time when the surface of the iron shows a lead-like colour, rapidly oxidize the lead-coloured substance to a brownish one, whereas they have no visible effect on iron not activated. The difference between activated and non-activated iron can, of course, be shown immediately after the activation by the addition of a suitable organic reagent, such as nitrosoguanidine. However, it can also be shown by the addition of an alkaline copper tartrate solution, which produces a brilliant coating, often in several colours, on the activated metal.

In most cases the concentration of the sodium hydroxide was 2–5 *N*. The activation was carried out either by mixing the iron material (screws, nails, pieces of soft iron wire) with small amounts of zinc or aluminium or by means of an external current. The experiments were made at room temperature. Further details will be published later.

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[1] [*Nature*, **157**, 550 (1946).]

Effect of Unsaturated Fatty Acids on the Acid Production of *Lactobacillus helveticus*

WE have recently described the effect of certain unsaturated fatty acids upon growth and acid production of *Lactobacillus helveticus* and other Gram-positive bacteria[1,2]. When added to the culture medium in a concentration of about 1 : 100,000, linolenic, linoleic and oleic acids, in that order of efficiency, inhibit the growth of these bacteria. The inhibition can be reversed by the addition of similar concentrations of certain surface-active agents, such as cholesterol or lecithin. We have suggested[2] that these phenomena may be of a physico-chemical nature.

In the case of two unsaturated fatty acids we have since tested for inhibitory activity the *trans*-isomers of the naturally occurring *cis*-forms. The *trans*-forms differ from the *cis*- in their molecular structure, length of crystal cell and surface area of monolayer.

Effect of α- and β-elaeostearic acids. It was not possible to test α-elaeostearic acid or its optical isomers by the methods originally described[1,2], as these substances did not remain stable during aerobic incubation at 37° C. The method adopted therefore was to dissolve the unsaturated fatty acid in ethanol, to add it with sterile precautions to autoclaved media, and to incubate the tubes anaerobically in carbon dioxide. Under these conditions reproducible results were obtained.

TABLE 1. ACID PRODUCTION OF *L. helv.* UNDER ANAEROBIC CONDITIONS (IN CARBON DIOXIDE).

	0·1 *N*-acid formed, ml. (24 hours)	0·1 *N*-acid formed, ml. (48 hours)
10 ml. $CHCl_3$-extracted medium*	4·9	15·6
10 ml. $CHCl_3$-extracted medium + 160 μgm. linoleic acid	0	0
10 ml. $CHCl_3$-extracted medium + 160 μgm. α-elaeostearic acid	1·8	10·9
10 ml. $CHCl_3$-extracted medium + 160 μgm. β-elaeostearic acid	6·1	16·4
10 ml. $CHCl_3$-extracted medium + 160 μgm. elaidic acid	6·1	15·4
10 ml. $CHCl_3$-extracted medium + 160 μgm. oleic acid	0	2·0

* The riboflavin-free medium used was improved[3] to give higher acid production than that used in experiments in Table 2. Riboflavin was added in amounts of 0·5 μgm. per tube.

As noted in Table 1, α-elaeostearic acid in concentrations of 160 μgm. per 10 ml. medium depressed the acid production of *L. helveticus* considerably for 48 hours, whereas the *trans*-isomer, β-elaeostearic acid, was without inhibitory activity. That α-elaeostearic acid was less efficient than linoleic acid may have been due to partial destruction of the former even under anaerobic conditions.

Effect of oleic and elaidic acids. When the lactobacilli were grown aerobically in presence of oleic acid and elaidic acid, respectively, the oleic acid inhibited growth and acid-production completely for the first 24 hr. of the incubation period (Table 2). Elaidic acid (the *trans*-form), however, did not show any inhibiting effect. A similar result was also obtained when both acids were added under the conditions described above, that is, after autoclaving and incubating anaerobically (see Table 1).

TABLE 2. ACID PRODUCTION OF *L. helv.* UNDER AEROBIC CONDITIONS.

	0·1 *N*-acid formed, ml. (24 hours)	0·1 *N*-acid formed, ml. (48 hours)
10 ml. $CHCl_3$-extracted medium*	2·8	7·9
10 ml. $CHCl_3$-extracted medium + 160 μgm. oleic acid	0·4	2·3
10 ml. $CHCl_3$-extracted medium + 160 μgm. elaidic acid	2·3	8·7

* The riboflavin-free medium used was that of Kodicek and Worden[2]. Riboflavin was added in amounts of 0·5 μgm. per tube.

In the case of these two pairs of optical isomers, therefore, the *trans*-forms do not inhibit the acid-production of *L. helveticus* under conditions which permit the naturally occurring *cis*-isomers to do so. The behaviour of the *trans*-forms on surfaces would differ from that of the *cis*-forms, and the results obtained in these experiments are therefore in keeping with the physico-chemical hypothesis of the inhibitory effect[2], although they do not necessarily exclude a purely chemical interaction.

We are indebted to Prof. T. P. Hilditch for suggesting these experiments and for supplying us with specimens of elaidic, α-elaeostearic (m.p. 46·5° C.; $E^{1\ per\ cent}_{1\ cm.}$ at 268 mμ = 1770), and β-elaeostearic (m.p. 71·2° C.) acids. Dr. T. Moore very kindly helped us with the preparation of a second specimen of α-elaeostearic acid (m.p. 46·3° C.) since the original sample deteriorated rapidly as the result of polymerization or oxidation.

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[1] Kodicek, E., and Worden, A. N., *Nature*, **154**, 17 (1944).
[2] Kodicek, E., and Worden, A. N., *Biochem. J.*, **39**, 78 (1945).
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Alcohol-Soluble Osteogenetic Substance from Bone Marrow

IN an interesting communication in *Nature*[1], Prof. Lacroix has reviewed his investigations of an osteogenetic substance which can be extracted from the bone tissue with alcohol, and for which he proposes the name 'osteogenin'. We should like, however, to point out that this substance has already been reported by Levander[2], Annersten[3] and Bertelsen[4]. We are at present engaged in a more detailed investigation of its chemical nature. In experiments performed in collaboration with Dr. Hans Bohr, we have confirmed Annersten's finding that the osteogenetic substance can be shaken out from its alcoholic solution with benzene. Furthermore, by saponification we have divided the lipid substances in the bone marrow into (*a*) the unsaponifiable fraction, (*b*) the fatty acid fraction and (*c*) the residual solution after saponification and extraction of (*a*) and (*b*). The osteogenetic activity in (*a*) was very low, that in (*b*) somewhat higher, while fraction (*c*) was completely inactive. The investigation is being continued.

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[1] *Nature*, **156**, 576 (1945).
[2] *Surg. Gynecol. Obst.*, **67** (1938). *Nature*, **155**, 148 (1945).
[3] *Acta Chirurg. Scand.*, **84**, Suppl. 60 (1940).
[4] *Acta Orthoped. Scand.*, **15**, 139 (1945).

Choline Esterase and its Specificity

A SPECIFIC choline esterase is an enzyme which hydrolyses acetylcholine at a higher rate than any other esters[1]. Richards and Cutkomp[2], however, have found that choline esterase from insect nerves is more active on acetyl-β-methylcholine than on acetylcholine. I have also arrived at similar results with other invertebrates[3]. Thus the whole animal of *Patella vulgata*, and the ampulla and podia of *Asterias rubens* from the west coast of Sweden, split acetyl-β-methylcholine at a higher rate than acetylcholine. Benzoylcholine is very little split. In contrast to this, *Terebratulina caput serpentis* hydrolyses acetylcholine but neither acetyl-β-methylcholine nor benzoylcholine.

Characteristic of the specific choline esterase is said to be the inhibition of the enzyme by excess of substrate. Moreover, acetyl-β-methylcholine but not benzoylcholine is hydrolysed by it. If we compare the activity-*pS* curves (*pS* is − log molar concentration of the substrate) with acetylcholine and acetyl-β-methylcholine as substrates, these curves might have the same shape. I have done this with different materials and have found that it is actually the case for the choline esterase of brain and red blood cells. It is not so, however, for the choline esterase in the blood of *Helix pomatia*. Thus hydrolysis is depressed when the substrate concentration is above the optimum when the substrate is acetylcholine but not when it is acetyl-β-methylcholine. The optimum substrate concentration for acetylcholine is about $2{\cdot}6 \times 10^{-3}$ *M*, for acetyl-β-methylcholine 10^{-1} *M*. In another respect also the choline esterase in *Helix* blood seems to be distinguished from the specific esterases in, for example, brain and erythrocytes from higher animals or in other tissues from *Helix*. The hydrolysis of acetylcholine by blood from the edible snail seems to be a zero-order reaction, that of acetyl-β-methylcholine a first-order reaction. Acetylaneurin is split at the same rate as acetyl-β-methylcholine, tributyrin at a lower rate; benzoylcholine and ethyl acetate are practically not hydrolysed at all.

Among other tissues investigated from *Helix pomatia*, the dart sac is surprisingly active as regards choline esterase; thus *Q* is 10; for comparison, *Q* for the choline esterase of frog muscle is 0·4–0·6[4]. The activity is very distinct from that of the snail blood. The hydrolysis of acetylcholine by choline esterase in the dart sac is not depressed by high substrate concentrations; thus this enzyme should be called 'pseudo'-choline esterase. Benzoylcholine is not at all or only slightly hydrolysed, while acetyl-β-methylcholine is even somewhat more attached. Extracts of this tissue do not split acetylaneurin and ethyl acetate. The activity against tributyrin, on the other hand, is higher relatively than that of choline esterase in *Helix* blood.

These results will shortly be discussed more fully elsewhere. We may, however, conclude that the last word has not been said regard-