not measure the B.M.R.'s of these dogs, studies on other dogs showed that this amount of thyroid usually increases the B.M.R. from 15 to 20%.

Unbeknown to us, Hepler and Simonds[2] had reported that dogs which were fed thyroid showed a greater drop in blood pressure than did normal dogs, when the hepatic veins were occluded for short intervals.

Further studies are being carried on to determine what changes occur that make it possible to produce traumatic shock in experimental hyperthyroid dogs.

Summary. The authors found that prolonged manipulation of the intestines of normal anesthetized dogs, does not produce shock. However, when dogs are fed .4 g of desiccated thyroid per kg of body weight per day for 1 week, these animals on manipulation of the intestines from 15 to 20 minutes, go into shock quite readily.

We wish to thank Dr. Carlson for his interest and advice during the course of these experiments.

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Accessory Growth Factor Requirements of Some Members of the Pasteurella Group.

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Most members of the Pasteurella group of bacteria develop satisfactorily in meat infusion-peptone media but fail to grow in simpler media made from hydrolyzed purified protein or in synthetic media. The substances in infusions of meat, other tissues or yeast which are needed for growth have not been previously identified. Accordingly we wish to report preliminary results of a study of the accessory growth factor requirements of some members of this group of organisms in which it will be shown that nicotinamide, pantothenic acid and, in some cases, the butyl factor for Clostridia are needed for prompt development.*

Seventeen typical *Pasteurella* strains were used. These were stock

2 Hepler, Opal E., and Simonds, J. P., *Arch. Path.*, 1938, **25**, 149.

* We are indebted to Dr. E. E. Snell and Dr. R. J. Williams for the samples of pantothenic acid used in these experiments and to Dr. W. H. Peterson for the butyl factor.

laboratory cultures which had been secured from different sources. They were isolated originally from hemorrhagic septicemias in various species of animals. The results presented in this report apply only to the typical strains of animal origin and not to other species at times included in this genus.

The basal medium consisted of a 0.5% solution of hydrolyzed purified gelatin to which was added a supplement of 8 amino acids, 0.3% glucose, 0.5% NaCl, 0.2% K_2HPO_4, 0.005% $MgSO_4$ and 0.001% $CaCl_2$. To this was added 1 cc of Hoagland salt mixture per liter of medium. The amino acid supplement consisted of 20 mg each of valine, tyrosine, tryptophane, cystine, methionine and histidine and 15 mg each of serine and threonine per liter. The medium was adjusted to pH 7.0 with *N* NaOH solution and tubed in 5 cc quantities.

The accessory growth factors were sterilized by filtration and added aseptically to the basal medium. In the first tests a mixture of known substances was used on the assumption that perhaps some of them might be required by these organisms. This mixture consisted of nicotinamide, diphosphopyridine nucleotide (cozymase), thiamine, thiamine diphosphate (cocarboxylase), riboflavin, beta-alanine, pantothenic acid, vitamin B_6 hydrochloride, nicotinamide methiodide, inositol, glutamine and sodium pyrophosphate.

The *Pasteurella* strains did not develop in the basal medium. Upon addition of the accessory mixture most of the cultures developed readily. Evidently one or more of the added factors was needed by these types. By simplifying the accessory mixture it was found that nicotinamide (or cozymase) and pantothenic acid were required for growth. Neither alone was effective. Two samples of pantothenic acid were used. Results obtained with one sample of 20% purity were duplicated with another of 70% purity. Pantothenic acid could not be replaced by beta-alanine. Tests with several characteristic strains showed that continuous cultivation through successive transplants was accomplished readily in the presence of nicotinamide and pantothenic acid.

Thirteen of the 17 cultures gave results essentially similar to those of *P. avicida* and *P. boviseptica* I shown in Table I. The other 4 cultures produced a scantier though still distinct growth, indicating that other factors or conditions were needed for ready cell multiplication. It was found that addition of the butyl factor for Clostridia[1] (probably biotin[2]) caused prompt and vigorous growth of

[1] McDaniel, L. E., Woolley, D. W., and Peterson, W. H., *J. Bact.*, 1939, **37**, 259; Woolley, D. W., McDaniel, L. E., and Peterson, W. H., *J. Biol. Chem.*, 1939, **131**, 381.

[2] Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1939, **61**, 3594.

TABLE I.
Effect of Growth Factors upon Development of Several *Pasteurella* Species of Animal Origin.

Basal medium with addition of:	Amt added, μg per cc of medium	*Pasteurella* avicida days* 1	avicida days* 2	boviseptica I days 1	boviseptica I days 2	boviseptica 18 days 1	boviseptica 18 days 2
Nothing (control)	—	—	—	—	—	—	—
Nicotinamide	0.1	—	—	—	—	—	—
Pantothenic acid	0.1	—	—	—	—	—	—
Nicotinamide plus pantothenic acid	0.1 each	+++	+++	+++	+++	±	+
Nicotinamide plus beta-alanine	0.1 each	—	—	—	—	—	—
Butyl factor	0.15	—	—	—	—	—	—
Nicotinamide plus pantothenic acid plus butyl factor	0.1 0.1 0.15	+++	+++	+++	+++	++	+++

— = No visible growth, ± = very light turbidity just at point of visibility, + to +++ = light to pronounced turbidity.
*All cultures were held for 10 days at 37° and observed at frequent intervals. Usually there was no change after the second day.

3 of the remaining 4 cultures. The growth of *P. boviseptica* 18 (Table I) is an example of the effect of addition of butyl factor.

On substitution of a mixture of 18 amino acids for the hydrolyzed gelatin solution it was found that with but two exceptions all of the cultures could be grown in the presence of nicotinamide, pantothenic acid and the butyl factor. As far as we are aware cultivation of these organisms in an amino acid medium with the addition of known accessory substances has not hitherto been accomplished.

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Normal and Abnormal Prothrombin Levels.

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While low fibrinogen levels have been reported in pernicious anemia, scurvy, pellagra, acute yellow atrophy, and myeloid dyscrasias, there are not available adequate data as to the prothrombin levels of such diseases in man. Quick[1] postulates that the blood in acute yellow atrophy of the liver may be as deficient in

[1] Quick, A. J., *Am. J. Med. Sci.*, 1940, **199**, 123.