

Preparation of Cell-free Plasma Coagulase of *Staphylococcus aureus*

IN 1908, Much reported that certain strains of *Staphylococcus* had the property of coagulating oxalated or citrated human blood plasma. This is nowadays considered as one of the most characteristic features of pathogenic *Staphylococcus aureus*, and is used to differentiate it from various other cocci. To my knowledge, attempts to separate the plasma coagulase from the bacterial cells have so far failed; for example, in personal experiments by filtration of broth cultures through Seitz and Chamberland filters, separation of cells by the centrifuge, killing the staphylococci by heat or the vapour of ether or chloroform.

In order to explain the inactivity of the filtrate or centrifugate, it has been taken as a working hypothesis that coagulase is not produced in such cultures, but is formed only in presence of plasma. This also had another basis in my finding that the rate of coagulation was independent of the age of broth cultures and was uninfluenced by repeated washing of the suspensions. Such results were opposed to the existence of preformed coagulase in cultures on ordinary media.

To verify the hypothesis that coagulase is produced only in contact with plasma, 10–25 per cent of human citrated plasma was added to a series of flasks containing nutrient broth. Control sets contained broth, and broth with sodium citrate only. All were inoculated with *Staphylococcus aureus*, three separate strains being tested. After twelve hours incubation at 37° C., the cultures were centrifuged and the supernatant fluid filtered through Chamberland L3 filters. The filtrates, which were tested for sterility, were added in various amounts to separate 1 c.c.'s of concentrations of human citrated plasma ranging from full strength to 1:40 in 0.85 per cent sodium chloride solution. While filtrates of broth and broth plus citrate were inactive, the filtrates of plasma-broth cultures showed a very strong coagulating power; for example, one drop of 1:10 dilution coagulated concentrated plasma in two hours and 1:10 plasma in fifteen minutes. Thus it appears that cell-free plasma coagulase can be obtained when *Staphylococcus aureus* is grown in presence of human plasma.

There is also abundant production *in vivo* by *Staphylococcus aureus* of plasma coagulase, which can be demonstrated readily in pus, etc. This 'direct coagulase test', which will be described elsewhere, affords a very rapid and reliable method of detecting staphylococcal infections.

After the above letter was written, my attention was directed to a paper by W. Smith and J. H. Hale¹, who found that by means of 'Gradocol' membranes coagulase could be demonstrated in broth culture filtrates. It is of interest that, as I observed, the addition of plasma to a broth culture led to the appearance of coagulase which was easily filterable through filters of the Chamberland type, whereas in absence of plasma neither centrifugates nor filtrates were active.

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¹ *Brit. J. Exp. Path.*, 25, 101; 1944.

Ailanthus, Source of a Peculiar London Honey

THERE can be few beekeepers in the heart of London, and one would not expect the Metropolitan area to be a promising locality for honey production. I therefore welcomed an opportunity of examining a sample of honey from an apiary in Kensington not far from Kensington Gardens, which was brought to my notice in 1943, on account of its unusual flavour. This honey was of a pale greenish-brown colour and after about three months in store set with a fine granulation. The first impression on tasting it was of a mild floral bouquet, but this was followed by a persistent after-taste reminiscent of cats. This flavour recalls exactly the cat-like odour given off by elder flowers (*Sambucus nigra*) when they are drying, and at first suggested that elder might be responsible. The pollens obtained by dilution in water and sedimentation were examined, but *Sambucus* pollen was absent. The most abundant, forming 44 per cent of the total, came from the Tree of Heaven, *Ailanthus altissima*, which is common as a street tree in Kensington. This species is dioecious, and the male flowers, especially, have a strong rather unpleasant odour recalling that of elder. A second major constituent was the sweet chestnut, *Castanea sativa*, the flowers of which also have a strong unpleasant aroma.

The fact that the peculiar after-taste is attributable to the *Ailanthus* was confirmed by the examination of a 1944 honey sample from the same apiary. In this (see table) *Ailanthus* pollen again preponderates, but *Castanea* forms rather less than 4 per cent of the total. The other major constituent in this season was privet, *Ligustrum vulgare*, which yields a coarse-flavoured honey, but this is of not uncommon occurrence and it is not responsible for the after-flavour.

POLLEN ANALYSES OF KENSINGTON HONEYS. THE FREQUENCY OF THE MAIN CONSTITUENTS IS EXPRESSED AS A PERCENTAGE OF THE TOTAL NUMBER OF POLLEN GRAINS.

Pollen type	Season	
	1943	1944
Tree of Heaven, <i>Ailanthus altissima</i>	44.0	37.7
Sweet chestnut, <i>Castanea sativa</i>	26.0	3.7
Privet, <i>Ligustrum vulgare</i>	6.2	28.8
Limes, <i>Tilia</i> spp.	6.6	4.0
Willow herb, <i>Epilobium angustifolium</i>	0.6	8.8
Horse chestnut, <i>Aesculus Hippocastanum</i>	0.6	1.7
Miscellaneous pollens	16.0	15.3
	100.0	100.0

Beekeepers are apt to attribute unpleasant flavours of unknown origin to the presence of honeydew in the honey. Off flavours from this cause are due not to the honeydew itself but to the sooty moulds that grow in it. Several fungi are concerned, probably the most abundant in Great Britain being *Cladosporium herbarum*. On the evidence of the sooty mould spores, there was very little honeydew in the honeys under review, the mould spores being 5.0 and 9.1 per cent as numerous as the pollen grains in 1943 and 1944 respectively.

The cat-like odour of elder flowers is lost when they are quite dry, and gives way to a pleasant aroma. In addition to medicinal and cosmetic uses, the flowers have been employed in food products to impart a muscatel flavour. The substances responsible for this flavour presumably are derived from the unpleasant smelling constituents of the essential oil. The nature