EFFECT OF SOME FATTY ACIDS ON THE OXYGEN UPTAKE OF *MYCOBACT. TUBERCUL. HUM.* IN RELATION TO THEIR BACTERICIDAL ACTION

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IN connexion with our work¹ on antibiotics against *Mycobact. tubercul.* the need of a rapid standardization method led us to try the Warburg technique for measuring the oxygen consumption. This method has been generally used for similar investigations on faster growing bacteria. We have found the method very satisfactory with *Mycobact. tubercul.* if a large amount of bacteria (1.5–6 mgm. wet weight) is used in each vessel. The slow growth of these bacteria seems to have discouraged previous workers on anti-tuberculous substances from using this method. However, it has been shown that the oxygen consumption of *Mycobact. tubercul.* is conveniently measurable with the Warburg technique^{2,3}.

In our standard procedure we used ordinary Warburg cups with one side-bulb. The main space of the sterilized vessels contained 2.25 ml. 5 per cent glycerol broth (pH 7.4) and 1 ml. of the solution containing the substance to be tested. The central cup contained 0.1 ml. 5 per cent potassium hydroxide and the side-bulb 0.3 ml. of a 1 per cent (wet weight) suspension of *Mycobact. tubercul. hum.* in saline. The vessels were shaken (80 strokes per minute) for one hour at 37° to ensure equilibration before admixing the bacteria from the side-bulb. It was generally sufficient to follow the oxygen consumption for eighteen hours.

The bacteria were collected from three-weeks old cultures on Löwenstein egg medium. The strain was isolated in 1944 from a case of human tuberculosis.

The oxygen uptake was in most cases approximately constant during the experiment (0.5–2.5 mm.³ oxygen per hour and mgm. (bacteria) (cf. Fig. 1 (6) and Fig. 2 (12)).

Sometimes it happened that it decreased slightly, but more frequently the oxygen consumption increased quite rapidly up to many times its initial values, resembling the oxygen uptake during the logarithmic growth-phase of faster growing bacteria. This speed-up was entirely due to the tubercle bacteria, for at the end of the experiments the fluid was found not to be contaminated with other bacteria. In fifteen of our 155 standardization series to date, each comprising twenty-two vessels, the oxygen uptake was so slow that no results could be obtained.

When the observation of the oxygen uptake was ended in the Warburg apparatus we transferred the contents into sterilized tubes that were centrifuged. The sediment was inoculated on Löwenstein egg medium and inspected after 2–4 weeks incubation. The results of this incubation thus show the bactericidal effect caused by the compound tested in the Warburg vessel.

Fig. 1. OXYGEN UPTAKE OF 3 MGm. TUBERCLE BACILLI IN THE PRESENCE OF DI-*n*-HEPTYLACETIC ACID.

(1), 1:7,000 (0.5 mgm.); (2), 1:14,000 (0.25 mgm.); (3), 1:28,000 (0.125 mgm.); (4), 1:56,000 (0.063 mgm.); (5), 1:112,000 (0.031 mgm.); (6), control.

A typical experiment is shown in Fig. 1, illustrating the effect of diheptylacetic acid. This acid is reported to be bacteriostatic against tubercle at approximately 1:50,000⁴. Fig. 1 shows that this acid decreased the oxygen uptake more and more at higher concentrations. The results of the incubation of the bacteria from this experiment are shown in the following table.

Vessel	1	2	3	4	5	6
Bacterial growth	(+)	+	+(+)	++	+(+)	++
Dilution	1:7,000	1:14,000	1:28,000	1:56,000	1:112,000	1:∞

(+), 1–8 colonies; ++, fair growth; ++, strong growth.

We have also tested other types of fatty acids and found that the unsaturated ones, namely, oleic, linoleic and linolenic, had a very marked effect on the oxygen uptake of the bacilli. Fig. 2 shows the influence of various concentrations of linolenic acid.

Fig. 2. OXYGEN UPTAKE OF 3 MGm. TUBERCLE BACILLI IN THE PRESENCE OF LINOLENIC ACID.

(7), 1:7,000 (0.5 mgm.); (8), 1:14,000 (0.25 mgm.); (9), 1:28,000 (0.125 mgm.); (10), 1:56,000 (0.063 mgm.); (11), 1:112,000 (0.031 mgm.); (12), control.

Incubation results from this experiment were as follows:

Vessel	7	8	9	10	11	12
Bacterial growth	0	0	(+)	++	+	++
Dilution	1:7,000	1:14,000	1:28,000	1:56,000	1:112,000	1:∞

0, no growth; (+), 1-8 colonies; +, fair growth; ++, strong growth.

The effect of these unsaturated acids was different from that of the saturated ones (Fig. 1). The unsaturated acids, unlike diheptylacetic acid, regularly showed a certain limit concentration above which the oxygen consumption of the bacteria was practically abolished and below which the bacteria were unaffected. The effect is caused by the double bonds, as stearic acid was completely inactive.

The results of the incubation tests show that linolenic acid had a very strong bactericidal effect, all out of 3 mgm. bacteria being killed at 1:14,000 and almost all at 1:28,000 after eighteen hours. As will be seen from Fig. 2, the abolition of the oxygen uptake runs parallel with the bactericidal effect. The bacteriostatically most active synthetic acid of Adams *et al.* (loc. cit.) was thus in our experiments considerably less active than the common unsaturated fatty acids occurring in the body.

In five series the following dilutions were found on an average to cause abolition of the oxygen uptake of 3 mgm. bacteria in 3.5 ml. volume:

Oleic acid	..	..	1:10,000
Linoleic acid	..	..	1:15,000
Linolenic acid	..	..	1:30,000

We found that this dilution may vary somewhat with different batches of bacteria, but the relative strengths of the acids are very constant and we were able to obtain reproducible values by always including a 'standard' acid in each series. We also found that bacteria with a greater oxygen uptake are regularly less susceptible to the effect of these acids than bacteria with a small oxygen uptake.

The unsaturated fatty acids obviously get adsorbed to the tubercle bacilli. We observed that the bactericidal activity was considerably decreased in the supernatant liquid after centrifuging off the bacteria after a Warburg experiment.

In correspondence herewith there was a direct proportion between the amount of bacteria added and the quantity of fatty acid needed for the abolition of their oxygen consumption.

Addition of 0.5 ml. of blood plasma reduced the activity 4-8 times. It seems quite likely that the fatty acids are partly adsorbed on the serum proteins, thus decreasing the action on the bacteria.

We have also tested the bacteriostatic activity of the acids mentioned above with the technique of Pryce⁵ and Muller⁶. The bacteria, fixed on glass plates, were incubated for seven days in citrated blood, haemolysed by the addition of an equal volume of water, in which the compound to be tested had been dissolved. Some results are given in the following table:

Dilution	1:4,000	1:8,000	1:16,000	1:32,000	1:64,000	1:128,000	1:256,000
Di-n-heptylacetic acid	+	+	++	++	++	++	++
Oleic acid	-	(+)	+	++	++	++	++
Linoleic acid	-	-	(+)	(+)	+	++	++
Linolenic acid	-	(+)	(+)	+	++	++	++

-, no colonies; (+), few, small colonies; +, few colonies, but size equal to control; ++, equal to control.

As will be seen from the table, the bacteriostatic activity of di-n-heptylacetic acid is rather weak in the presence of blood. The common unsaturated fatty acids are more active also in this test. The fact that linolenic acid is less active than linoleic acid might be explained by its more rapid destruction by autoxidation during the incubation.

It seems interesting that these unsaturated acids, occurring normally in the animal body, are more active against tubercle bacilli than the most active saturated acid synthesized hitherto. It is also interesting in view of the numerous reports especially in older literature about treatment of various tuberculous lesions with soaps or oils mainly consisting of these unsaturated fatty acids⁷.

During the course of our experiments on the purification of the antibiotica against *Mycobact. tubercul.* mentioned above⁸ the Warburg technique proved to be very useful. However, it should be pointed out that the results of Davide¹ cannot be ascribed to the common unsaturated fatty acids.

¹ Davide, *Arkiv Kemi, Min., Geol.*, B, 19, No. 7 (1945).

² Kempner, *Amer. Rev. Tub.*, 46, 157 (1945).

³ Dieckmann and Menzel, *Z. Hyg. Inf. Krankh.*, 113, 709 (1932).

⁴ Stanley, Coleman, Greer, Sacks and Roger Adams, *J. Pharmacol.*, 45, 121 (1932).

⁵ Pryce, *J. Path. Bact.*, 53, 327 (1941).

⁶ Muller, *J. Path. Bact.*, 56, 429 (1944).

⁷ Lindenberg and Pestana, *Z. Immun. Forsch.*, 32, 66 (1921).

⁸ To be published later.

DISCOVERY OF ELEMENTS 95 AND 96 AND THE CHEMICAL PROPERTIES OF THE TRANSURANIC ELEMENTS

THE discovery of the elements 95 and 96 was announced on November 16, 1945, by Prof. G. T. Seaborg, of the University of California, now working at the 'Metallurgical Laboratory' of Chicago University (*Ind. and Eng. Chem.*, News Edition, 2085 and 2190; 1945). Isotopes of the two elements—as yet nameless, apparently owing to lack of 'transplutonic' planets—have been produced by bombarding uranium 238 and plutonium 239 with 40 MeV. α -particles in the Berkeley 60-inch cyclotron. Enough material has been made for chemical studies by tracer technique in the way in which the properties of element 93 (neptunium; Np) and 94 (plutonium; Pu) were first investigated in 1940. Nuclear properties of the new elements, including action of neutrons on them, are not discussed in the lecture.

According to Seaborg, the elements beginning from 89 (actinium) have a sufficiently similar electronic structure to warrant their consideration as a group ('actinide series') in the way in which the rare earths are so considered ('lanthanide series'). Although the first members of the actinide group (actinium, thorium, protoactinium, uranium) show great chemical resemblance to the elements in the vertical columns of the Periodic Table, in which they have generally so far been included (yttrium, zirconium, niobium, molybdenum, and lanthanum, hafnium, tantalum, tungsten), later members behave differently: neptunium is quite unlike rhenium, plutonium quite unlike ruthenium or osmium. Seaborg suggests that, beginning with thorium, the 5f rather