

gen intake, and from the output of nitrogen in the urine, that probably less than 5% of the amino acids were retained, except perhaps during the first few days of administration. It seems clear that in the second case, the intravenous administration of amino acids initiated regeneration of plasma protein. Evidently sufficient amounts were retained to form new plasma protein. Farr and MacFadyen⁵ have shown in careful nitrogen balance studies that nephrotic children are able to utilize intravenously administered amino acids but do not build plasma proteins. According to Whipple's⁶ concept of protein storage, it is likely that the amino acids replenish depleted protein stores.

Conclusion. In one of 2 hypoproteinemic individuals exhibiting the "nephrotic stage" of chronic nephritis, intravenous administration of amino acids was, on two occasions, followed by a well marked rise in the level of the serum proteins. The rise occurred in the albumin fraction. The other subject failed to respond.

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Production of *Cl. welchii* Toxin in Peptone-free Medium.

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Clostridium welchii is customarily cultivated in glucose-peptone beef-infusion broth for toxin production. Many workers have found highly variable yields of toxin, which is usually due to the variation in different lots of peptone. Reed and his coworkers¹ have grown various strains of *Clostridia* in a medium consisting of 5% gelatin and 1% peptone with maximal hemotoxin production by

⁵ Farr, Lee E., and MacFadyen, Douglas A., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 444.

⁶ Whipple, George H., *Am. J. Med. Sc.*, 1938, **196**, 609.

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

Cl. welchii ranging from 1:1000 to 1:4000 dilutions which hemolyzed 50% of a 2% suspension of rabbit's red blood cells. Recently Gladstone and Fildes² have reported successful cultivation of various *Clostridia* in C.C.Y. media (casein acid-hydrolysate, casein tryptic-digest, yeast extract) without reference to toxin-production.

The writers have undertaken a study of the nutritional requirements and toxin-production of *Cl. welchii* in a simplified medium with the object of producing consistent yields of toxin. The basal medium consists of:

MgSO ₄	0.02 g
Na ₂ HPO ₄ · 12H ₂ O	5.76 "
KH ₂ PO ₄	0.24 "
l-tryptophane	0.10 "
Casein acid-hydrolysate 0.28% total N	1000 ml

pH adjusted to 7.9; autoclaved at 10 lb for 10 min.

After autoclaving, glucosamine hydrochloride and glucose were added to give a final concentration of 0.1% and 0.2% respectively.

Casein acid-hydrolysate was prepared according to the method employed by Mueller and his coworkers.^{3, 4} As expected, the basal medium alone did not support the growth of *Cl. welchii*. The U. S. Public Health strain of *Cl. welchii* SR12 was used throughout the experiments.

Growth was successfully obtained by addition to the medium of 0.1% liver extract.[†] Equally good growth was obtained by substituting pantothenic and pimelic acids for the liver-extract (Table I). Addition of riboflavin and nicotinic acid was found necessary for toxin-production (Table I).

After 5 or 6 passages through pigeons the organisms were cultured in glucose beef-infusion broth for 5 hours or less. Bacterial cells were transferred into a sterile tube, centrifuged and washed with sterile saline twice before inoculation into the medium for toxin production. Unless otherwise stated the cultures were incubated at 37°C for 17 hours. There was sufficient buffer in the medium to prevent a fall in pH to less than 6.1 to 6.2 in 17 to 22 hours' cultivation. Although small amounts of liver-extract (0.01 to 0.05%) gave satisfactory growth, more uniform toxin-production followed the addition of 0.1% liver-extract to the medium.

Toxins have so far been produced in batches of media not ex-

² Gladstone, G. P., and Fildes, P., *Brit. J. Exp. Path.*, 1940, **21**, 161.

³ Mueller, J. H., *J. Immunol.*, 1939, **37**, 103.

⁴ Mueller, J. H., and Johnson, E. R., *J. Immunol.*, 1941, **40**, 33.

† Mother-liquor from the alcohol-precipitation of crude liver-extracts. One g of the extract equals 300 g of fresh liver.

ceeding 40 ml in volume. To avoid loss of toxin through adsorption by filtration, the cultures were centrifuged at 4000 rpm for one hour and the supernates carefully pipetted off. The toxins were tested in mice weighing 17 to 22 g, and in pigeons of 275 to 300 g; 50% hemolysis of a 2% suspension of rabbit's red blood-cells in the highest dilution was taken as the titer of hemotoxin.

Since almost all the iron had first been removed from the casein acid-hydrolysate, various concentrations of FeSO_4 were added to the medium. The optimal concentration for consistent production of toxin was found to be 0.20 to 0.25 mg Fe per liter. A definite amount of iron in the media has previously been found necessary for production of diphtheric toxin by Pappenheimer and Johnson.⁵ Variation in the phosphate content of the medium greatly influenced morphology, growth, and toxin-production. When the phosphate content of the final medium was increased from 0.56 to 1.11 mg P/ml the organisms became coccoid in form, and growth and toxin-production decreased.

Quantitative estimates of growth were made by determinations of bacterial nitrogen in twice-washed packed cells from given volumes of culture. These values are recorded in Table I.

TABLE I.
Representative Growth and Toxin-production of *Cl. welchii* in Peptone-free medium.

Basal medium*	Accessory factors. Liver extract, %	Final pH	Bacterial N, mg/40 ml	Hemotoxin titration 50% hemolysis of 2% rabbit, r.b.c.	M.L.D. for mouse, ml	M.L.D. for pigeon per 100 g, ml
1	.1	6.2		1:20000	.05	
2	.1	6.2	2.3	1:10240	.1	.03
3	.2	6.1	2.4	1:20480	.1	.05
4	.2	6.2	2.3	1:10240	.1	.05
5†	.2	6.4	2.1	1:10240	.1	.06
6†	.1	6.6	2.0	1:10240		.08
7	‡	6.3	2.0	1:640	none	none
8	§	6.2	2.3	1:12800	.1	.08
Peptone beef-infusion broth with 0.25% glucose		5.2 to 5.7	2.9	1:5120	.1	.08

*Formula is given in the text.

† $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 12.6 g, KH_2PO_4 , 0.4 g per liter.

‡Calcium d-pantothenate and pimelic acid, $2\mu\text{g}/\text{ml}$.

§Calcium d-pantothenate and pimelic acid, $2\mu\text{g}/\text{ml}$, nicotinic acid, $5\mu\text{g}/\text{ml}$, riboflavin, $0.2\mu\text{g}/\text{ml}$.

⁵ Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1936, 17, 335.

Summary and Conclusions. *Cl. welchii* was grown in a medium free from peptone or other substances of large molecular weight. Toxins were consistently produced which were equal to or more potent than those produced in glucose peptone beef infusion broth. The pH of the culture media did not fall below 6.0. This may in part be responsible for the higher potency of toxin obtained in this medium. The basal medium alone did not support growth; when it was supplemented with pantothenic acid, pimelic acid, nicotinic acid, riboflavin, or liver-extract, there was a marked increase of growth and toxin-production.

The fact that the highest bacterial-nitrogen value was obtained in glucose-peptone beef-infusion broth, appears to indicate that the casein hydrolysate still lacks one or more chemical ingredients required for maximal growth.

The work is being continued in an effort to identify a chemically defined medium for maximal growth and consistent toxin production by *Cl. welchii*.

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Retention of Radiophosphorus in Whole and Aliquot Portions of Tissues of Patient Dead of Leukemia.

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This note presents the radioactive phosphorus (P^{32}) and the non-radioactive phosphorus (P^{31}) content of tissues obtained from a patient dead of chronic lymphoid leukemia, who had received orally 19 days before death a single administration of a sodium phosphate solution, containing radio-phosphorus, which emitted 20 millicuries of beta radiation on the day of administration.¹

Materials and Methods. The radio-phosphorus was produced by the Berkeley cyclotron.² The patient was a 65-year-old carpenter

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¹ Lawrence, J. H., Scott, K. G., and Tuttle, L. W., *New International Clinics*, 1939, **3**, 33.

² Lawrence, E. O., and Cooksey, D., *Phys. Rev.*, 1936, **50**, 1131.