CXIX. THE INFLUENCE OF TEMPERATURE ON THE NATURE OF THE FAT FORMED BY LIVING ORGANISMS.

By LEONORE KLETZ PEARSON
AND HENRY STANLEY RAPER.

From the Physiology Department, University of Manchester.

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LEATHES and RAPER [1925, p. 119] have put forward the view that the temperature at which fats are formed in nature is one of the factors which determine their degree of unsaturation. This suggestion is based mainly on three lines of reasoning. Firstly, a general survey of the fats found in animals and plants indicates that the highly unsaturated fats are most commonly found in plants growing in temperate or cold regions and in the poikilothermic animals which live under relatively cold conditions, whilst the most saturated fats are found in tropical or subtropical plants and in warm-blooded animals. The carnivora should not be included in this generalisation because the composition of their adipose tissue fat is partly, at any rate, determined by the fat which they take as food, and even in animals such as the pig, which produce fat easily from carbohydrate, differences have been observed in the composition of their stored fat which may be referred to differences in the composition of the fats present in the cereals on which they have been fed. Secondly, it is usual to find that the fat present in living organisms is fluid at the temperature at which the organisms exist. This would preclude the formation of saturated acids to any great extent in organisms living under temperate or cold conditions because such acids would tend to raise the melting points even of mixed glycerides which contained them. The higher melting point of the saturated acids would be less important from this point of view in the warm-blooded animals or tropical plants and it might be more likely therefore to expect saturated acids to occur to a greater extent in organisms living under warmer conditions. Thirdly, various hypotheses have been made regarding the chemical reactions by which fatty acids are formed in nature, *viz.* autocondensation of acetaldehyde [Leathes, 1906], condensation of pyruvic acid and acetaldehyde [Smedley and Lubrzynska, 1913], or autocondensation of pyruvic acid [Neuberg and Arinstein, 1921]. All these admit of the possibility of the formation of long chains of carbon atoms with unsaturated linkages as products of the condensation reactions which produce them and if they are to give rise to saturated carbon chains a reduction must take place subsequently.

If this represents what takes place, then the unsaturated acids would be the precursors of the saturated. Two different types of reaction are involved, namely, condensation and reduction. It seems possible that both these reactions would not necessarily take place with the same ease at low temperatures and, if it be assumed that the condensation takes place readily at low temperatures whereas the reduction requires a higher temperature, an explanation of the distribution in nature of the saturated and unsaturated acids referred to above might be found on these lines. This would not imply, as might at first sight be thought, that at any given temperature the exact degree of unsaturation of a fat would be determined solely by that temperature. It would also depend on other factors, *e.g.* the amount of the catalyst present which brings about the reduction, or the extent to which the synthesis into glycerides was taking place, thus possibly inhibiting further reduction.

It seemed to us that it would be possible to test the main hypothesis as to the influence of temperature, experimentally, by growing an organism at different temperatures, all other conditions being kept the same. Certain of the fungi seemed likely to be suitable subjects since they grow fairly readily at different temperatures and some of them contain notable amounts of fat. The work was actually begun three years ago but, owing to one of us (L. K. P.) having to withdraw owing to unforeseen circumstances, it had to be discontinued in 1926 and a further opportunity to continue it has not yet arisen. In the meantime, Terroine, Bonnet, Kopp and Véchot [1927] working on almost the same lines have obtained results in complete accord with our own. We have therefore thought it advisable to publish the data we have obtained up to the present. The work will be extended when a suitable opportunity offers.

EXPERIMENTAL.

Two fungi were used, namely, *Aspergillus niger* and *Rhizopus nigricans*. The former was grown on Czapek's solution to which 1 % ammonium sulphate and 3 % glucose were added. The latter would not grow on this medium but grew well in Meyer's solution with the addition of 1 % asparagine and 3 % glucose. The fungi were grown in litre flasks containing 500 cc. of the medium which were sterilised in the usual way. It was found possible to grow *Aspergillus niger* at three temperatures, namely, 18°, 25° and 35°, whereas *Rhizopus nigricans* was grown only at 12° and 25°, it being found impossible to grow this organism at 35°. The rate of growth varied with the temperature and it was allowed to continue till a good mat of the mould covered the surface of the medium. In some instances the fungus was left for some time after this had occurred in order to see what effect this would have on the amount of fat present and on its iodine value.

The methods used for extracting the fat from the mould were as follows. The mat of fungus was broken up, strained and pressed on a Büchner funnel and well washed with distilled water; after roughly drying between filterpaper it was weighed. It was next covered with alcohol in a wide-necked flask and allowed to stand at least 24 hours. The alcohol was then removed by filtration and the fungus extracted with hot alcohol by suspending it in a thimble from the lower end of a reflux condenser which was attached to a flask containing 75 cc. boiling absolute alcohol. After extracting for 3 hours, the alcohol was removed from the flask and a further extraction with a fresh portion of 75 cc. alcohol was carried out. The three alcoholic extracts were combined, 15 cc. of 50 % aqueous KOH were added and most of the alcohol was distilled off on the water-bath, the fat undergoing saponification during this process. The fatty acids and unsaponifiable matter soluble in light petroleum were obtained from this solution by acidification and shaking with a measured quantity of light petroleum as in the usual method of fat estimation by the modified Liebermann method [cf. Leathes and Raper, 1925, p. 58]. From an aliquot part of the light petroleum extract, the fatty acids were removed by shaking with dilute sodium hydroxide in 50 % alcohol and then, after liberating the acids again with sulphuric acid, they were taken up in light petroleum, the solvent was distilled off in an atmosphere of CO_2, the flask finally evacuated for half an hour whilst in a boiling water-bath and the fatty acids weighed. The mean molecular weight was determined by titration in excess of alcohol with aqueous $N/10$ NaOH and, after recovery of the fatty acids from this solution by acidifying and extracting with light petroleum, their iodine value was determined by Wijs' method.

The results obtained are given in the accompanying table.

Fungus	Temp.	Time of growth (days)	Moist wt. of fungus (g.)	Total wt. of fatty acids (g.)	Mean molecular wt.	Iodine number	Mean iodine number
Aspergillus niger	18°	17	49·8	0·323	303	146·7	149
		17	46·3	0·206	300	153·6	
		56	39·9	0·084	323	150·2	
		56	41·5	0·086	330	145·9	
	25°	10	59·4	0·276	287	124	129
		14	50·3	0·302	304	132·5	
		17	51·1	0·254	290	131·3	
		35	40·5	0·252	287	127	
	35°	6	39·2	0·468	293	92·1	95
		7	38·8	0·527	285	92·3	
		9	37·8	0·633	290	99·9	
Rhizopus nigricans	12°	30	34·6	1·705	281	88·7	88
		30	31·0	1·669	289	87·2	
	25°	10	32·5	1·082	288	79	78
		13	35·4	1·168	287	77·3	

Discussion.

It will be seen, with both fungi, that the more unsaturated fats are produced at the lower temperatures, and, in the case of *Aspergillus niger*, the fat produced at a temperature intermediate between the highest and lowest had also an intermediate iodine value. The period of growth does not appear to affect the results at any given temperature. Comparable results have been obtained by Terroine, Bonnet, Kopp and Véchot [1927] with the mould *Sterigmatocystis nigra*[1]. When grown at 35° the iodine value of its fatty acids was 85, whereas when grown at 17° it was 114. With the Timothy Grass bacillus the iodine value of the fatty acids was 33 when grown at 35° and 58 at 14°. These observers also determined the iodine value of the total fatty acids of a mollusc (*Melanopsis donneti*) and a fish (*Astatotilapia desfontainesi*) living at a temperature of 30°, the figures obtained being 91 and 87 respectively, whereas the fatty acids of a mollusc (*Limnaea peregra*) living at 8° had an iodine value of 125. These results all support the view that the temperature of the environment has an influence on the degree of unsaturation of the fatty acids which organisms produce. This however does not prove that all the deductions are correct which led to the hypothesis that temperature influences the nature of the fat formed in living organisms. Although in our own work, and that of Terroine and his colleagues, temperature was the only variable imposed by the experimenters on the different organisms, it was not necessarily the only variable in the experiments. It has been shown by the French workers that the composition of the organisms grown at different temperatures is not absolutely the same, though the differences are not very great. Then, difference in conditions of growth, *e.g.* temperature, the rate of growth, the type of medium, the exhaustion of the medium, affect the morphological character of fungi and have some influence on spore formation. These modifications of the life processes of the organisms imply that the chemical reactions which are normally taking place in them will be considerably modified. For these reasons it cannot be considered that the few experiments which have been carried out in this field of work prove that the changes observed in the composition of the fatty acids in organisms grown at different temperatures are simply the result of the effect of temperature on the two chemical reactions which have been thought to play a great part in fat formation, *i.e.* condensation of some simple aliphatic substance and reduction of the unsaturated compounds produced. Terroine and his colleagues, by comparing the energy loss from the medium during growth with the potential energy of the fungus formed at different temperatures, have shown that the expenditure of energy is somewhat greater at the higher temperatures. This indicates that the production of the more saturated acids under these conditions involves a greater utilisation of the potential energy of the medium and suggests that reduction succeeds condensation in the series of reactions by which fatty acids are produced, and thereby involves a greater expenditure of energy.

SUMMARY.

1. The iodine value of the fatty acids produced by *Aspergillus niger* and *Rhizopus nigricans* varies with the temperature at which these organisms are grown.

[1] A synonym of *Aspergillus niger*.

2. At higher temperatures the fatty acids produced are more saturated, this being in conformity with the observation that, in general, the more saturated fats found in nature are usually produced at higher temperatures than the less saturated ones.

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