Esterification of Fatty Acids with Glycerol

Biswas and Ganguly[1] have recently published observations on the esterification of fatty acids with glycerol which are not in full agreement with the results of the work carried out at present in this Laboratory. It is hoped to publish the details of this work elsewhere, but it might be of interest to discuss a few salient points here.

Biswas and Ganguly state that since esterification of fatty acids with glycerol and glycerolysis of fats yield appreciable amounts of β-monoglycerides the ignoring of their presence could lead to a wrong computation of diglycerides and hence also of triglycerides based on the estimation of monoglycerides and hydroxyl values. (Incidentally the results for β-monoglyceride contents obtained by Biswas and Ganguly in the esterification of oleic acid, namely, 0·04 per cent, differ greatly from the value of 3 per cent obtained under similar conditions in this Laboratory, and are probably much too low.) The occurrence of β-monoesters in technical monoglycerides prepared by alkali-catalysed glycerolysis of fats has been repeatedly reported in the past few years[2–4] and Biswas and Ganguly's advice as to the need of accounting for their presence appears reasonable. However, there is here a curious anomaly. Some years ago Feuge and Bailey[5] suggested that glycerolysis proceeds according to chance inasmuch as the amounts of mono-, di- and tri-glycerides found experimentally in equilibrated products follow closely compositions calculated on the basis of random distribution of acyl groups. These results were confirmed recently by Choudhury[6] in a number of oils. In his investigation the conclusions were based on the estimation of α-monoglycerides by the periodate method and on hydroxyl values, the presence of β-monoglycerides being dismissed as insignificant. In reality β-monoesters in freshly prepared products may amount to as much as 10 per cent of total monoglycerides and normally to about 3 per cent absolute. Since on the basis of hydroxyl values 1 per cent of monoglyceride with an average hydroxyl value of 320 is equivalent to 3·5 per cent of diglyceride with an average hydroxyl value of 90 it would follow that Choudhury's conclusions regarding glycerolysis were wrong. If in his calculations the hydroxyl values due to β-monoglycerides were taken into consideration, the contents of diglycerides would, in most cases, be reduced by some 10 per cent and the contents of triglycerides increased by a similar amount. The results would be incompatible with random esterification.

However, recent investigations[4,7] in which total mono- di- and tri-glycerides were determined chromatographically point clearly towards random or near random esterification. It would seem therefore justifiable to suggest that Choudhury's estimations of α-monoglycerides or more likely of hydroxyl values were inexact, and that this error led to the correct assumption of random esterification. The computation of di- and tri-glycerides from hydroxyl values is thus open to criticism whether the amount of β-monoglycerides is known or not. A direct estimation of mono-, di- and tri-glycerides seems to be required.

Another issue raised by Biswas and Ganguly is the esterification-rate of various fatty acids. Their experiments show that fatty acids of medium molecular weight (such as lauric) and unsaturated acids (oleic, linoleic) react with glycerol faster than high molecular saturated acids (stearic). They attribute these differences to the higher solubility of glycerol in lauric and oleic acids than in stearic acid. This opinion and similar views on the effects of the solubility of glycerol in fats[8] are based on a fallacy. Admittedly, on the weight basis the solubility of glycerol in fats rich in stearic acid is lower by about one-tenth than in fats rich in lauric acid[9]. On the basis of molar equivalents, however—and this is the basis of Biswas and Ganguly's own kinetic measurements—the solubility of glycerol in stearic acid is by one-tenth higher than in lauric and equal to that in oleic acid, which seems to dispose of Biswas and Ganguly's explanation. According to earlier investigations[10,11], there is no appreciable difference between the reactivities of various fatty acids with methanol except those with less than four carbon atoms. This has now been confirmed in this Laboratory in the case of glycerol by carrying out the esterification in high-boiling inert solvents. The different rates of esterification of various fatty acids with glycerol in the absence of co-solvents appear thus to be due to factors other than the reactivity of fatty acids. The investigation of these factors is in progress.

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BIOCHEMISTRY

Actinonin : an Antibiotic Substance produced by an Actinomycete

During a programme of screening of soil and other materials to select antibiotically active micro-organisms, Drs. R. Green and R. Bhagwan Singh, of the Institute for Medical Research, Kuala Lumpur, isolated a new species of an actinomycete, later classified under the serial number *Streptomyces* Cutter *C*/2 (N.C.I.B. 8845). It was active against a number of bacterial strains, including *Mycobacterium phlei* and *Staphylococcus aureus*.

We have found that the organism grows well in deep culture at 30°–32° in a medium containing a source of nitrogen (for example, dried autolysed yeast) and a carbohydrate (for example, starch) in stirred, aerated flasks or fermentation vessels; such cultures show the same type of antibacterial activity as on plate culture, the activity being present practically exclusively in the medium.

Isolation of the active substance (named 'actinonin') on a large scale (that is, from 300 l. of culture) proved to be a relatively straightforward matter. The material was completely transferable from the culture filtrate into *n*-butanol from which, after concentration, it was further purified by a series of partitions between aqueous solution and chloroform