurium dioxid in concentrations of 1:10,000 was also reduced by *B. coli*. The colonies alone showed up in grayish-white circles, in the center of which dark gray metallic tellurium spots were especially noticeable.

IS BACTERIAL REDUCTION CAUSED BY HYDROGEN SULFID?

Desiring to confirm the statement made by Gloger that only organisms that produce sulfuretted hydrogen were able to cause reduction, the author repeated Gloger's experiments using, however, selenium dioxid and sodium selenite instead of potassium tellurite.

On growing the organisms used by Gloger, *B. acidi-lactici*, *B. diphtheriae*, *B. pseudodiphtheriae*, in a sugar-free medium to which had been added five or six drops of 1 per cent solution of neutralized sodium selenite, faint reduction was found to have occurred at the end of twenty-four hours along the path of the

stab of the cultures of *B. pseudodiphtheriae*. Cultures of *B. coli*, serving as controls, showed remarkable profuseness of reduction. At the end of two weeks, pigmentation was not found in the *B. diphtheriae* and *B. acidi-lactici* cultures, although control cultures, which had indicated reduction at the end of one day, showed greater reduction at the end of the fortnight.

Believing that reduction did not take place because of the high concentration of sodium selenite, the experiments with *B. acidi-lactici* and *B. diphtheriae* were repeated. This time only two drops or 0.1 cc. of a 1 per cent solution of selenium dioxid or 0.1 cc. of a 0.2 per cent solution of sodium selenite were used. At the end of twenty-four hours the two organisms showed good reduction along the line of growth, in both the selenium dioxid and in the sodium selenite media.

Tabulating the results of different workers, we find the following condition in respect to Gloger's statement:

NAME OF ORGANISM	H_2S PRODUCTION	GOSIO 1905— Na_2TeO_3	GLOGER 1906— K_2TeO_3	LEVINE 1913— Na_2SeO_3	KLIGLER 1913— SeO_2	BELFANTI 1914— Na_2TeO_3
B. tuberculosis-hominis....		Faint reduction	Negative	Reduction		Reduction
B. tuberculosis-avium... ..		Faint reduction	Negative			Reduction
B. diphtheriae.....	Traces	Faint reduction	Negative	Reduction	Reduction	
B. pseudotuberculosis (Pfeiffer).....		No reduction	Negative			
B. pseudodiphtheriae.....	Traces	Faint reduction	Negative	Reduction	Reduction	
B. acidi-lactici.....		Strong reduction	Negative	Strong reduction		
Spirillum rubrum.....		Not tried	Negative	Not tried		

Kligler, in looking for a rapid and convenient method of differentiating *B. diphtheriae* from *B. pseudodiphtheriae*, attempted to grow these organisms on media containing one part of selenium dioxid in fifty thousand. He found reduction in both organisms. When grown on slant cultures, those organisms on the surface of the slants were usually unpigmented, but reductions were always observed within the medium, the reduced selenium accumulating as a rule in the water of condensation.

REDUCTION OF ANAEROBES

Klett found that sodium selenite so markedly arrested the growth of the anaerobes, of malignant edema and symptomatic anthrax, that no reduction was observed. They, however, reduced sodium tellurite and in the presence of this salt growth continued vigorously. Gosio in his first paper dealing with selenite and tellurite reduction found that the bacilli of oedema, tetanus and symptomatic anthrax hardly gave any growth in his special media and manifested no reducing power. He used very high concentrations, a few drops of a 20 per cent solution in each tube. W. Kruse in his text book stated that while selenites and tellurites are reduced by aerobic bacteria no such action can be induced by anaerobic cultures. It seems important to ascertain these points, for if selenium is to be used as an index of bacterial contamination, growth of anaerobes would pass unnoticed.

In order to put these facts to the test, there were made some preliminary experiments with the organisms of symptomatic anthrax, oedema and tetanus. It was found that on the addition of five or six drops of 2 per cent sodium selenite to a sugar-free medium no growth was obtained. It seems that the concentration of selenite was too great and the experiment was therefore repeated, using 0.15 cc. of a 1 per cent sodium selenite. Other experiments were tried with 0.15 cc. of 1 per cent potassium selenocyanid and 0.1 cc. of a 0.5 per cent selenium dioxid. At the end of twenty-four hours the potassium selenocyanid did not inhibit the growth of these organisms, and gas formation had taken place in the tetanus tube. No red pigmentation was

TABLE 2

Reduction of sodium selenite: anaerobes

NAME OF ORGANISM	CONCENTRATIONS											
	1:100,000			1:50,000			1:25,000			1:10,000		
	Reduction at end of			Reduction at end of			Reduction at end of			Reduction at end of		
	24 hours	48 hours	96 hours	24 hours	48 hours	96 hours	24 hours	48 hours	96 hours	24 hours	48 hours	96 hours
B. welchii, 521*	+	−	−	+	+	+	+	+	+	+	++	++
B. welchii, 500	+	+	−	+	+	+	+	+	+	++	++	++
B. welchii, 20	+	−	−	+	+	+	+	+	+	++	++	++
B. welchii, 13	+	+	−	+	++	++	+	+	+	−	−	−
B. feseri, 48	−	−	−	+	+	+	−	+	+	+	+	+
B. feseri, 53	+	−	−	+	+	+	−	+	+	+	+	++
B. tetani, 274	+	−	−	+	+	+	−	++	+	+	+	+
B. tetani, 1	+	−	−	+	+	++	+	+	+	−	−	−
B. sporogenes, 120	+	−	−	+	+	+	+	+	+	++	++	++
B. sporogenes, 138	+	−	−	+	+	+	++	++	+	++	++	++
B. sporogenes, 425	−	−	−	+	+	+	+	+	+	++	++	++
B. oedematis, 421	+	−	−	+	++	++	−	+	+	−	−	−
B. oedematis maligni, 558	−	+	+	+	+	++	+	+	+	−	−	−
B. oedematis maligni, 485	+	−	−	+	+	++	−	+	+	−	+	+
B. botulinus, 595	+	−	−	+	+	+	+	+	+	+	+	+
B. putrificus, 459	−	+	−	+	+	+	−	++	+	−	−	−

* The number after the name of the bacillus represents the number of the organism in the bacteriological collection at the Museum of Natural History.

noted in any of the selenocyanid tubes. In the oedema culture containing sodium selenite and in the one containing selenium dioxid there was very slight growth. In both tubes reduction alone the line of meager growth had taken place. In the tetanus tubes there was no growth and no reduction in the selenium dioxid or in the sodium selenite medium. Selenium dioxid and sodium selenite tubes inoculated with symptomatic anthrax displayed no growth and consequently no reduction.

At the end of the second day the reduction observed with the oedema bacillus was more profuse. There was greater growth in the sodium selenite tube. With the tetanus organism, reduction was noticeable in the selenium dioxid tube after two days, but no growth and no reduction in the sodium selenite tube. After the fifth day slight reduction was observed in the sodium selenite tube inoculated with symptomatic anthrax. After eight days the selenocyanid showed no free selenium formation.

It was considered probable that the selenocyanid in greater amounts would form brick-red selenium along the path of growth. *B. coli,* and the bacilli of symptomatic anthrax, oedema and tetanus were grown in a potassium selenocyanid medium containing 1 per cent lactose and also in a sugar-free medium. To each tube was added either 0.5 or 1 cc. of the selenocyanid. It was noted that the presence of these relatively large amounts of potassium selenocyanid caused a diminution in the growth of the anaerobic organisms and in the volume of the gas liberated. There occurred less growth in the tubes containing the larger amounts of the salt. No pigmentation, however, was observed at the end of the week, either in the sugar-free or in the sugar-containing medium; *B. coli,* however, showed reduction along the stab in the lactose medium containing 1 cc. of potassium selenocyanid. A sugar-free medium inoculated with *B. coli,* gave evidence of reduction at the end of three days. Evidently the presence of lactose favored acid production by *B. coli* and therefore acid decomposition of the selenium compound. When the tubes were allowed to stand the selenium tint diffused gradually through the medium.

A more systematic study of the anaerobes was determined

upon. Having found that the concentration of the selenium compounds was an important factor in growth and in reduction, sodium selenite was added to the medium employed in the following concentrations: 1:100,000; 1:50,000; 1:25,000; and 1:10,000.

Fifteen anaerobes, including *B. Welchii*, *B. tetani*, *B. chauvoei*, *B. putrificans*, *B. oedematis* and *B. botulinus* were tested in ordinary agar stabs. The tubes were inverted in alkaline pyrogallol solution and incubated, the results being recorded after one, two, three and four days respectively. An abundance of both growth and reduction was noted. As in the other experiments the intensity of reduction was a function of luxuriancy of growth. In all instances reduction was obtained along the line of growth only.

We find here a very striking corroboration of the fact long reported by Cahen, Smith, Kitasato and Weyl that anaerobes reduce as a result of the biogenic activity of the cell. These investigators, however, worked with dyes and the relation of the cell to the reducing process was not as strikingly brought out as in the case of the selenium compounds. Here reduction is localized and seems intimately related to the growth of the organism, while the presence of the free chocolate-red or brick-red selenium granules in the cell points strongly to the fact that the reduction is an intracellular phenomenon. The anaerobic bacteria evidently utilize oxygen for their metabolic processes just as the anaerobes do, and it is only oxygen in the free state that is inimical to the life of the organism.

No appreciable inhibition of growth was observed except in some cases in concentrations of 1:10,000. Reduction in dilutions of 1:100,000 was found to have taken place with most strains within forty-eight hours. At the end of two days the red selenium streak following the path of growth began to disappear and at the end of three days no sign of free selenium was visible except in the case of one strain of *B. oedematis-maligni* and *B. putrificans*. The higher selenite concentrations showed excellent reduction but there was less tendency for the precipitated selenium to disappear. At the end of three months

the brick-red selenium had completely disappeared in all the culture tubes except the ones containing sodium selenite in the proportion of 1:10,000. For practical purposes, concentrations of 1:25,000 give the best results, as all the strains tried reduced within twenty-four to forty-eight hours, while in 1:10,000 selenite agar, one strain of *B. Welchii*, one strain of *B. tetani*, *B. oedematis* and *B. oedematis-maligni* failed to reduce even at the end of four days.

REDUCTIONS WITH THE DIPHTHERIA GROUP

Gloger reported failure of *B. diphtheriae* and *B. pseudodiphtheriae* to reduce. This was probably due to the high concentration of selenium compound used. Joachimoglu and Hirose report that the following concentrations will kill diphtheria; 1:1160 for sodium selenite; 1:420 for sodium tellurite; 1:666 for selenate; 1:125 for tellurate. They employed a culture medium containing 4 per cent sugar. Conradi, on the other hand, made use of the reducing action of *B. diphtheriae* on tellurium salts for the isolation of these organisms. It seemed likely, therefore, that a more thorough study of the reducing powers of the different members of this group might reveal characteristics that would be of value in differentiating them from one another.

Four strains of *B. diphtheriae*, seven strains of pseudodiphtheriae and three strains of diphtheroid organisms from Hodgkin's disease were used. These were grown on agar slants containing 0.0001, 0.00004, 0.000001 gms. of sodium selenite or selenium dioxid, in 1000 grams of agar. Observations were made after five hours, twenty-four hours, four days and ten days. The nature of the growth and the intensity of the reduction were recorded.

In general, reduction was obtained with all the strains, the best reaction appearing in the 1:10,000 dilution of sodium selenite and in the 1:25,000 of selenium dioxid. It is evident that the concentration of the selenium reagent is the important factor in determining the course of reaction. It is also interesting to note in this connection that while reduction was obtained in dilutions as high as 1:100,000 and in some cases even in

TABLE 3

Selenium dioxid: diphtheriae group

Growth and reduction at end of five hours (*A*), twenty-four hours (*B*), four days (*C*), ten days (*D*)

NAME OF ORGANISM	CONCENTRATIONS																											
	1:200,000						1:100,000						1:50,000						1:25,000						1:10,000			
	A		B		C	D	A		B		C	D	A		B		C	D	A		B		C	D	A		B		C	D
	Reduction	Growth	Reduction	Growth	Reduction	Reduction	Reduction	Growth	Reduction	Growth	Reduction	Reduction	Reduction	Growth	Reduction	Growth	Reduction	Reduction	Reduction	Growth	Reduction	Growth	Reduction	Reduction	Reduction	Growth	Reduction	Growth	Reduction	Reduction
B. diphtheriae, 61	−	+	−	++	−	−	+	+	+	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. diphtheriae, 96	−	+	−	++	−	−	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B. diphtheriae, 97	−	+	−	++	−	−	+	+	+	+	++	++	++	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
B. pseudodiphtheriae, 2	−	+	−	−	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++
B. pseudodiphtheriae, 99	−	−	+	+	+	−	+	+	−	+	++	+	+	+	+	+	+	+	+	+	+	+	++	+	−	−	−	−	+	++
B. pseudodiphtheriae, 100	−	+	−	++	−	+	+	+	+	++	++	+	+	+	+	+	+	+	+	+	+	+	+	++	+	+	+	++	+	++
B. pseudodiphtheriae, 494	−	−	−	++	−	?	+	+	+	++	++	+	+	++	+	+	+	+	+	+	++	+	+	+	−	−	+	+	++	++
B. pseudodiphtheriae, 495	−	+	−	++	+	?	+	+	+	++	++	+	+	++	+	++	+	+	+	+	++	+	++	+	+	+	+	+	++	++
B. Hodgkini, 640	−	+	+	++	+	−	−	+	+	++	++	+	−	+	+	+	+	+	−	−	++	+	+	++	−	−	++	+	++	++
B. Hodgkini, 641	−	+	−	++	−	−	+	+	++	+	++	+	+	+	+	+	+	+	−	−	++	+	++	++	−	−	++	+	++	++
B. Hodgkini, 642	−	+	−	++	−	−	−	+	−	++	++	−	−	−	+	+	+	+	−	−	−	−	+	+	−	−	−	−	+	+
B. Hoffmani, 21	−	+	−	++	−	−	−	+	+	+	++	+	+	++	++	+	+	+	−	−	−	−	−	+	−	−	−	−	−	+
B. Hoagi, 497	−	+	+	++	−	−	−	+	+	++	++	+	+	+	+	+	+	+	+	+	+	+	++	+	−	−	+	+	++	++
B. serosis, 570	−	+	−	++	+	−	−	+	+	++	++	+	+	++	+	+	+	+	+	+	++	+	++	+	−	−	+	+	+	+

* The number after the name of the bacillus represents the number of the organism in the bacteriological collection at the Museum of Natural History.

1:200,000 the red color due to the deposition of selenium gradually faded away and disappeared completely after a few days. In the higher concentrations the fading was not as marked and the intensity of the color was not appreciably diminished after a month.

Gosio, Maassen and also Rosenheim have proven the formation of a volatile organic selenid or tellurid when microörganisms are grown in cultures containing compounds of selenium. Maassen showed that the alkyl selenid or tellurid produced by microorganisms was ethyl selenid or tellurid, while Hofmeister working with tissues of higher animals found the alkyl to be methyl. The fading, which was also observed with anaerobes, is probably due to volatilization of the selenium in the form of an organic compound.

Reduction is intimately associated with vigor of growth. Too high a concentration inhibits growth and gives diminished reduction. Too low a concentration, on the other hand, does not inhibit growth and may give no evidence of reduction; apparently it has no effect on the bacterial cell since the latter does not avail itself of the oxygen in this compound, as indicated by the absence of reduction. The diphtheria bacilli are more sensitive to the toxic action of the selenium compounds than are the diphtheroids. A marked inhibition is observed in the growth of the former in a dilution of 1:10,000 of the selenium dioxid, while the latter are affected but little by that concentration.

The abundance of oxygen likewise influences the intensity of reduction. Thus but little reduction is obtained on the upper part of the streak, while the reduction is more marked in the water of condensation and still more evident in the stab. Apparently these cells utilize the combined oxygen only when the oxygen pressure of the medium falls below a certain point.

Reduction of selenite and selenium dioxid is an intracellular phenomenon. The reduction always follows the line of growth in the stab culture. On streak growths only the discrete colonies (or the entire growth if the growth is confluent) are colored red while the rest of the medium is not colored. Reduction is al-

ways more intense in the center of the colony and fades away toward the edge. A microscopic examination reveals the red granules of elemental selenium deposited within the cell.

Aside from the difference in sensitiveness to the toxic action of the selenium dioxid, the diphtheria and pseudodiphtheria bacilli also show very distinct differences in the character of their growth on the streak. This characteristic difference most likely results from the inhibitive action of the selenium dioxid. The diphtheria bacilli grow in the form of small discrete colonies, of brick-red color (due to a deposition of selenium), while the pseudodiphtheria bacilli grow in larger colonies or in a continuous streak along the line of inoculation.

EXPERIMENTS WITH THE COLON-TYPHOID GROUP

All the members of the colon-typhoid group were tested by Kligler, Greenberg and the author, in a manner similar to that employed in the study of the diphtheria organisms with the exception that only one dilution (1:10,000) of sodium selenite was used. The cultures were streaked on agar slants containing the selenite and observations made after twenty-four hours, forty-eight and ninety-six hours, respectively. Forty-five colon-organisms, ten *dysenteriae* and twenty *B. typhi* were tested.

In brief, the results were as follows: After twenty-four hours all organisms gave distinct reduction except the *B. dysenteriae*, *paratyphi* A and the fowl cholera bacilli.[2] The coli, para-coli and enteritidis types and some *B. typhi* gave strongest reduction, the typhi and paratyphi B. followed closely. That *B. coli* is a better reducer than *B. typhi* has also been demonstrated with nitrate (Dieudonné, Lunkevicz), and with ammonium molybdate (Capaldi, Proskauer). After forty-eight hours *B. cholerae* showed reduction, while the dysenteriae and paratyphi A still failed to show any or but faint traces of reduction. Practically the same result was obtained after ninety-six hours. These results are significant in that they strengthen the view that the colon para-colon enteridis group is closely associated,

[2] With lower concentration of sodium selenite Gosio obtained reduction with dysentery and paratyphoid organisms.

that *B. typhi* and *B. paratyphi* B are more intimately related, while the *paratyphi* A and *dysenteriae* form a third division of the family.

Handel and Theodorascu observed that most strains of *B. coli* were more inhibited in their growth by sodium selenite than were typhoid bacilli; Guth was able to confirm this and found that the selectivity for the latter organism was increased by an alkaline reaction. The optimum concentration of the selenium salt he found to be 0.15 per cent. A comparison with malachite green and Endo agar showed the superiority of selenium agar as a culture medium for the selective growth of typhoid bacilli. As a practical medium Guth used selenium-agar to which was added 0.1 per cent crystal violet.

SELENIUM COMPOUNDS AS INDICATORS OF MICROBIAL LIFE

The use of dyes, such as methylene blue, as indicators of biologic reducing processes is by no means ideal. Methylene blue is very easily reduced by a great many compounds which may happen to be in the culture medium. Hasse has shown that among the amino acids, glycine is the most potent in bringing about the reduction of methylene blue. The leuco form of the dye, moreover, is very easily re-oxidized to methylene blue by exposure to air or by shaking. This fact can be readily illustrated by the following experiment. Leuco-methylene blue can be prepared by boiling with zinc dust an aqueous solution of the dye made alkaline with sodium hydroxide. The colorless liquid which results becomes blue on the addition of tap water or on slight shaking.

Selenium compounds as indicators are of greater value. The reduction is localized and for that reason cannot be ascribed to bacterial metabolites. Furthermore, reduction of the selenium compound is an irreversible reaction. The precipitated selenium shows no tendency to reoxidize.

In order to serve as indicators of microbial life selenium dioxid or sodium selenite should be reduced by all microörganisms. Those, which at first evinced no such power, did not reduce owing to the presence in the medium of too high a concentration of the

selenium compound. It will be remembered that selenium dioxid or sodium selenite in too great amounts can prevent growth entirely. Without growth there is no reduction, for the intensity of the latter process is directly proportional to that of the former. The result of retardation in growth depends not only upon the concentration of the selenium compound but also upon the nature of the organism. Concentrations which have no effect upon one individual or a whole type will prevent growth and therefore reduction in another individual or another type. This explanation holds for members of the anaerobe group, the tuberculosis group, the diphtheria group, the colon-typhoid group and a few isolated individuals. In the cases of anaerobes, selenium dioxid or sodium selenite proves very inimical to growth. When, however, the medium contains very minute amounts of the selenium compound growth and reduction invariably result.

Proteus mirabilis and *B. phosphorescens*, according to Gosio's first communication, gave uncertain reactions. It was found that in repeating experiments with these two organisms, *P. mirabilis* grown on a sugar-free medium containing five or six drops of neutralized sodium selenite brought about good reduction within twenty-four hours. *B. phosphorescens* under similar conditions did not cause reduction.

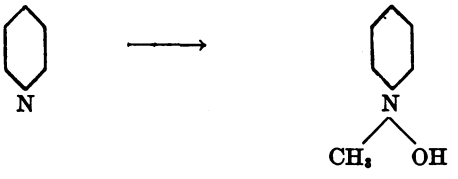
The experiment with B. *phosphorescens* was repeated with smaller amounts of sodium selenite. Selenium dioxid was also tried as indicator. To tubes containing sugar-free medium was added 0.1 cc. of 0.2 per cent sodium selenite solution. Tubes were also made up with 0.1 cc. of 0.1 per cent selenium dioxid solution. At the end of three days reduction was noticeable in the phosphorescens culture treated with sodium selenite but not in the one containing the more toxic selenium dioxid. Evidently this organism responds very sensitively to selenium compounds, a fact which accounts for Gosio's negative finding. Growth was hardly noticeable.

ALKYLATION BY MICROÖRGANISMS

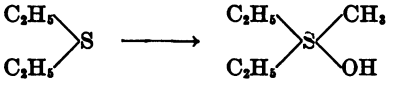
We have seen that certain bacteria produce volatile compounds of characteristic odor from culture media containing

sodium selenite and that in some instances the brick red selenium formed by reduction is entirely removed by a biologic process of volatilization. This disappearance, which we believe is the result of alkylation, takes place very readily in cultures having extremely low concentrations of sodium selenite.

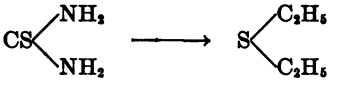
The researches of several investigators have established the fact that alkylation is not an unusual biologic process. His found that pyridine is converted in the biologic organism to methyl pyridyl ammonium hydroxid.



Neuberg and Grosser observed the formation of diethyl methyl sulfinium base from diethyl sulfid.



Pohl demonstrated the production from thiourea of diethyl sulfid.



Gosio was the first to show that certain molds grown upon media containing minute quantities of arsenic produce volatile compounds characterized by a garlic-like odor. Biginelli proved that the gas generated from arsenic cultures was completely absorbed by mercuric chlorid solution with the formation of colorless crystals of a double compound of diethyl arsine and mercuric chlorid, represented by the formula

$$As\,H(C_2H_5)_2\,2HgCl_2$$

Penicillium brevicaule, which Gosio isolated from the air, possessed this property in the highest degree. Indeed, this mold

may be regarded as a living reagent for arsenic. According to Abel and Buttenberg, quantities as minute as 0.000001 gram may be recognized with certainty. This test is so delicate that it has been found of great value in toxicological analysis in the preliminary examination for arsenic. It is considerably more delicate than Bettendorf's test and it may equal in sensitiveness the Marsh and the Gutzeit tests. The great advantage of the biological over the chemical method lies in the fact that less time is required to get results since the tedious destruction of organic matter is rendered unnecessary, and since, furthermore, a number of tests may be made at the same time.

The "arsenic molds" do not produce odoriferous volatile compounds from sulphur, phosphorus, boron, bismuth or antimony compounds, but they possess the power of converting selenium and tellurium compounds into volatile substances of characteristic odor. The odor from tellurium compounds resembles that arising from arsenic compounds, and is garlic-like, while the odor from selenium compounds is mercaptan-like. Selenium and tellurium may be differentiated from arsenic by the use of molds which do not alkylate the latter. According to Maassen 0.1 gram of sodium selenite or tellurite in 50 grams of culture medium inhibits or prevents the formation of the volatile compound, and 0.001 gram of sodium selenite or tellurite does not produce the odor. In order to get a positive test 50 grams of the nutrient medium should contain no less than 0.005 gram and not more than 0.1 gram of selenite or tellurite. The odor persists for months.

A number of workers—Japha, Rabuteau, Czapek and Weil, Hofmeister, Woodruff and Gies, Maassen, Jones, Filippi and Levine—have observed after the administration of selenium compounds to animals the presence of a volatile compound of characteristic odor emanating from the expired air. Gmelin, Hausen, Heeren, Rabuteau, Czapek and Weil, Hofmeister, Mead and Gies and others have observed a peculiar odor about their clothing, about the animals and in the room when experimenting with tellurium compounds. The odor was attributed by some (Rabuteau, Fillipi) to hydrogen selenid or tellurid while

others (Wöhler and his pupils) believed it to be due to ethyl selenid or tellurid. Hofmeister in an extensive research found methyl tellurid in the expired air of experimental animals. Reasoning by analogy, he concluded that the substance exhaled by animals dosed with compounds of selenium, was methyl selenid. Maassen corroborated the findings of Hofmeister in so far as higher animals were concerned. He proved, however, that while animals synthesized the methyl selenid or tellurid, microörganisms produced the ethyl selenid or tellurid.

MICROÖRGANISMS AS REAGENTS FOR SELENIUM COMPOUNDS

1. Alkylation through molds

There are two type methods in toxicological procedures for the detection of selenium. One type method depends upon purely chemical means. The tissue under investigation is oxidized, using as the oxidizing reagent any of the following: potassium hydroxid and potassium nitrate (Kletzinsky), aqua regia (Vohl), nitric acid (Quarelli, Levine), nitric and sulphuric acids (Duhamel and Juillard), potassium chlorate and hydrochloric acid (Levine). After removal of excess of the oxidant the resulting clear solution is boiled with hydrochloric acid to reduce any selenate to selenite.

$$Na_2SeO_4 + 2HCl \rightarrow Na_2SeO_3 + H_2O + Cl_2$$

The selenite is finally reduced to free selenium with sulphur dioxid (Kletzinsky, Quarelli, Vohl, Levine) or hydrazine hydrate (Duhamel and Juillard).

The other type method is biological. There are three distinct ways of detecting selenium biologically. One of these is through the use of molds. The detection of arsenic employing molds as biologic reagent has become a well established method as a result of the researches of Gosio, Abel and Buttenberg and others, and has now found its way into standard text books on toxicology. This mold method also works for selenium. It is necessary, however, to subject the tissues to oxidation before using the microörganic reagent. According to Rosenheim, free

selenium or tellurium, unlike free arsenic, is not alkylated by molds. Administration of selenium compounds to animals sets up according to Levine two processes of detoxication: one, removal of the poison by volatilization through a process of alkylation; the other, the reduction of the compound to inert, nontoxic, free selenium which deposits in the tissues. Before applying a mold like *Penicillium brevicaule*, the tissue is oxidized in order to convert any free selenium to the selenite form.

Quarelli was the first to make actual application of the mold method. He employed it in the analysis of tissues of a human being who had died of cancer, and who had received while still living, injections of colloidal selenium, a substance for which in cancer some clinicians claimed therapeutic value. The tissues were dried in a thermostat, powdered, suspended in water and treated with nitric acid. The selenium was thus converted to selenious acid. The excess of nitric acid was removed by evaporation. The mixture was finally neutralized with 0.1 N sodium hydroxid. Portions of the solution of tissue thus prepared were inoculated with mold, while other portions were acidified with hydrochloric acid and reduced to free, brick-red selenium by means of sulfur dioxid. The chemical and the biological method gave similar results. With both methods Quarelli obtained the greatest quantity of selenium in blood and liver and relatively smaller quantities in gall bladder, heart, aorta, stomach, pancreas and duodenum.

2. Reduction by bacteria

Scheuerlen, Klett, Gosio, Maassen and others have shown that sodium selenite is reduced by bacteria to brick-red selenium. This reduction we have also found to occur with selenious acid and with selenic acid, but not with its salts, the selenates. Potassium selenocyanid also yields free selenium, but this decomposition is not a vital phenomenon since acids in general and specifically acid metabolites in the culture medium bring about this result.

With sodium selenite the precipitated selenium can be very prettily observed in the medium along the path of growth.

Glucose accelerates the reducing process. One part of sodium selenite may be detected by reduction in 200,000 parts of culture medium. *B. coli* is especially suitable as a biologic reducer. The tissue containing selenium is digested with nitric acid. The excess thereof is removed by evaporation. The residue is boiled with hydrochloric acid to reduce any selenate to selenite. The resulting solution is evaporated to a small volume, neutralized and incorporated into glucose agar medium, which is finally inoculated with *B. coli*. The efficacy of the procedure was proven as follows: to tissues in 5 gram portions—muscle, liver, spleen and blood—were added 0.0005, 0.001 and 0.002 gram of sodium selenite. These were treated in the manner described and the final residues mixed with 10 grams of culture medium. Inoculated with *B. coli* the media showed a streak of red selenium following the line of growth in the stab culture.

Experiments *in vivo* were also performed. One rabbit was given subcutaneously 0.0005 gram, while another was given 0.001 gram of sodium selenite per kilo. Portions of the liver of these animals were oxidized. Selenium was detected by the formation of brick-red selenium through reduction with sulphur dioxid of one-half of the oxidized tissue residue acidified with hydrochloric acid. The other half was incorporated in culture media, which were inoculated with *B. coli*. The brick-red line following the path of growth indicated the presence of selenium. Portions of the liver were put in 5 per cent formaldehyde, sectioned and stained with eosin and hematoxylin. The liver cells had degenerated to such an extent that they stained very poorly. Chocolate red granules of selenium were found intracellularly and extracellulary.

The histological method, which constitutes a third biological method, was used by Jones to demonstrate the reduction of selenium compounds in the animal organism. Levine has elaborated this method as a toxicological procedure. Dogs and rabbits given sublethal and lethal doses of selenium compounds show reduction in some tissues, especially in the liver. He recommends the histological examination of the liver and other tissues as a routine procedure in the toxicological analysis of tissue for selenium.

THE NATURE OF BACTERIAL REDUCTION

Reduction is brought about by the bacterial cell. This fact was proven by Spina and Rothberger. According to these investigators, heating cultures at 60° to 70°C. destroys the reducing power. Gosio found no reduction in dead cultures. Smith did not observe reduction in filtered culture although Hahn and Cathcart, working with methylene blue, found filtrates to be reducing in action. According to Cahen, Spina, Smith, Rothberger, Klett, Beijerinck and Maassen, reduction is due to the direct activity of the bacterial cell. Roszahegyi, Baginsky, Müller, Wolff, and Wichern conclude, however, that reduction results not directly from the bacterial protoplasm but from its products of metabolism. The later findings, those of Kanaido, indicate that reduction is a vital process and is inhibited by rabbit's normal serum and to a very marked degree by homologous immune sera.

The reducing power of bacteria has been ascribed by recent investigators to an enzyme named reductase. Sterile bacterial extracts are known to have reducing power. Avery and Neill obtained reduction of methylene blue from sterile broth extracts of unwashed pneumococci entirely free from living or intact cells. There has been a great deal of discussion regarding the nature of bacterial reductase. The question whether it is an exo-enzyme or an endo-enzyme or whether such a thing as bacterial reductase exists, has been answered both affirmatively and negatively. Experiments undertaken by Kligler and Levine with the intention of determining these points, indicated that reduction was closely associated with the life of the cell, and that the more vigorous the growth the more marked was the reduction. The filtrate of a broth culture of *B. coli* passed through a Berkefeld failed to reduce selenium dioxid. In answer to the objection that the Berkefeld withheld certain substances, such as a co-activator or the enzyme possessing reduction properties, we point to the lack of reduction in the agar tube outside of the zone of growth. If there were such a substance as a soluble bacterial reductase or some metabolic by-product of reducing nature, its gradual diffusion throughout the

medium should cause diffuse reduction. Furthermore, a killed broth culture of *B. coli* added to the solution of selenite failed to show any reduction even after two weeks. Finally a large mass growth of an actively reducing strain of *B. coli* was dried in a desiccator triturated with sand, taken up with physiological salt solution and centrifuged. The supernatant liquid (1) kept under toluol to prevent growth, together with a solution of sodium selenite to make up a concentration of 1:10,000, failed to bring about any reduction. The residue (2) was extracted with a mixture of glycerol and water. This extract (3) had no reducing action on sodium selenite. The mass (4) remaining after the glycerol extraction was also brought into contact with sodium selenite but no reducing action was apparent. However, a combination of supernatant liquid (1) and residue (2) showed decided ability to reduce.

Harden and Zilva believe that the reducing enzyme is not a single principle but a system consisting of enzyme plus acceptor or activator. They found that by washing with saline *Bacillus coli-communis* loses its activator or acceptor and is no longer able to reduce methylene blue. Addition of the boiled washings restores, however, the lost function. Avery and Neill found that washing pneumococci in phosphate solution also renders them incapable of reducing methylene blue. Upon the addition of meat infusion or extract these washed bacterial cells recovered their reducing power. To determine the nature of the activator or acceptor, Harden and Zilva added a great variety of substances to their washed organisms. They obtained positive results with a great number of the substances used. The following list gives the results with the compounds and mixtures tested:

Positive

Boiled washings	Mannitol
Bouillon	Alanine
Horse serum	Asparagine
Liebig's extract	Albumin
Arabinose	Globulin
Xylose	Peptone (Witte)
Glucose	Sodium acetate

Levulose	Sodium formate
Galactose	Sodium succinate
Mannose	Sodium lactate
Maltose	Sodium pyruvate
Inulin	Sodium glycerate
Glycerol	Isobutyric acid

Negative

Formaldehyd	Potassium oxalate
Acetaldehyd	Rochelle Salts
Propylaldehyd	Sodium citrate
Isovaleraldehyd	Hydroxybutyric acid
Cinnamylaldehyd	Linolinic acid
Salicylaldehyd	Butyl alcohol
Lactose	Phloroglucinol
Raffinose	Creatin
Erythrol	Guanidin hydrochlorid
Dulcitol	Hypoxanthin
Sorbitol	Xanthin
Oenanthol	Adenin
Glycocol	

Bach maintains that simple aldehydes possess the power to activate. According to him, amino acids may also function, since these are readily converted to aldehyd. Harden and Zilva's experiments, however, proved negative with aldehydes. Bach claims that complex aldehydes like carbohydrates do not activate. Harden and Zilva found a number of carbohydrates capable of restoring reducing power.

Abelous and Aloy found that many substances besides aldehydes act as activators or co-ferments. Among others may be mentioned benzylamin, dibenzylamin, substances with heterocyclic groups, such as quinolin, the terpene hydrocarbons and such inorganic compounds as manganese salts. Harden and Norris found with washed yeast a number of substances capable of playing the rôle of activator or acceptor. Such were benzaldehyd, salicylaldehyd, anisicaldehyd, isovaleraldehyd, dihydroxyacetone, succinic acid, glycollic acid, lactic acid, sodium lactate, citric acid, glyceric acid, bouillon, boiled yeast extract, normal horse serum and sterile milk. Negative results were obtained with glycol, propylene glycol, 2-3 butylene glycol, glycerol, pyrogallol, resorcinol, quinol, acetone, formaldehyd, acetaldehyd, methylglyoxal, citral, glucose, levulose, pyruvic

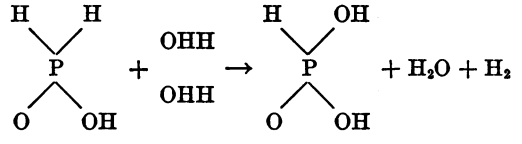
acid, formic acid, acetic acid, malic acid, tartaric acid, mandelic acid, yeast nucleic acid, creatine, guanidin, p-phenylenediamin, glycocol, alanine, aspargine, tyrosine, and Witte's peptone.

The number of compounds that have the power to re-activate the reducing enzyme present such a variety of chemical types that it is difficult to determine a specific chemical nature for the compounds functioning as activators. It is very likely that the rôle of acceptor or activator may be played by any compound that has the power to take up with ease the oxygen removed from the substrate or zymolyte undergoing reduction.

There seem to be some discrepancies in the results of the investigators cited. Harden and Norris found that acetaldehyd activates washed muscle but not washed yeast. Harden and Zilva found that benzaldehyd, anisealdehyd and salicylaldehyd activate washed yeast, although Harden and Norris report that these same compounds show a negative behavior towards washed *Bacillus coli*. Glycerol, glucose, levulose, alanine, asparagine and peptone were negative with yeast but positive with *Bacillus coli*. The experiments of Harden and his collaborators were performed qualitatively, without regard to concentration of added substance or of hydrogen ions. It may be possible that by regulating these factors, the results of Bach and of Harden and his associates would attain some semblance of agreement. The point in reference to concentration may be illustrated from the very experiments of Harden and Zilva. They found that the addition of increasing quantities of broth or of glycerol increased the rate of reduction until a certain optimum, after which any further increase in the quantity of broth or glycerol diminished the rate of reduction.

Bach attributes the reducing action of plant or animal organism to a system in which enzyme, substrate, acceptor and water in a state of dissociation each plays a specific rôle. The dissociating process furnishes the hydrogen to reduce the methylene blue or nitrate as well as the oxygen to oxidize the acceptor (aldehyd). In other words, biologic reduction is a complex phenomenon, a hydrolytic-oxidative-reduction process, of which the Cannizzaro reaction is an example.

Bach's explanation becomes more plausible if we consider an analogous case—the oxidation of hyposulfites, which he himself investigated. In water alone hyposulfites do not undergo oxidation, at least at any measurable rate. But in the presence of a catalyst (finely divided palladium) this does take place. The water is decomposed and its HO used for oxidation of the hyposulfite. The hydrogen is taken up temporarily by the palladium and then set free. An easily reducible substance, if present, will be reduced by the nascent hydrogen.



When aldehydes are taken instead of hyposulfite, it is observed that the metals of the platinum group do not to any appreciable extent accelerate the decomposition of water and hence the oxidation of the aldehyd. It is only after the addition of an easily reducible substance, such as methylene blue, indigo or nitrate, which acts as an acceptor for nascent hydrogen, that acceleration occurs. Thus, formaldehyd in the presence of a catalytic agent and acceptor like methylene blue is of some theoretical interest. Since this dye contains no oxygen it follows that the atom of this element necessary to convert formaldehyd into formic acid must come from water, the only source of oxygen in the reaction system.

The reducing enzyme bears an interesting analogy to the oxidizing enzyme. If we regard the oxidizing system as

peroxidase + peroxide

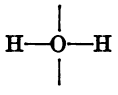
we may look upon the reducing system as

Schardinger enzyme + aldehyd

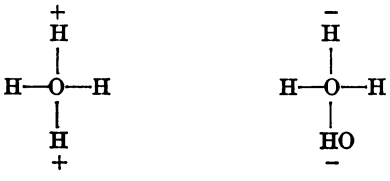
An oxidase produces its oxidation by the aid of gaseous oxygen, while a reductase acts through the intermediation of the combined oxygen in water.

Bach's explanation of the part taken by water in the reducing

phenomenon lies in the consideration of water as an unsaturated compound in which oxygen is tetravalent.



Since ions are believed to be associated with water molecules to form unstable complexes, and since hydrogen and hydrogen ions are present in water, it is probable that two compounds are formed, one, water in combination with hydrogen ions, the other, water in combination with hydroxyl ions.



The first may be named hydrogen suboxide or oxygen perhydride. It bears analogy to the metallic salts of the type M_4O, an example of which is Ag_4O. The second is the hydrate of hydrogen peroxide ($H_2O\ (OH')_2$).

The acceleration of the oxidation of aldehydes in the presence of platinum, Engler and Wöhler explain on the ground that the colloidal metal unites with oxygen to form a peroxide, PtO_2, which reacts with water to form the hydrate, $HO - Pt - O - O$, which acts as a powerful oxidizing agent. This compound is also supposed to be produced by the interaction of platinum and the $H_2O\ (OH')_2$ present in water. Since the latter is present in a small amount, it is quickly used up, the equilibrium is disturbed, and more is therefore formed, with the result that the catalytic process continues. It is not unlikely that the platinum metals would form strongly reducing hydrides by interaction with oxygen perhydride (H_4O) if its presence in water is admitted.

Bach believes that the reducing enzyme or reductase activates oxygen perhydride just as peroxidase causes the activation of peroxide oxygen. For this reason he calls the reducing enzyme,

perhydridase. Bach's view, although it explains the occurrence of active hydrogen, is built on a weak foundation for it supposes the existence of compounds of doubtful nature.

Differing somewhat from Bach's conception of biologic reduction is that formulated by Wieland. This investigator sought the essential cause of certain typical biologic oxidations in the activation of oxygen, not in the activation of hydrogen. According to his conclusions oxidases, reductases and mutases have no separate existence.

His views may be illustrated from his conclusions derived from a quantitative study of the action of Schardinger's enzyme on salicylaldehyd. The aldehyd is assumed to act in the form of its hydrate and the enzyme activates two of its hydrogen atoms which are thus rendered available for an acceptor. This acceptor may be (1) molecular oxygen, (2) an easily reducible substance like methylene blue, or (3) a molecule of the aldehyd in the unhydrated form. If molecular oxygen is the acceptor, the result is the oxidation of the aldehyd. If an easily reducible substance is the acceptor, its reduction is associated with indirect oxidation of the aldehyd. If unhydrated salicylaldehyd is the acceptor, the Cannizzaro reaction, the mutase effect, is observed.

$$R\,CH\begin{matrix}OH\\OH\end{matrix} + R\,CHO \longrightarrow R\,COOH + R\,CH_2OH$$

Whether any one of these results is obtained or a mixture of all three, depends upon the concentration and speed of reaction of the various acceptors. The most important hydrogen acceptor is oxygen but other acceptors may be equally important for other aspects of cell activity. Thunberg, from a series of experiments, has been led to adopt Wieland's conception. According to the latter methylene blue acts as a hydrogen acceptor. The hydrogen comes from a substance in the tissue, which acts as a hydrogen donator and the enzyme itself plays the rôle of a hydrogen transportase.

Some investigators hold that reduction through the intervention of enzymes is not the only mechanism for biologic re-

duction. Heffter is of the belief that a great part of the phenomenon relating to the oxidizing and reducing properties of protoplasm is inherent in the sulfhydryl group. Mathews and Walker have demonstrated the ease with which cystein, $CH_2(SH)$ $CH(NH_2)$ COOH undergoes oxidation. Arnold has shown that tissues give the sodium nitroprussid reaction indicating the presence of the sulfhydryl grouping (SH). He has also shown that a number of proteins give this reaction and that in the absence of protein, a positive response is elicited from cystein.

Very recently, F. G. Hopkins has isolated from yeast, muscle and liver a compound giving the nitroprussid reaction. It is a dipeptide of glutamic acid and cystein. It has reducing properties similar to those ascribed by de Rey-Pailhade to his philothin of yeast. Hopkins by way of analogy calls his dipeptide glutathione. The sulfhydryl group (SH) of its cystein fraction readily gives up its labile hydrogen. It, therefore, acts as an oxygen acceptor or hydrogen donator. The dipeptide, when in the oxidized or disulfid form (S-S) may act as a hydrogen acceptor.

$$2RSH + O_2 \longrightarrow R{-}S{-}S{-}R + H_2O_2$$

$$2RSH + H_2O_2 \longrightarrow R{-}S{-}S{-}R + 2H_2O$$

$$R{-}S{-}S{-}R + H_2 \longrightarrow 2RSH$$

It is in this play of oxidizing and reducing effects of cystein and cystine that may be found, at least in part, the explanation for the affinity of the cells for oxygen and the possibility of the formation of peroxid. Avery and Neill in their work on the pneumococcus suggest that peroxid formation and methylene blue reduction are functions of the same or closely related systems, though the particular action induced depends upon whether molecular oxygen or methylene blue serves as a hydrogen acceptor or oxygen donator.

Nascent hydrogen itself may account for some biologic reductions. Hoppe-Seyler has demonstrated by bacterial intervention the formation of carbon dioxid and the very potent reducing agent, hydrogen, from formic acid, glycolic acid, lactic and glyceric acids. He found that those compounds which did

not evolve free hydrogen, like malic acid, tartaric acid and asparagin, were reduced in part to succinic acid. A number of workers have proven the reduction by anaerobes of amino acids with the formation of saturated fatty acid and ammonia. This calls into interaction two atoms of hydrogen.

$$R\,CHNH_2\,COOH + H_2 \longrightarrow R\,CH_2\,COOH + NH_3$$

Very recently, Neuberg and Neuberg and Rewald have shown that among the products of bacterial decomposition of α-ketonic acids may be found formic acid, carbon dioxid and hydrogen. Carbohydrates may also yield hydrogen since they have the power to produce as a result of biologic fragmentation, the very acids that evolve this reducing gas.

Certain tissue compounds also possess reducing power. Hasse has shown that amino acids, particularly glycine, have the power to reduce methylene blue. Of special interest are the investigations of Fränkel and his associates in relation to the strong reducing action of tissue phosphatids. Compounds associated with intermediate metabolism—formic acid, lactic acid, aldehydes and others—are known for their strong reducing action. Formaldehyd, in minute quantities, is found in plant tissue and acetaldehyd has been proven by Neuberg and Nord to be an intermediate product in the bacterial fermentation of glucose, mannitol or glycerol.

Several investigators have shown that in the presence of carbohydrates bacteria show greater reducing capacity. This accelerated effect may be due to the carbohydrate itself or to its fragmentation products. The reducing action at 37.5°C. of carbohydrates with a free carbonyl group in the absence of tissue has been demonstrated by Levine by the use of sodium selenite in alkaline medium and even by the use of the ordinary Fehling and Fehling-Benedict reagents. Liver pulp, boiled and unboiled, also reduced sodium selenite. Compounds closely associated with carbohydrates as products of their decomposition—aldehydes, ketones, formic and lactic acids—reduced sodium selenite in the presence or absence of tissue but in a slightly acid medium.

Indeed, it may be stated that most of the organic compounds apt to be present in biologic material show greater or less reducing tendencies, although these are not manifested with the ordinary reagents nor at biologic temperatures. Levine with the use of sodium orthovanadate and Levine and Jahr with the use of ammonium molybdate have shown that almost all types of organic compounds of biologic significance—unsaturated fatty acids, glycerol, lipins, amino acids, proteins, carbohydrates, aldehydes, ketones, phenols, uric acid and creatinin—are capable of reducing. Plant and animal tissues heated or unheated, kept at 37.5°C. easily reduce ammonium molybdate. The reduction of potassium permanganate is not necessarily enzymatic in character. Levine has shown that boiled or unboiled diffusate from yeast suspension, bacterial cultures or liver, as well as the filtrate obtained by alcoholic precipitation, bring about at ordinary temperature the instantaneous reduction of permanganate. Yet in spite of all that may be said in favor of the reducing action of the various chemical components of tissue, we must not overlook the presence or underestimate the importance of enzymes responsible for reduction.

The author gratefully acknowledges his indebtedness to Professor William J. Gies for his valuable suggestions and helpful criticisms and to Dr. I. J. Kligler and Mr. William Rothberg for their valuable coöperation.

SUMMARY

Living bacteria bring about the reduction of selenious acid (Chabrié and Lapicque, Levine), selenic acid (Levine) and sodium selenite (Scheuerlen, Klett, Gosio, Levine). Sodium selenate is not reduced (Klett, Levine); nor is potassium selenocyanid (Levine). Due to decomposition induced by acids, metabolically produced, selenium may, however, be deposited from potassium selenocyanid, as in the case of *B. coli* cultures (Levine). Heated cultures do not reduce (Klett, Gosio, Levine). The media employed should not contain chemical reducing substances, such as glucose or lactose (Gosio, Gloger, Levine).

Reduction is a vital process (Cahen, Spina, Smith, Roth-

berger, Klett, Maassen, Gosio, Levine); it is an intracellular process (Kligler, Levine, Harden, Zilva, Kanaido). The reductase elaborated by the bacterial cell is an endo-enzyme reducing energetically in the presence of an activating substance or coenzyme, which is capable of being dissolved out from the cell or of being removed by Berkefeld filtration (Kligler and Levine, Harden and Zilva).

Selenious acid, sodium selenite and selenic acid retard growth. The extent of retardation depends upon the concentration and chemical nature of the selenium compound and upon the individuality of the organism. *Streptococcus pyogenes* is more sensitive than *B. coli*. The anaerobes of symptomatic anthrax, oedema and tetanus are extremely sensitive and growth does not take place except in minute concentrations of the above selenium compounds (Levine).

Sodium selenate and potassium selenocyanid in the quantities used show but slight retarding effect on growth (Levine).

Reduction is directly proportional to the intensity of growth (Klett, Gosio, Levine). When there is no growth there is no reduction (Klett, Gosio, Levine), but when the concentration of selenium compound is very small (1:200,000) there may be growth without visible evidence of reduction (Levine). This lack of visible selenium may be due to its removal by volatilization. With higher concentration of selenium compounds in the culture medium the activity of reduction outbalances that of alkylation (Levine).

Selenium dioxid or sodium selenite cannot be used as a differential test between aerobes and anaerobes, since both types reduce (Levine).

There is no specific relation between reduction and formation of hydrogen sulfid, as Gloger maintained, since organisms such as *B. acidilactici*, *B. pseudodiphtheriae* or *B. tuberculosis*, that produce no hydrogen sulfid or only faint traces, are capable of reducing selenium dioxid or sodium selenite (Levine).

The diphtheria organisms have been tested with different concentrations of selenium dioxid and have been found to be efficient reducers. In the very high concentrations some or-

ganisms failed to grow and therefore gave no evidence of reduction (Levine).

The reducing action on sodium selenite in very high concentration (1:10,000) by the various organisms in the colon typhoid group may be of practical value in differentiating one type from another. *B. paratyphi* B reduces while *B. paratyphi* A does not (Levine). This difference in action harmonizes with the findings of Burnet and Weissenbach, Jordan and Victorson and also Kligler. These investigators distinguished these two types of organisms by the use of lead acetate media, which made apparent the difference in the reducing action as manifested by the production of hydrogen sulfid.

Microörganisms can be used as living reagents in the toxicological analysis for selenium. With the aid of certain alkylating molds, selenium can be detected by means of the characteristic and persistent odor of ethyl selenid (Quarelli). With the aid of bacteria that possess intense reducing activity, selenium compounds, in the form of selenite ion, can be identified by the brick-red line or streak following the path of growth in a stab culture (Levine).

Selenium compounds serve as better indicators for reducing enzymes than organic dyes. Since the reduction is localized in the bacterial zone of growth, it cannot be ascribed to metabolic products. Unlike the reduction of dyes, the decomposition of selenium compounds to free selenium is an irreversible reaction and the precipitated element shows no tendency to re-oxidize (Levine).

For practical purposes selenium dioxid or sodium selenite in a concentration of 1:50,000 or 1:25,000 can be used to demonstrate bacterial reduction in a solid sugar-free culture medium (Levine).

Selenium agar (0.15 per cent Na_2SeO_3) as a culture medium for the selective growth of typhoid bacilli is superior to malachite green or Endo agar according to Guth.

REFERENCES

ABEL, R., AND BUTTENBERG, J. 1899 Ueber die Einwirkung von Schimmelpilzen auf Arsen und seine Verbindungen. Zeitschr. f. Hyg. u. Infektionskrankh., **32**, 449.

ABELOUS AND ALOY 1917 Sur les phénomenès biochimique d'oxydo-réduction. Comptes rendus des séances de l'académie des sciences, **165**, 270.

ACKERMANN, D. 1908 Ein Faülnisversuch mit Arginin. Zeitschr. f. physiol. Chemie, **56**, 305.

ACKERMANN, D. 1909 Ueber die Entstehung von Faülnisbasen. Zeitschr. f. physiol. Chemie, **60**, 482.

ACKERMANN, D. 1910 Ueber den bakteriellen Abbau des Histidins. Zeitschr. f. physiol. Chemie, **65**, 504.

ACKERMAN, D. 1911 Die Sprèngung des Pyrrolidinringes durch Bakterien. Zeitschr. f. Biol., **57**, 104.

ACKERMAN, D., AND KUTSCHER, F. 1910 Ueber die Aporrhegmen. Zeitschr. f. physiol. Chemie, **69**, 273.

ALSBERG, C. L. 1915 Note on the reduction of oxyhemocyanin in the serum of *Limulus polyhemus* L. Jour. Biol. Chem., **23**, 495.

ARNOLD, V. 1911 Eine Farbenreaktion von Eiweisskörpern mit Nitroprussidnatrium. Zeitschr. f. physiol. Chemie, **70**, 300.

ARNOLD, V. 1911 Ueber den Cysteingehalt der thierischen Organe. Zeitschr. f. physiol. Chemie, **70**, 314.

AVERY, O. T., AND NEILL, J. W. 1924 Studies on oxidation and reduction by pneumococcus. III. Reduction of methylene blue by sterile extracts of pneumococcus. Jour. Exper. Med. **39**, 543.

BACH, A. 1911 Zur Kenntnis der Reduktionsfermente. I Mitteilung. Ueber das Schardinger Enzym (Perhydridase). Biochem. Zeitschr., **31**, 443.

BACH, A. 1911 Zur Kenntnis der Reduktionsfermente. II Mitteilung. Reduktion der Nitrate durch das System Perhydridase-Aldehyd-Wasser. Biochem. Zeitschr., **33**, 282.

BACH, A. 1912 Zur Kenntnis der Reduktionsfermente. III Mitteilung. Vorkommen eines Kofermentes in tierischen Geweben. Biochem. Zeitschr., **38**, 154.

BACH, A. 1913 Zur Kenntnis der Reduktionsfermente. IV Mitteilung. Pflanzliche Perhydridase. Biochem. Zeitschr. **52**, 412.

BACH, A. 1914 Zur Kenntnis der Reduktionsfermente. V Mitteilung. Bildung von Aldehyden aus Aminosaüren. Biochemi. Zeitschr. **58**, 205.

BAGINSKY, A. 1888 Ueber Gährunsvorgänge im kindlichen Darmcanal. Deutsche medizin. Wochenschr. **14**, 391.

BARR AND BUCHANAN. 1912 Iowa Stage College Engineering Experiment Station, Bulletin 26.

BEHRING. 1889 Beiträge zur Aetiologie des Milzbrandes. VII. Ueber Lackmusgefärbte Agarnährboden. Zeitschr. f. Hyg. u. Infektionskrankh. **7**, 177.

BELFANTI, S. 1912 Die vitale Reakton nach Gosio beim Tuberkelbazillus. Zeitschr. f. Chemotherapie u. verwande Gebiete **1**, 113.

BRASCH, W. 1909 Ueber den bakteriellen Abbau primärer Eiweissspaltungsprodukte. Biochem. Zeitschr. **18**, 380.

BRASCH, W. 1909 Weitere Untersuchungen über den bakteriellen Abbau primärer Eiweissspaltungsprodukte. Biochemi. Zeitschr. **22**, 403.

BRENNER, W. 1916 Züchtungsversuche einiger in Schlamm lebenden Bakterien Auf Selenhaltigen Nahrboden. Jahrb. f. wissenschaftliche Botanik, 19, **57**, 95.

CAHEN, F. 1887 Ueber das Reduktionsvermögen der Bakerien. Zeitschr. f. Hyg. u. Infektionskrankh. **2**, 386.

CAPALDI, A., AND PROSKAUER, B. 1896 Beiträge zur Kenntnis der Säurebildung bei Typhusbacillen und *Bacterium coli*. Zeitschr. f. Hyg. u. Infektionskrankh., **23**, 452.

CATHCART, E., AND HAHN, M. 1902 Ueber die reduzirenden Wirkungen der Bakterien. Arch. f. Hyg. **44**, 295.

CHABRIÉ, C., AND LAPICQUE, L. 1890 Sur l'action physiologique de l'acide sélénieux. Compt. rend. des séances de l'académie des sciences, **110**, 152.

CONRADI, H. 1913 Ueber ein neues Prinzip der elektiven Züchtung und seine Anwendung bei Diptherie. Centralblatt für Bakteriologie, II Abtheilung, Referate **57**, 93.

CZAPEK, F., AND WEIL, J. 1893 Ueber die Wirkung des Selens und Tellurs auf den thierischen Organismus. Arch. f. exper. Pathol. u. Pharmokol., **32**, 438.

DAKIN, H. D. 1922 Oxidations and Reductions in the Animal Body, p. 98.

VAN DELDEN, A. 1903 Beitrage zur Kenntnis der Sulfatreduktion durch Bakterien. Centralbl. f. Bakteriol., 11 Abt., **11**, 81.

DIEUDONNÉ. 1894 Zusammenfassende Uebersicht über die in den letzer zwei Jahren gefunden cholerasähnlichen Vibrionen. Centralbl. f. Bakteriol., Abt. 1, **16**, 363.

DUHAMEL, B. G., AND JUILLARD, M. 1912 Localization du sélénium colloidal électriques dans les organes. Comptes rendus de la société de biologie, **72**, 714.

ENGLER, C., AND WÖHLER, L. 1901 Pseudokatalytische Sauerstoffübertragung. Zeitschr. f. anorganische Chemie, **29**, 1.

ELSNER 1894 Untersuchungen uber electives Wachsthum der Bacterien coliarten und des cholerasähnlichen Vibrionen. Centralbl. f. Bakteriol. Abt. 1. **16**, 363.

FILIPPI, E. 1913 Recherche farmocologicle sopra il selenio e i suoi composte Lo Sperimentale, Firenze, **67**, 565.

GASSER, J. 1890 Culture du bacille typhique sur milieux nutfitifs colorés. Arch. du médecin experimentale et d'anatome pathologique **2**, 750.

GAYON, U., AND DUBOURGE, E. 1894 Sur les vins mannités. Annales de l'institut Pasteur, **8**, 108.

GAYON, U., AND DUBOURGE, E. 1901 Nouvelles recherches sur le ferment mannitique. Annales de l'institut Pasteur, **15**, 525.

GIES, W. 1902 Chemical changes in the body in which the methyl group may be involved. Therapeutic Monthly, **2**, 144.

GLOGER, R. 1906 Kallium tellurosum in der Medizin und Hygiene. Centralbl. f. Bakteriol. **40**, 584.

GMELIN, CHI. 1824 Versuche über die Wirkungen des Baryts, Strontians u.s.w. auf den thierischen Organismus, Tubingen, p. 43.

GOPPELSRÖDER, F. 1862 Beitrage zum Studium der Salpeterbildungen. Poggendorf's Annalen, **115**, 125.

GOSIO, B. 1904 Sulla decomposizione di sali del telurio per opera dei microorganismi. Rendice Revista Accadamia dei Lincei. Seduita del 24 aprile; seduita del 4 guigno.

GOSIO, B. 1905 1 telluriti e seleniti come rivelatori d'inquinamenti batterici. Rendice Revista Accademia dei Lincei. Seduita del 6 agosto.

GOSIO, B. 1905 Indikatoren des Bakterien Lebens und ihre praktische Bedeutung. Zeitschr. f. Hyg. u. Infektionskrankh. **51**, 65.

GOSIO, B. 1892 Azione di alcune muffe sui composti fissi d'arsenico. Rivista d'igiene, e sanita publica **3**, 201.

GUTH, F. 1916 Selennährboden für die elektive Züchtung von Typhus-bacillen. Centralbl. f. Bakteriol., 1 Abt., **77**, 487.

HÄENDEL, AND THEODORASCU. Unpublished findings cited by Guth.

HAGGARD, H. W. 1921 The fate of sulfides in the blood. Jour. Biol. Chem., **49**, 519.

HARDEN, A., AND NORRIS, R. V. 1914 The enzymes of washed zymin and dried yeast (Lebedoff). II. Reductase. Biochem. Jour. **8**, 100.

HARDEN, A., AND ZILVA, S. S. 1915 The reducing enzyme of *Bacillus coli communis*. Biochem. Jour. **9**, 379.

HASSE, F. 1919 Ueber die Methylenblaureduktion durch Glycin. Biochem. Zeitschr. **98**, 159.

HAUSEN, K. 1853 Versuche über die Wirkung des Tellurs auf den lebenden Organismus. Annalen der Chemie und Pharmacie, **86**, 208.

HEEREN, M. 1861 Ueber Telluräthyl und Tellurmethylverbindungen. Chem. Centralbl., **6**, 916.

HEFFTER, A. 1907 Die reduzierenden Bestandteile der Zellen. Medizinisches naturwissenschaftliche Archiv., **1**, 82.

HELMOHOLTZ. 1843 Ueber das Wesen der Fäulnis und Gährung. Archiv. f. Anatomie, Physiologie und wissenschaftlichte Medicin, p. 453.

HIS, W. 1887 Ueber das Stoffwechselsproduct des Pyridins. Arch. f. exper. Pathol. u. Pharmakol., **22**, 253.

HOFMEISTER, F. 1904 Ueber Methyliering im Thierkörper. Archiv für exper. Pathol. u. Pharmakol. **12**, 457.

HOPKINS, F. G. 1921 On an auto-oxidisable constituent of the cell. Biochem. Jour. **15**, 286.

HOPPE-SEYLER, F. 1878 Ueber Gärungsprozesse. Zeitschr. f. physiol. Chemie, **79**, 2, 1.

JAPHA, A. 1842 Experiementa nonulla de vi selenii in organismum animalem. Dissertation, Halle.

JOACHIMOGLU, G. 1920 Zur Pharmakologie des Selens und Tellurs. I. Mitteilung. Die Wirkung ihrer Säuren auf Bakterien. Biochem. Zeitschr., **107**, 300.

JOACHIMOGLU, G., AND HIROSE, W. 1921 Zur Pharmakologie des Selens und Tellurs II. Mitteilung. Wirkung ihrer Säuren auf Diptheriebacillen. Biochem. Zeitschr., **125**, 1.

JONES, C. O. 1909 The physiological effects of selenium compounds with relation to their action on glycogen and sugar derivatives. Brit. Biochem. Jour. **4**, 405.

JORDAN, E. O., AND VICTORSON, R. 1917 Differentiation of the paratyphoid enteritides group. Jour. Infect. Dis. **21**, 554.

KANAIDO, S. 1916 Reduction of methylene blue by bacteria inhibited by immune sera. Saikiu Gaku (Zasshi, No. 243, 169; Japanese Medical Literature 2 (Part 1), 2-3 (1917).

KING, W. E., AND DAVIS, L. 1914 Potassium tellurite as an indicator of microbial life. Amer. Jour. Pub. Health **4**, 917.

KITASATO, S., AND WEYL, Th. 1890 Zur Kenntnis der Anaeroben. Zeitschr. f. Hyg. u. Infektionskrankh., **8**, 41; **9**, 97.

KLETT, A. 1900 Zur Kenntnis der reduzirenden Eigenschaften der Bakterien. Zeitschr. f. Hyg. u. Infektionskrankh. **33**, 137.

KLETZINSKY, V. 1858 Ueber die Ausscheidung der Metalle in den Secreten. Wiener medizin. Wochenschr. **1**, 356.

KLIGLER, I. J. 1917 Simple medium for the rapid differentiation of typhoid and paratyphoid bacilli. Amer. Jour. Pub. Health **7**, 805, 1042.

KLIGLER, I. J. 1913 The source of ammonia and nitrate in nitrate-peptone solutions, Amer. Jour. Pub. Health, **3**, 788.

KLIGLER, I. J., AND LEVINE, V. E. 1915 The Scheurelen-Klett selenium reaction in the diphtheria group. Biochem. Bull., **4**, 196.

KLIGLER, I. J., AND LEVINE, V. E. 1915 The nature of bacterial reductase. Unpublished communication.

KLIGLER, I. J., GREENBERG, D., AND LEVINE, V. E. Experiments with colon-typhoid bacilli grown on selenium medium. Unpublished communication.

KOCHMANN, R. 1920 Uber Schwefelwasserstoffbildung aus Sulfaten durch Faeces. Biochem. Zeitschr. **112**, 1255.

KRUSE, W. 1910 Allegemeine Mikrobiologie, Leipzig, p. 659.

LABBÉ, M. 1903 Action comparée des microbes at des toxines microbiennes sur le sang défibriné. Compt. rend. de la société de biologie **55**, 201.

LAURENT, E. 1890 Expérience sur la réduction des nitrates par les végétaux. Annals de l'institut Pasteur, **4**, 722.

LEVINE, V. E. 1913 Biochemical studies of selenium. Biochem. Bull., **2**, 552.

LEVINE, V. E. 1914 Ph. D. Dissertation, Columbia University.

LEVINE, V. E. 1914 Reduction of selenium compounds in the living organism. Biochem. Bull. **3**, 463.

LEVINE, V. E. 1915 Ann. New York Acad. Sci., **26**, 385.

LEVINE, V. E. 1915 Sodium selenite as a laboratory reagent for reducing substances. Biochem. Bull., **4**, 217.

LEVINE, V. E. 1915 The reducing power of anaerobes. Biochem. Bull., **4**, 215.

LEVINE, V. E. Reduction of permanganate by biologic tissue. Unpublished communication.

LEVINE, V. E. Reductions with compounds of the rarer elements II Sodium ortho-vanadate. Unpublished communication.

LEVINE, V. E., AND AHANA, W. W. Potassium selenocyanid as an indicator for free acidity. Unpublished communication.

LEVINE, V. E., AND JAHR, H. M. 1915 Reductions with compounds of the rarer elements. I Ammonium molybdate. Biochem. Bull., **4**, 224.

LEVINE, V. E., AND JAHR, H. M. 1921 The reducing action of micro-organisms on ammonium molybdate. Abstr. Bacteriol., **5**, 4.

LIPMAN, J. G., AND WAKSMAN, S. A. 1923 The oxidation of selenium by a new group of autotrophic microörganisms. Science, **58**, 60.

LUNKEWICZ, M. 1894 Eine Farbenreaktion auf die salpetrige Säure der Kulturen der Cholerbacillen und einiger anderen Bakterien. Centralbl. f. Bakteriol., Abt. 1, **16**, 9, 945.

MAASSEN, A. 1901 Die Zersetzung der Nitrate und der Nitrite durch die Bakterien. Arbeit. aus dem kaiserlichen Gesundheitsamte, **18**, 1.

MAASEN, A. 1901 Die biologische Methode Gosios zum Nachweis des Arsens und die Bildung organischer Arsen-Selen-und Tellurverbindungen. Arbeit. aus dem Kaiserlichen Gesundheitsamte, **18**, 1.

MAASSEN, A. 1904 Uber das Reduktionsvermogen der Bakterien und uber reduzirende Stoffe in pflanzlichen und tierischen Zellen. Arbeit. aus dem kaiserlichen Gesundheitsamte, **21**, 377.

MARPMANN. 1894 Zur Unterscheidung des *Bacillus typhi abdominalis* vom *Bacillus coli commune*. Centralbl. f. Bakteriol., **16**, 817.

MATTHEWS, A. P., AND WALKER, S. 1909 The spontaneous oxidation of cystein. Jour. Biol. Chem. **6**, 21.

MAYMONE, B. 1916 Sulla diagnosi differenziale dei paratifi tipo A e B. Archiv. per le scienze mediche, Torino, **40**, 202.

MEAD, L. D., AND GIES, W. J. 1901 Physiological and toxicological effects of tellurium compounds with a special study of their influence on nutrition. Amer. Jour. Physiol., **5**, 104.

MEUSEL, E. 1875 Nitritbildung duch Bakterien. Berichte des deutschen chemischen Gesellschaft **8**, 1214 and 1653.

MEYERS, JOHN T. 1920 The production of hydrogen sulfid by bacteria. Jour. Bacteriol., **5**, 231.

NĚMEC, A., AND KÁS, V. 1921 Ueber die Einfluss des Selens auf die Entwickelung einiger Scheimmelpilze aus der Gattüng Penicillicum. Biochem. Zeitschr., **114**, 12.

NENCKI. 1878 Uber den chemischen Mechanismus der Fäulnis. Jour. f. praktische Chemie, **17**, 105.

NEUBERG, C. 1914 Das Verhalten der α-Ketosäuren zu Mikroorganismen. I Mitteilung. Die Fäulnis von Brenztraubensäure und Oxalessigsäure. Biochem. Zeitschr., **67**, 90.

NEUBERG, C. 1914 Das Verhalten der α-Ketosäuren zu Mikroorganism. II Mitteilung. Die Faulnis von α-Ketobuttersäure. Biochem. Zeitschr., **67**, 122.

NEUBERG, C., AND GROSSER 1905 Eine neue schwefelhaltige Substanz aus dem Hundeharn. Centralbl. f. Physiol., **19**, 316.

NEUBERG, C., AND NORD, F. F. 1919 Anwendungen der Abfangmethode auf die Bakteriengärungen I. Acetaldehyde als Zwischinstufie bei der Vergärung von Zucker, Mannit und glycerin durch *Bacterium coli*, u. s. w. Biochem. Zeitschr., **96**, 133.

NEUBERG, C., AND NORD, F. F. 1919 Anwendungen die Abfangmethode auf die Bakteriengärungen. II Festleguuing den Aldehydestufie bei der Essiggärung. Biochem. Zeitschr., **96**, 158.

NEUBERG, C., AND REWALD, B. 1915 Das Verhalten der α-Ketosäuren zu Mikroorganismen. III Die Faulnis der d, l-methyl ethyl Brenztraubens äure. Biochem. Zeitschr., **71**, 122.

NEUBERG, C., AND ROSENBERG, E. 1907 Ueber die bei der Eiweissfäulnis auftretenden Fettsäuren sowie uber die optisch aktive Valeriansäure und Kapronsäure. Biochem. Zeitschr., **7**, 178.

PAKES, W. C. C., AND JOLLYMAN, W. H. 1901 Bacterial decomposition of formic acid into carbon dioxid and hydrogen. Jour. London Chemical Society **79**, 386.

PETRIE, R. J., AND MAASSEN, A. 1893 Beiträge zur Biologie der krankheitserregenden. Bakterien insbesonderes über die Bildung Von Schwefelwasserstoff durch dieselben unter vornehmlicher Berücksichtigung des Schweinerothlaufs. Arbeit. aus dem kaiserlichen Gesundheitsamte, **8**, 318.

PETRIE, R. J., AND MAASSEN, A. 1893 Weitere Beitrage zur Schwefelwasserstoff bildung aërober Bakterien und kurze Angaben uber Merkaptanbildung derselben. Arbeit. aus dem kaiserlichen Gesundheitsamte **8**, 490.

PETRUSCKY, J. 1889 Bakteriochemische Untersuchungen. Centralbl. f. Bakteriol. **6**, 63, 625 and 657.

PLIMMER, R. H. 1918 Citation in reference to coprosterol. Pract. Organic and Physiol. Chem., p. 34.

POEHL, A. 1886 Ueber einige biologisch-chemische Eigenschaften der Mikroorganismen in Allgemeinen und uber die Bildung der Ptomaine durch die Cholerabacillen in Speciellen. Berichte der deutschen chemischen Gesellschaft, **19**, 1159.

POHL, J. 1904 Ueber eine Alkylsynthese nach Thioharnstoffaufnahme. Archiv f. exper. Pathol. u. Pharmakol., **51**, 341.

QUARELLI, G. 1913 Sulla chemoterapia col selenio. Pensiero medico, **3**, 209.

RABUTEAU. 1869 Recherches sur les propriétés et sur l'élimination des composés oxygénés du sélénium et du tellure. Gazette hebdomodaire de médicin et de chirurgie, **6**, 194 and 241.

DE REY-PAILHADE, J. 1894 Études sur les propriétés chimique de l'extrait alcoolique de levûre de bière; formation de l'acide carbonique et absorption d'oxygène. Compt. rend. des séances de l'académie des sciences, **118**, 201.

DE REY-PAILHADE, J. 1888 Bulletin de la société chimique, **106**, 1683; 1890, 171.

REDFIELD. 1912 Hydrogen sulfid production and its significance in the sanitary examination of water. Ph. D. Thesis, Cornell.

ROSENHEIM, O. 1902 The decomposition of the compounds of selenium and tellurium by molds and its influence on the biologic test for arsenic. Proc. Chem. Soc. London, **18**, 138.

ROSZAHEGYI, A. 1898, 1899 Ueber das Züchten von Bakterien in gefärbter Nährgelatine. Centralbl. f. Bakteriol., **24**, 513; **25**, 15, also 69.

SALKOWSKI, E., AND H. 1883 Ueber die Entstehung der Homologender Benzoesäure bei der Fäulnis. Zeitschr. f. physiol. Chemie, **7**, 450.

SALKOWSKI, E., AND H. 1883 Zur Kenntnis der Eisweissfäulnis. II Die Skatolcarbonsäure. Zeitschr. f. physiol. Chemie, **9**, 8.

SALTET, R. H. 1900 Ueber Reduktion von Sulfaten in Brackwasser durch Bakterien. Centralbl. f. Bakteriol., II Abt., **6**, 648, 695.

SENATOR. 1918 Citation in reference to auto-intoxication. Wells' Chemical Pathology, 3rd edition, p. 581.

SCHEUERLEN. 1900 Die Verwendung der selenigen säure in der Bakteriologie. Zeitschr. f. Hyg. u. Infektionskrankh., **33**, 135.

SCHARDINGER, F. 1902 Ueber das Verhalten der Kuhmilch gegen Methylenblau Zeitschrift für Untersuchung der Nährung-und Genussmittel., **5**, 1113.

SCHARDINGER, F. 1894 Beitrage zur hygienischen Beurteilung des Trinkwassers. Centralbl. f. Bakteriol., **16**, 853.

SMITH, TH. 1896 Reduktionserscheinungen bei Bakterien und ihre Beziehung über Reduktionserscheinungen in steriler Bouillon. Centralbl. f. Bakteriol., **19**, 181.

SOMMARUGA, E. 1892 Ueber Stoffwechselproducte von Mikroorganismen. Zeitschr. f. Hyg. u. Infektionskrankh., **12**, 273.

SPINA, A. 1887 Bakteriologische Versuche mit gefärbten Nährsubstanzen. Centralbl. f. Bakteriol., **2**, 71.

STEPANOW, A. 1902 Ueber die Zersetzung des Jodkalium im Organismus durch Nitrite. Archiv. f. exper. Pathol. **47**, 411.

STOKVIS, C. S. 1899 Bijdrage tot verklaring der Zeavelwaterstofvorming in het Amsterdamsche grachwater. (Academisch proefschrift).

TANNER, F. W. 1917 Studies of the bacterial metabolism of sulfur. Formation of hydrogen sulfid from certain sulfur compounds under aerobic conditions. Jour. Bacteriol., **2**, 585.

THUNBERG, T. 1917 Die biologische Bedeutung der Sulfhydrylgrippe. Ergebnisse der Physiologie **11**, 328.

THUNBERG, T. 1920 Zur Kenntniss des intermediären Stoffwechsels und der dabei wirksamen Enzyme. Skandinavisches Archiv für Physiologie **40**, 1.

UFFELMAN, J. 1891 Ueber den Nachweiss der Typhusbacillen. Berlin. klin. Wochenschr. **28**, 857.

VOHL 1865 Citation from Eulenberg, H. Die Lehre von den schädlichen und giftigen Gasen, p. 454.

WICHERN, H. 1910 Quantitative Untersuchungen ueber die Reduktionswirkung der Typhus-Coli Gruppe. Archiv f. Hyg., **72**, 1.

WIELAND, H. 1914 Ueber den Mechanismus der Oxydationsvorgänge. III Berichte der deutschen Chemischen Gesellschaft, **47**, 2085.

WÖHLER, F. 1840 Telluraethyl. Annalen der Chemie und Pharmacie, **35**, 11.

WOLFF, K. 1899 Ueber Dentrifikation. Hygienische Rundschau, **9**, 546.

WOLFF, J. 1909 Sur quelques propriétés nouvelles des oxydases de Russala Delica. Compt. rend. des séances de l'académie des sciences, **148**, 500.

WODDRUFF, I. O., AND GIES, W. J. 1902 On the toxicology of selenium and its compounds. Amer. Jour. Physiol. Proc. of the Amer. Physiol. Soc., **6**, 29.

WROBLEWSKI, A. 1898 Zusammensetzung des Buchner'schen Hefepressaftes. Berichte der deutschen chemischen Gesellschaft, **31**, 3218.

WURTZ 1891 Bacille d'Eberth et coli-bacille. Le bulletin médical **5**, 1155.

PLATE 1

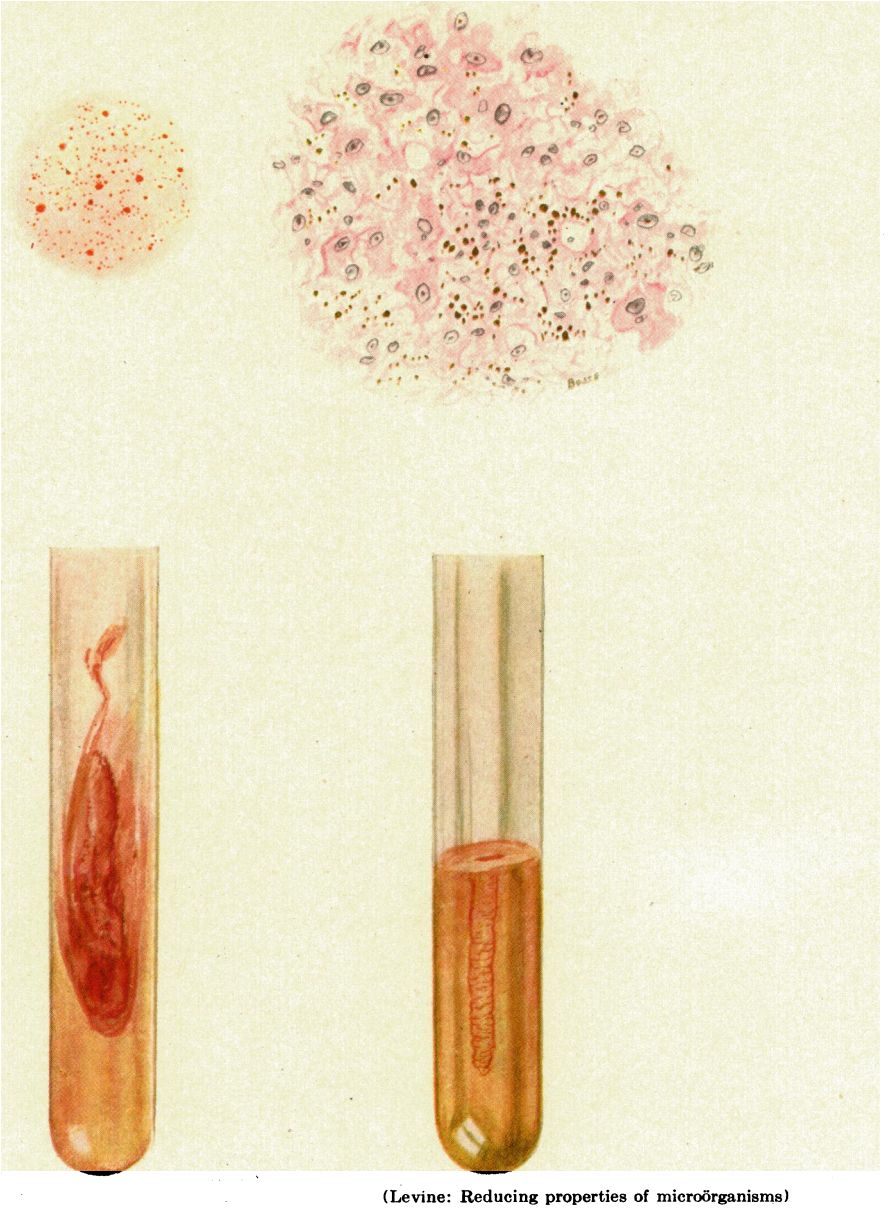
ILLUSTRATIONS OF BIOLOGICAL REDUCTIONS OF SELENIUM COMPOUNDS

FIG. 1. *B. coli* grown on a sodium selenite culture medium. The colonies appear as red spots.

FIG. 2. Another culture of *B. coli* grown on a slant.

FIG. 3. Section of the liver of a dog that had been subcutaneously treated with 2 mgm. selenium dioxid per kilo of body weight. The chocolate red granules represent deposited selenium. The cells, especially the nuclei, have suffered degeneration to the extent that they no longer stain readily.

FIG. 4. Stab culture of *Streptococcus pyogenes*.



(Levine: Reducing properties of microörganisms)