Since lactic acid production is increased in traumatic shock[6] and corticoids raise the blood sugar only in the presence of the liver,[3] it is tempting to assume that in shock, the blood sugar decreases in the absence of the adrenals because the lactic acid produced fails to be resynthesized into glucose. If, on the other hand, carbohydrate-metabolism-active corticoids are administered, the blood sugar can be raised above normal by resynthesis of the excess lactic acid. In any case, these experiments give further support to the assumption that changes in carbohydrate metabolism play an important part in the pathogenesis of the shock syndrome.

13381

Gas Gangrene-Toxin Production in Gelatin-Thioglycollate Medium.*

G. B. REED AND J. H. ORR.

From Queen's University, Kingston, Canada.

In a recent note, Reed, Orr and Baker[1] showed that good yields of *Cl. welchii* toxin were produced in a simple gelatin-peptone medium. Since that work was done a further simplification of the medium has been found to give larger and more consistent yields of both hemotoxin and lethal toxin by the 4 principal gas gangrene species: *Cl. welchii, Cl. septicum, Cl. novyi* and *Cl. sordellii.*

1. This simplified medium was based on the observation of Koser, Chinn and Saunders[2] that certain brands of gelatin support growth of many of the more exacting aerobic species.

The growth of several species of the genus *Clostridium* was tested in a medium in which gelatin provided the only source of nitrogen. Four brands were tried. Five percent concentration of the 4 gelatins were dissolved in the following salt-sugar solution:

[6] Dosne, C., Proc. of the 7th Annual Meet., Canad. Physiol. Soc., Montebello, October, 1941.

* Part of an investigation aided financially by the Canadian National Research Council.

[1] Reed, G. B., Orr, J. H., and Baker, M. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 620.

[2] Koser, S. A., Chinn, B. D., and Saunders, F., *J. Bact.*, 1938, **36**, 57.

Na_2HPO_4	5 g
KH_2PO_4	1 "
NaCl	2 "
$MgSO_4$	0.1 "
Dextrose	2 "
Water	1000 ml

The reaction was adjusted to pH 7.6 and the media autoclaved in deep tubes.

Table I indicates that of the 4 gelatins tested, Difco and Grayslake (a domestic brand) support abundant growth of the 6 more important species of gas gangrene anaerobes. Eastman's "practical" permits a slight amount of growth in the first culture but none in subsequent transfers. Eastman's "purified gelatin (de-ashed)" fails to support growth of any of the 6 species. This corresponds with Koser, Chinn and Saunder's observations on aerobes, that highly purified photographic gelatin lacks some growth-promoting factor or factors present in certain laboratory and domestic gelatins.

TABLE I.

Comparative Growth Promoting Effect of 4 Brands of Gelatin in 5% Concentrations in a Salt-Sugar Solution.

	Difco		Grayslake		Eastman practical		Eastman purified	
	1st gen.	2nd gen.	1st gen.	2nd gen.	1st gen.	2nd gen.	1st gen.	2nd gen.
Cl. welchii	+	+	+	+	±	—	±	—
Cl. septicum	+	+	+	+	—	—	—	—
Cl. novyi	+	+	+	+	—	—	—	—
Cl. sordellii	+	+	+	+	—	—	—	—
Cl. histolyticum	+	+	+	+	—	—	—	—
Cl. sporogenes	+	+	+	+	±	—	±	—

2. The medium which has given most consistent yields of toxin is made as follows:

Gelatin (Bacto or Grayslake)	100 g
Na_2HPO_4	5 "
KH_2PO_4	1 "
NaCl	2 "
$MgSO_4$	0.1 "
Dextrose	2 "
Na thioglycollate	1 "
Water	1000 ml

The ingredients are dissolved together, adjusted to pH 7.6 before autoclaving, dispensed in tubes or flasks to a depth of not less than 6 cm and autoclaved at 15 lb for 25 minutes, in the case of tubes, or longer for larger containers.

Table II indicates the yields of hemotoxins and lethal toxins from the four principal toxigenic gas gangrene species. In this table the

TABLE II.
Hæmotoxin and Lethal Toxins Produced in Gelatin-Na-thioglycollate Medium.

	Hæmotoxin, active dilution	Lethal toxin, M.L.D. mouse
Cl. welchii	1-1020 to 1-480	0.5 to 1.0 ml
Cl. septicum	1-32 " 1-128	0.1 " 0.01 "
Cl. novyi	1-32 " 1-128	0.1 " 0.01 "
Cl. sordellii	1-4 " 1-16	0.1 " 0.01 "

figures for hemotoxin represent the highest dilution of the culture filtrate which will hemolyze 50% of a 2% suspension of washed rabbit red cells. The figures for lethal toxin represent the smallest volume of culture filtrate which kills a 20 g white mouse, after intraperitoneal injection in 5 to 7 hours. The range indicated in the table represents yields from several strains of each species mentioned. Any one strain maintained by day to day transfers in this gelatin medium shows very little variation in toxin yield on successive trials in the same batch or on different batches of the medium.

Several old laboratory strains of *Cl. welchii, Cl. septicum* and *Cl. novyi* which yielded negligible amounts of toxin gave good yields after 8 to 10 mouse passages.

The toxins produced in the gelatin medium are actively antigenic. Highly potent antitoxins have regularly been obtained in rabbits, after 8 to 10 doses of toxin. One ml of these antitoxins will neutralize 1000 or more lethal doses of the specific toxin, tested in white mice.

Ten days' treatment of the toxins with 0.3% formalin results in loss of toxicity. The resulting toxoids are as active or more active than fresh toxins in stimulating antitoxin formation in the rabbit.

Both the hemotoxin and the lethal toxin may be recovered by centrifuging at high speed, or by filtration through Seitz or sintered glass (Jena G5 on 3) filters. Filtration results in only a very slight decrease in toxin content, especially if the first 20 to 30 ml of toxin, in the case of small filters, are discarded.

3. In this medium the growth and toxin yield are closely related to the initial oxidation-reduction potential, which in turn is influenced by the concentration of gelatin and, of course, by the addition of reducing agents. Fig. 1 indicates the oxidation-reduction potential of media with 2 to 20% gelatin, with and without 0.1% sodium thioglycollate, before and after inoculation with *Cl. welchii*. It is apparent that the Eh of the medium, without thioglycollate, is progressively more negative with increasing concentration of gelatin. On the other hand, the addition of 0.1% sodium thioglycollate brings the several concentrations of gelatin to approximately the same Eh.

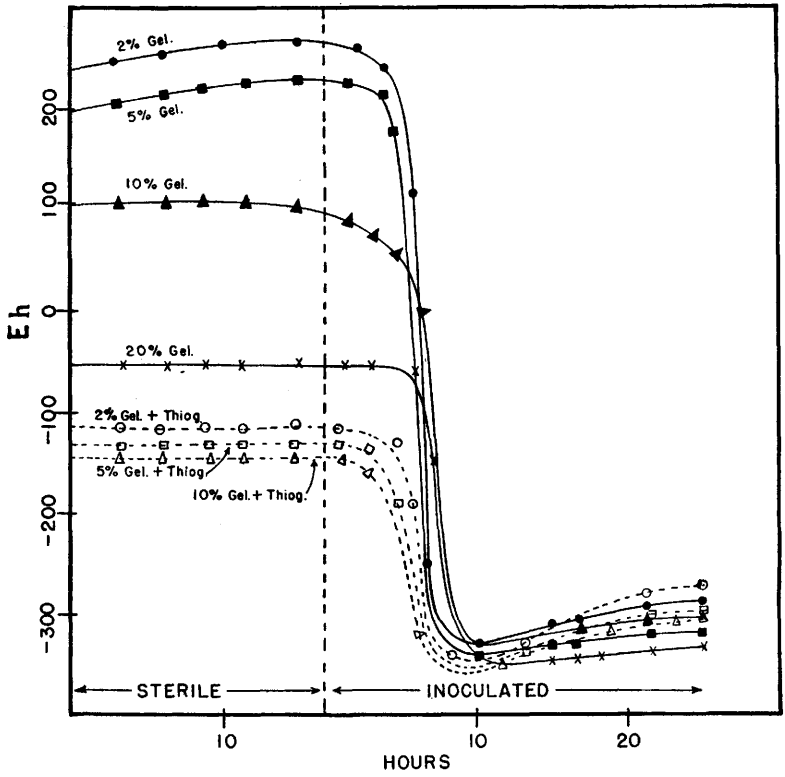

FIG. 1.

Graphs showing the Eh of gelatin medium, 2 to 20% gelatin, with and without sodium thioglycollate, before and after inoculation with *Cl. welchii*.

When heavily inoculated with *Cl. welchii* (0.01 ml of an 18-hour culture in 5% gelatin medium with thioglycollate) the fall in potential is similar in all the media, with and without thioglycollate. Ascorbic acid and cysteine in 0.1% concentrations affect the potential in essentially the same manner as sodium thioglycollate.

The influence on the medium of gelatin concentration and added reducing agents from the point of view of growth is indicated by the minimum effective inoculum of *Cl. welchii,* shown in Table III. It is apparent that in the absence of added reducing agent, growth is promoted by increasing the concentration of gelatin; this runs parallel with the more negative potential effected by the higher gelatin concentration. However, when the potential is rendered still more negative with sodium thioglycollate, the low gelatin concentrations still do not provide an efficient medium. It is, therefore, apparent that the higher gelatin concentrations provide some growth-promoting or nutritive factor in addition to shifting the Eh toward a more

TABLE III.

Minimum Inoculum of *Cl. welchii* to Initiate Growth in Gelatin Media with 2 to 20% Gelatin With and Without Sodium Thioglycollate.

Inoculum, ml. *Cl. welchii* culture	2% gelatin thioglycollate		5% gelatin thioglycollate		10% gelatin thioglycollate		20% gelatin thioglycollate	
	none	0.1%	none	0.1%	none	0.1%	none	0.1%
10^{-3}	+	+	+	+	+	+	+	+
10^{-4}	—	+	+	+	+	+	+	+
10^{-5}	—	—	—	+	+	+	+	+
10^{-6}	—	—	—	—	+	+	+	+
10^{-7}	—	—	—	—	—	+	—	+
10^{-8}	—	—	—	—	—	+	—	+
10^{-9}	—	—	—	—	—	—	—	—

+ indicates definite growth in 24 hours; — = no growth.

favorable level. It is further evident that the medium with 2 to 10% gelatin is more readily inoculated with *Cl. welchii* when the Eh is lowered to approximately 100 millivolts by the addition of sodium thioglycollate. Similar effects on growth are produced by the addition of cysteine or ascorbic acid.

The gelatin concentration in the medium also conspicuously influences toxin yield, as indicated in Table IV. Two and 5% concentrations give relatively low yields, 10 and 20% give much higher yields. This is probably proportional to growth. The addition of sodium thioglycollate increases toxin production in low concentrations of gelatin but has little influence in the higher concentrations provided the inoculum is large enough to initiate rapid growth.

TABLE IV.

Yield of Hæmotoxin in Medium with Increasing Concentrations of Gelatin, with and without Sodium Thioglycollate.

Medium	Hæmotoxin dilution					
	1-10	1-100	1-1000	1-2000	1-4000	1-8000
2% gelatin						
No thioglycollate	+	—	—	—	—	—
0.1% "	+	+	—	—	—	—
5% gelatin						
No thioglycollate	+	+	—	—	—	—
0.1% "	+	+	+	+	—	—
10% gelatin						
No thioglycollate	+	+	+	+	+	+
0.1% "	+	+	+	+	+	+
20% gelatin						
No thioglycollate	+	+	+	+	+	+
0.1% "	+	+	+	+	+	+

+ indicates haemolysis of 50% or more of a 2% suspension of red cells.
— indicates no haemolysis or less than 50% of the 2% red cell suspension.

Conclusions. The gas gangrene group of anaerobes grow luxuriantly in a medium consisting of a buffered salt-mixture with dextrose and gelatin. Highly purified gelatin is not suitable. In this medium all the toxigenic gas gangrene species produce a high and consistent yield of hemotoxin and lethal toxin. The toxins are actively antigenic and make effective toxoids.

13382

Effect of Quinine on Metabolism in Fasting Dogs and in Patients with Creatinuria.*

A. T. MILHORAT, W. E. BARTELS AND V. TOSCANI.

From the Russell Sage Institute of Pathology in Affiliation with the New York Hospital and the Departments of Medicine and Psychiatry, Cornell University Medical College, New York.

The observation that quinine diminishes the output of certain urinary substances has been confirmed by several investigators; however, practically all of the experiments heretofore reported were made on animals or human subjects receiving an adequate diet. Prior[1] appears to have been the only investigator who studied these effects of quinine during fasting and in his studies, which were made on only one dog, urea was the only urinary substance that was determined. Aoki[2] found a diminution in the output of creatine and creatinine when quinine was given to patients with hyperthyroidism and animals which had received desiccated thyroid gland. Since quinine is now used in the management of certain muscular disorders, *e. g., myotonia congenita* and muscular rigidity of paralysis agitans, it seemed of interest to further investigate the metabolic effects in an attempt to elucidate the action of the drug in these muscular syndromes.

Methods. There were two series of experiments, one on fasting dogs, the other on patients with muscular wasting and creatinuria. Female dogs were fasted for a preliminary period of at least 10 days and throughout the experiments but were allowed free access to drinking water. The animals were kept in metabolism cages

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Prior, *Arch. f. d. ges. Physiol.*, 1884, **34**, 237.

2 Aoki, K., *Folia endocrin. Jap.*, 1928, **3**, 1331 (abstr. *Jap. J. Med. Sci.*, 1931, **4**, 13).