indicated that liberation of the hypophyseal luteinizing factor was minimal in the absence of estrogen; the factor remaining stored within the pituitary gland.

Release of the luteinizing factor by estrogen was suggested also by the type of ovarian response produced by pituitaries from oophorectomized adult female rats which had been injected with estradiol benzoate daily for 30 to 45 days. These pituitaries produced follicular development but no corpora lutea, due to removal of the luteinizing factor by the estrogen treatment.

15491 P

Effect of Long Chain Fatty Acids on Bacterial Growth.

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We have shown elsewhere that certain water soluble lipids promote diffuse growth of tubercle bacilli in synthetic media, especially in the presence of serum albumin.[1,2] These same substances have now been found to enhance the growth of other microbial species, in particular of an unidentified micrococcus (strain C), recently isolated in our laboratory. Some understanding has also been gained of the conditions under which long chain fatty acids can stimulate bacterial growth.

It is known that the soaps of fatty acids exert a bacteriostatic and bactericidal effect on certain micro-organisms, particularly on the Gram-positive and acid-fast species, and that unsaturated acids are more toxic than the corresponding saturated compounds.[3-9] For example, concentrations of oleic acid as low as 0.000001-0.00001% are sufficient to cause inhibition or retardation of growth of small inocula of human tubercle bacilli in synthetic liquid media. On the other hand, fatty acid esters (methyl oleate, triethanolamine oleate, phosphatides) exhibit little or no primary toxicity. That the lack of toxicity is not due to poor solubility of the esters is indicated by the fact that the polyoxyethylene derivatives of oleic acid are essentially nontoxic, even though they are completely dispersible in water.[1,2] Thus, tubercle bacilli grow readily in synthetic media to which has been added 0.1-1.0% of Tween 80 (a polyoxyethylene derivative of sorbitan monooleate) purified to remove unesterified fatty acid.[10] Detoxification of the fatty acids can also be achieved by adding to the medium native serum albumin. When an adequate amount of this protein is added to an opalescent soap emulsion (at neutral pH), there occurs an immediate clearing of the emulsion with concomitant disappearance of toxicity. It takes approximately 40 parts by weight of albumin to achieve complete detoxification of 1 part of oleic acid; however, growth of tubercle bacilli can be obtained in media containing 0.01% oleic acid and 0.5% serum albumin if a sufficiently large inoculum is used.

When rendered nontoxic, either by ester-

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TABLE I.
Effect of Oleic and Stearic Acid on Bacterial Growth.

Added to casein hydrolysate medium				Growth in mg/10 ml*—7 days incubation	
Glucose %	Oleic acid %	Stearic acid %	Albumin %	Micrococcus C mg	Avian tubercle bacillus mg
0	0	0	0	0.2	0.1
0	0.001	0	0	1.0	0
0	0.01	0	0	0	0
0.5	0	0	0	0.3	0.1
0.5	0.001	0	0	2.1	0
0.5	0.01	0	0	0	0
0	0	0	0.5	0	0.2
0	0.001	0	0.5	1.0	0.3
0	0.01	0	0.5	3.9	1.1
0.5	0	0	0.5	0	0.3
0.5	0.001	0	0.5	4.1	0.5
0.5	0.01	0	0.5	5.1	1.1
0	0	0.001	0.5	0	0.3
0	0	0.01	0.5	0	0.8
0.5	0	0.001	0.5	0	0.6
0.5	0	0.01	0.5	0	1.2

* The amount of growth was evaluated from measurements of optical density and of sediments obtained by centrifugation in comparison with suspensions containing known weights of bacteria.

ification or by admixture with serum albumin, a number of long chain fatty acids are found able to enhance the growth of certain bacteria; the different bacterial species, however, differ markedly in their response to the various acids. The comparative behavior of tubercle bacilli and of micrococcus C in this respect can be summarized in the following statements.

Enhancement of growth of tubercle bacilli can be obtained by adding 0.01% of any of a variety of long chain fatty acids—saturated or unsaturated—to a medium containing 0.5% crystalline serum albumin. The presence of glucose or of other readily available carbon compounds is not necessary for the development of growth or for demonstration of the enhancing effect of the fatty acid. This finding is in agreement with the fact that several long chain fatty acids have been found to stimulate oxygen uptake by tubercle bacilli.[11] In the case of micrococcus C, on the other hand, abundant growth results from the addition of oleic, linoleic, linolenic or arachidonic acids (0.0001-0.001%) to a mineral medium containing glucose as the sole source of carbon. Saturated fatty acids, on the contrary, appear completely unable to permit growth. In this respect the nutritional requirements of micrococcus C are similar to those of diphtheria and tetanus bacilli.[12,13] Although none of the other substances tested (yeast extract, hydrolysates of casein, protein, etc.) can take the place of the unsaturated fatty acids in initiating growth of the micrococcus, the abundance of the growth is markedly increased by the addition of glucose to a medium containing an adequate concentration of unsaturated acid. Finally, addition of crystalline albumin to media containing only minute concentrations of unsaturated acids completely inhibits the small amount of growth of micrococcus which would have taken place in the absence of the protein; this inhibitory effect can be neutralized by adequate addition

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of oleic, linoleic, linolenic or arachidonic acid. No similar growth inhibitory effect by albumin has been observed in the case of tubercle bacilli.

These different phenomena are illustrated in Table I in which are compared the growths of a strain of avian tubercle bacilli and of micrococcus C in a casein hydrolysate medium to which oleic acid, stearic acid, glucose and serum albumin were added as indicated.

More extensive data concerning the effect of different fatty acids on bacteria—both in liquid and on agar media—will be presented in a forthcoming publication; it will be shown also that, at equal concentrations of long chain fatty acids, the water soluble esters are more efficient than the corresponding soaps in supporting bacterial growth.

It appears worth pointing out at this time that, under the proper cultural conditions, the amount of growth yielded by micrococcus C seems to be directly related to the amount of unsaturated fatty acids present in the medium (between 0.00001 and 0.0001%). This property suggests that the culture might lend itself to the development of a microbiological assay method for these lipids.

15492

Heart Rate of the Albino Rat.*

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Hoskins, Lee and Durrant[1] counted the heart rates of rats by stethoscope while the animals lay dozing in their cages, and they reported a basal rate of 281±18 beats per minute. Fishburne and Cunningham[2] wrapped their rats in a towel for auscultation of the heart sounds; the rates they obtained fell in the neighborhood of 300 beats per minute. Meyer and Yost[3] held their rats in the crook of the arm while palpating the apex beat, and they found the rates of adult rats to vary between 300 and 330 beats per minute.

Drury, Harris and Maudsley,[4] Robertson and Doyle,[5] and Leblond and Hoff[6] recorded electrocardiograms in rats and thus avoided subjective errors, but they did so while forcibly restraining their rats in the supine position to allow insertion of needle electrodes through the skin. Kniazuk[7] described an oscillographic method for obtaining electrocardiograms from rats placed in specially constructed cages. The method developed independently by the present author follows the same principle as that of Kniazuk but has the advantage of making inexpensive permanent records. It involves registration of the electrocardiogram from the surfaces of the rat's feet.

At first, the record was transcribed with the usual electrocardiograph, but, aside from the cost of bromide paper and the time lost in developing the film, that procedure was unsatisfactory in that it failed to tap the heart's potential through plantar callouses that the rats acquired in their mesh-floored cages.

The apparatus finally adopted was that developed for the recording of brain waves

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