careful search made for traces of thyroid tissue under a binocular dissecting microscope. In animal No. 54, one relatively small piece, and in animal No. 34, two small pieces of thyroid material were found. In the remaining 4 animals no traces of the gland tissue were observed.

That these animals were definitely hypothyroid is shown by the decrease in their rate of oxygen consumption per kilogram per hour. This rate was determined by a modified Benedict apparatus for 10 normal, non-pregnant rabbits, and the average for the group was taken as normal. The decrease, determined one month after delivery of young and shown in the table, varied from 14 to 33%, indicating that all experimental animals exhibited some degree of hypothyroidism.

These results are not in agreement with the work of Ukita, who reported an increase in the period of gestation to from 60 to 70 days in rabbits thyroidectomized from the 7th to 10th day of pregnancy.[2] Our results indicate that experimental hypothyroidism produced after mating apparently had little effect on the duration of the gestation period in the rabbit.

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Growth Factors in Relation to Development of Certain "Fastidious" Bacteria.

STEWART A. KOSER AND FELIX SAUNDERS.

From the Departments of Hygiene and Bacteriology and of Physiological Chemistry, University of Chicago.

It is well known that many of the more fastidious bacteria require special media such as fresh meat infusions for their development and refuse to grow, or at best grow poorly, on the ordinary standard agar and broth made with peptone and meat extract. These organisms, almost without exception, also refuse to grow in a chemically definite or synthetic medium. The nature of the necessary substances in the more complex media is largely a matter of conjecture at present, although it constitutes one of the very important problems of bacterial nutrition.

In the present investigation we have attempted to separate from

[2] Ukita, T., *Physiol. Abst.*, 1920, **5**, 39.

ordinary veal infusion the factors responsible for growth of some of the more exacting bacteria. It seems reasonable to suppose that a more exact knowledge of these substances would not only reveal many practical applications in bacteriological work but would also have a rather general biological significance.

The removal of growth factors from protein digests or infusions by the use of charcoal has been reported by Robinson and Rettger,[1] Thjotta and Avery,[2] Mueller,[3] and Freedman and Funk.[4] In our work it was found that treatment of an ordinary veal infusion with charcoal resulted in the removal of unknown substances necessary for the growth of certain microorganisms. Other commonly used adsorbents, such as Fuller's earth, kaolin, talc, Lloyd's reagent, filter paper, calcium phosphate, Supercel, or silica gel were relatively ineffective. The unknown substances or growth factors, after removal by charcoal from an infusion, could be recovered by extraction from the charcoal with hot ethyl alcohol or hot acetone.

These extracts, after evaporation of the alcohol or acetone and subsequent solution of the residue in water, were effective in "activating" an infusion from which growth factors had been removed,

TABLE I.
Addition of Growth Factors to Synthetic Medium and to a Mineral Salt Solution.

Organisms used	Extract added† cc.	Development in synthetic medium — days			Development in mineral solution (control) — days		
		1	2	7	1	2	7
Corynebacterium diphtheriae, Park 8	.1	—	+	+++	—	—	+
	.01	—	+	+++	—	—	—
	.001	—	—	++	—	—	—
	.0001	—	—	—‡			
	.00001	—	—	—			
	none	—	—	—	—	—	—
Corynebacterium diphtheriae, No. 5	.1	—	++	+++	—	—	—
	.01	—	++	++	—	—	—
	.001	—	—	+			
	.0001	—	—	—			
	none	—	—	—	—	—	—

Inoculations were made from salt solution suspensions of each organism, all tubes receiving 0.1 cc. The suspensions were prepared by lightly inoculating the salt solution from 24- or 48-hour growths on veal infusion agar.

†Amount of extract added to each 5 cc. tube of synthetic medium or mineral solution. The total solids, both organic and mineral, in this sample of extract amounted to 3 mg. per cc. of extract.

‡ In this case ++ growth had appeared after 14 days.

1 Robinson, H. C., and Rettger, L. F., *J. Bact.*, 1918, **3**, 209.

2 Thjotta, T., and Avery, O. T., *J. Exp. Med.*, 1921, **34**, 97.

3 Mueller, J. H., *J. Bact.*, 1922, **7**, 309, 325.

4 Freedman, L., and Funk, C., *J. Metabolic Res.*, 1922, **1**, 457.

or in rendering a standard beef extract-peptone broth more suitable for growth of certain exacting organisms.

The presence of essential growth factors in these extracts could also be shown by their addition to a synthetic medium containing several amino acids, dextrose, and mineral salts. An experiment of this nature is shown in Table I.

In the synthetic medium alone the diphtheria bacilli refused to develop. However, with the addition of small amounts of material extracted from veal infusion good growth was obtained. The amino acids and glucose were used as food and energy sources only when an additional factor was also present.

The total solids in the added extract amounted to only 3 mg. per cc. of extract. Thus, when 0.001 cc. or 0.0001 cc. of extract was added to a tube (5 cc.) of synthetic medium, the added total solids amounted to 0.0006 or 0.00006 mg. per cc. of synthetic medium. These small amounts of added material determined whether or not the diphtheria bacilli were able to develop in a synthetic medium which itself contained 5.3 mg. of organic matter per cc., or roughly ten thousand to one hundred thousand times as much. The extract evidently supplied very little actual food material, for when added to the mineral solution which was used for control purposes, a scanty, delayed growth occurred only where larger amounts, 0.1 cc. per tube, were used.

Quite similar results were obtained with several strains of dysentery bacilli, the typhoid bacillus and *Staphylococcus albus*. On the other hand, several strains of scarlet fever and septic sore throat streptococci, *Streptococcus lactis,* pneumococci and Pasteurella all refused to develop in the synthetic medium plus added extract. Further work showed that the factors responsible for growth were destroyed on ignition. A measured sample of extract was evaporated to dryness and the organic matter burned off. The ash which remained exerted no stimulating effect whatever.

The growth factors dealt with here are believed to belong in the class of growth accessory substances. Their addition to synthetic media, even though in the present impure state, presents a method for securing growth of at least some of the more fastidious bacteria in very simple culture solutions. The use of such solutions may offer another approach to the study of toxins and many other problems connected with the metabolism of these organisms.