Meat and Fish Flavors

Significance of Ribomononucleotides and Their Metabolites

N. R. Jones

A review paper covers the innate tastes of ribomononucleotides and their breakdown products, separately and in mixtures; flavor-enhancing properties of these compounds and composite effects in the presence of others; flavor-precursing qualities by reaction with other groups of compounds during cooling, processing, and storage; ribomononucleotides as key biochemical determinants of concentrations of other flavorous compounds; and estimation of 5′-ribomononucleotide and their breakdown products, particularly hypoxanthine as indices of freshness and quality.

While the ribomononucleotides have become increasingly important as flavor additives or enhancers during the past decade, their identification as flavorous compounds, naturally occurring in foodstuffs, extends back over 50 years. As long ago as 1913, Kodama isolated inosine 5′-monophosphate as a constituent to which could be attributed flavor characteristics of *Katsuobushi*, a molded, dried, Japanese bonito preparation. Furthermore, the early patents predating the microbiological and semisynthetic production of ribomononucleotides employed animal or fish sources of raw material.

About 10 years ago, Japanese and British workers became interested in the biochemical changes that occur in meat and fish prior to microbial spoilage and relate to quality change. At about the same time, American workers were separating flavor-precursing systems from meat and reported that nucleotide derivatives were present in active fractions. Subsequently, a body of information has become available on the significance of purine-containing compounds and their derivatives as constituents of fresh, stored, and processed flesh foods. Some of these reports have been at apparent variance with each other but such discrepancies are explicable on the basis of present knowledge.

Five distinct facets of research can be distinguished in the field:

The innate tastes of ribomononucleotides and their breakdown products, separately and in mixtures.

Flavor-enhancing properties of these compounds and composite effects in the presence of other compounds.

Flavor-precursing qualities by reaction with other groups of compounds during cooking, processing, and storage.

Ribomononucleotides as key biochemical determinants of concentrations of other potentially flavorous compounds in stored meat and fish.

Estimations of concentrations of 5′-ribomononucleotides and their breakdown products, particularly hypoxanthine, as indices of freshness and quality.

Failure to distinguish among such considerations can lead to errors in interpretation. For instance, Tarr (1966), commenting on the relevance of nucleotide degradation to flavor change and quality evaluation in the context of hypoxanthine production may not have appreciated that the monitoring of hypoxanthine concentration at subthreshold levels in flesh foods can nevertheless provide an index of changes in ribomononucleotide content that are relevant to flavor loss, rather than the appearance of off-flavor. Neither does he appear to recognize the deep significance in terms of composite effects with other compounds present in tissue of different stages of freshness of Spinelli's observation (1966) that added hypoxanthine confers bitterness to spoiling fish but not to fresh.

Workers in this field should also bear in mind that they may be dealing with biological material that is innately variable in the animal at the point of slaughter and variable in composition in dying tissue thereafter; and that processes such as freezing and thawing can introduce compositional changes in materials that may be considered to be stable and suitable for control purposes in their experimentation.

Tropical Products Institute, 56-62, Gray's Inn Road, London, United Kingdom.

COMPOSITION AND BREAKDOWN IN MUSCLE

In resting muscle, adenosine 5′-triphosphate (ATP) is commonly found at a concentration of 5 to 7 μmoles per gram wet weight and this contributes the major proportion of the muscle mononucleotide in the living animal. Smaller quantities of guanosine, cytidine, inosine, and uridine phosphates occur also (Jones and Murray, 1960). In some mammalian (but apparently not fish) muscles a significant proportion of inosine 5′-triphosphate (ITP) is found (Bendall and Davey, 1957).

In practice, however, the meat or fish technologist rarely sees such material. Almost invariably the animal is in a state of some exercise or stress at the point of slaughter on fishing vessels or in abattoirs. The labile phosphate residues are lost by the actions of the tissue adenosine triphosphatases and myokinase systems to form adenosine 5′-monophosphate (AMP). On the time scales of flavor change that we are to consider, these are rapid reactions. In the muscles of true fish and in mammals AMP undergoes rapid autolytic deamination to form inosine 5′-monophosphate (IMP) (Jones and Murray, 1961). In mammalian muscle a proportion of the IMP may also derive from ITP. In both fish and meat a little derives also from the cleavage of nicotinamide adenine dinucleotide (Jones and Murray, 1966). Under some conditions post-mortem there can be some delay in IMP accumulation depending on species and nutritional status. This may be associated with a transient resynthesis of ATP consequent upon the utilization of any residual glycogen in the muscle or even upon the operation of residual aerobic processes.

IMP is cleaved, in turn, considerably more slowly than in the above reactions, but still autolytically (5′-nucleotidase or nonspecific phosphomonoesterases) to form inosine (Ino). This compound is then converted either autolytically (riboside hydrolase or phosphorylase), or under the intervention of a

developing spoilage microflora, to form hypoxanthine (Hy) and either ribose or ribose 5-monophosphate (Jones, 1963; Jones and Murray, 1962; Kassemsarn *et al.*, 1963; Shewan and Jones, 1957; Tarr, 1966).

In the shellfish, the liberation of hypoxanthine often follows an alternative route (Arai, 1960). AMP is dephosphorylated (5′-nucleotidase) to form adenosine without prior deamination. This is then deaminated autolytically (adenosine deaminase) to form inosine and thereafter broken down as for true fish or mammals; or it is cleaved hydrolytically or phosphorylytically (muscle or bacterial riboside hydrolase or phosphorylases) to form adenine which is then deaminated autolytically or microbiologically to form hypoxanthine.

Whatever the route of formation, the hypoxanthine is then converted by a developing spoilage microflora in turn to xanthine, uric acid, and ring cleavage products (Kassemsarn *et al.*, 1963). However, the reviewer understands that some workers have failed to detect xanthine in some species. This need not necessarily indicate that it is not formed transiently en route to uric acid.

For the practical purposes of flavor control and quality assessment, this series of reactions may be summarized as a rapid pre-mortem, or autolytic post-mortem, degradation of ATP to IMP followed by a slower, initially autolytic but later bacterial cleavage of IMP to form Hy. The time courses of these reactions in "wet" muscle vary considerably from one species to another among the fish. Whereas the reaction through to hypoxanthine is almost complete within 5 days in red fish (an extreme case; Fraser *et al.*, 1968), it is commonly still in progress at the limit of edibility (say after 16 to 18 days of chill storage) in most commercial north Atlantic and Pacific species (Burt *et al.* 1967; Fraser *et al.*, 1968; Jones and Murray, 1962; Kassemsarn *et al.*, 1963; Spinelli *et al.*, 1964). In other species with a far longer shelf life—e.g., swordfish, halibut—and particularly in mammalian muscle, the sequence of reactions is far slower (Dannert and Pearson, 1967b; Dyer *et al.*, 1966; Spinelli, 1967 a,b).

Nucleotide breakdown reactions can also occur during freezing, cold storage, and thawing (Jones, 1965 a,b). A recent report (Connell and Howgate, 1968) that this is not the case may result from the use of biologically more variable control material, thus masking relatively small changes in concentration. Reactions can also occur in the dry state if the conditions of dehydration do not denature enzymes and sufficient water remains (above approximately 75% ERH).

The rates of reaction at different points of the sequence vary considerably according to species and condition. Here, concentrations of individual compounds range widely.

TASTES OF NUCLEOTIDES AND BREAKDOWN PRODUCTS, ALONE AND IN THE PRESENCE OF OTHER COMPOUNDS

While most recent attention has been devoted to the enhancing effects of certain of these compounds and synergistic effects with amino acids, there is evidence that they can contribute flavorous character themselves. Thus Kodama (1913) concluded from his early studies on dried bonito that the histidine salt of IMP was a key flavor component and a number of workers have reported that IMP can occur in fresh fish muscle and meat extracts at concentrations in which solutions are strongly meaty (Jones, 1960b, 1961; Saito, 1960; Wood, 1961). Opinion differs as to the relevance of inosine formed during the breakdown of ribomononucleotide. Jones (1961) found that concentrations occurring in chill-stored fish muscle were flavorless at the relevant pH, but Kazeniac (1961) considered that this compound contributed bitterness to chicken. In practice, concentrations of inosine can vary considerably in muscles and in some—e.g., lemon sole (Kassemsarn *et al.*, 1963), petrale sole (Spinelli *et al.*, 1964), and red fish (Fraser *et al.*, 1968)—the compound is broken down as rapidly as it is produced in muscle and appears only in traces.

Of greater significance perhaps is the contribution that hypoxanthine may make to flavor. Moncrieff (1951) reported that purines are bitter. Jones (1961) found that concentrations of hypoxanthine of the order of 2 μmoles per gram (such as occur in spoiling fish muscle at the point of which taste panels can detect the character) are bitter in solution at physiological pH. Kazeniac (1961) found that hypoxanthine contributed bitter flavor to chicken. However, Komata (1964) found that the purine was not a "positive taste substance" in unripe sea urchin gonad, a Japanese delicacy, and Udo and Sato (1962) reported the compound to be tasteless. This view was initially supported by Hashimoto (1964), but this worker subsequently changed his view (Jones, 1966). Current Japanese opinion appears to be in agreement with the Kazeniac and Jones findings (Kobayashi, 1966). A report by Spinelli (1966) has clarified this issue. He showed that solutions of the purine were bitter at concentrations such as occur in muscle post-mortem but found, however, that effects of the purine when added to fish muscle were detectable organoleptically only when the bacterial counts indicated incipient spoilage. As indicated above, the original report (Jones, 1961) referred to material subject to bacterial attack.

Tarr (1966) in a review of glycolysis and nucleotide degradation pointed out the apparent difference between Jones' observations (1961) on the bitterness of staling fish and Spinelli's (1966) on the absence of bitterness in fresh fish with added hypoxanthine. He omits reference to Spinelli's observation in the same paper that the added purine confers bitterness to staling material, and that it is bitter in solution. On the available evidence there need be no confusion.

No definitive explanation can be advanced for the differing effects of hypoxanthine in fresh and staling muscle. If the reviewer may be permitted a conjecture, it would appear that the development of autolysis and the spoilage microflora either removes compounds—e.g., possibly IMP, which together with others exerts masking effects on off-flavors—or produces compounds which enhance hypoxanthine bitterness.

While most reports suggest that nucleotide degradation is related to flavor change, Fraser *et al.* (1968) found no correlation for red fish, and Rhodes (1965) none for mammalian muscle. Interpretation of the latter report is open to question, since there was no direct correlation of the results with organoleptic evaluations and the controls were frozen. However, the red fish work is not open to such objections. It is, at first sight, difficult to reconcile on observation that some 3 μmoles per gram of IMP can disappear from muscle with no flavor change while other workers have reported significant improvement on the addition of lesser quantities of IMP to a widely ranging group of food commodities—e.g., sausage, sole, soup, sardines, and water (Caul and Raymond, 1964; Dannert and Pearson, 1967a,b; Kuninaka, 1967). Perhaps now unexplained masking effects are responsible (Spinelli, 1966), or considerable change occurred in some species during cooking (Groniger and Spinelli, 1968).

Alternatively, it may be possible that the general environment (or perhaps particularly that contributed by amino residues) may be exerting an over-all overwhelming synergistic effect on the specific nucleotide-type function and that in the fresher fish the correct amino environment is not al-

ways available. Certainly amino acid composition does vary considerably post-mortem and Hashimoto (1964) and later workers have confirmed an early observation (Toi *et al.*, 1961) that there is a strong mutual enhancement of flavors between mononucleotide and glutamate (Kuninaka, 1967). The earlier reports that the OH function of the purine was necessary for flavor-enhancing activity may be misleading in the context of some natural flavor situations, since adenosine compounds now appear to be equally effective (Jones, 1967a, review). These observations are relevant to the situation in some shellfish (Arai, 1960) which lack adenylate deaminase.

Naturally occurring concentrations of IMP in muscle (whether of fish or mammalian origin) commonly exceed considerably those suggested by manufacturing companies and in the literature as possessing significant, desirable, flavor-enhancing properties. Furthermore, the amino acid environment, in which these compounds exercise any activity naturally, also is apparently highly suitable for mutual enhancement as indicated above (Bramstedt, 1962; Hornstein, 1967; Jones, 1967a; references to amino acid compositions of flesh foods). Consequently, there is probably a fruitful field for technico-economic research in the comparative optimal uses of either commercial preparations or cheap flesh foods as flavor enhancers. Equally, the supplementation of the dearer flesh foods with flavor enhancers with a view to upgrading quality in deteriorating material remains a potentially useful but largely unexplored field.

NUCLEOTIDES AND DERIVATIVES AS FLAVOR PRECURSORS

While volatile degradation products of lipids apparently can play a considerable part in determining the flavorous quality of some flesh foods (Pippen, 1967), particularly perhaps as components of high-fat fish species, there is much evidence to show that flavor-precursing character of meat and fish resides also in the water-soluble fractions of muscles.

Hornstein (1967) has recently compared the results of Batzer *et al.* (1960), on the fractionation of meat flavor precursors by dialysis and ion exchange chromatography, with those of Wasserman and Gray (1965), who used similar procedures but obtained a differing pattern of distribution. By reference also to earlier work, he concluded that high molecular weight material was not relevant, that similarities in amino acid and carbohydrate composition were reflected in similar lean meat odor, that a specific glycoprotein and inosinic acid may be precursors of beef flavors or parts thereof, and that a browning-type reaction may not be the sole mechanism responsible for the production of lean meaty flavors, since in at least one instance meat flavor was produced by a mixture of amino acids, polypeptides, and hypoxanthine.

Perhaps much importance should not be attached to relatively minor discrepancies between reported findings of different groups in such experimentation. Apart from considerations of direct effects of variations in composition of raw material, depending on storage history, etc., secondary considerations may be relevant. For instance, ion exchange phenomena (and other types of binding) of mononucleotides and other phosphorylated compounds onto protein and peptide do not appear to have been considered in these approaches. These effects can be highly pH-dependent; and pH, in turn, is a major determinant of flavor (Brant and Hanson, 1962; Jones, 1961; Pippen, 1967).

Sugar-amino reactions play a major part in the development of flesh-food flavors (Hornstein, 1967; Macy *et al.*, 1964). It would not be appropriate to consider these in any detail in this paper, but in the context of the significance of nucleotide derivatives as flavor precursors, the courses of nucleotide metabolism and glycogenolysis in muscle are intimately related biochemically and their products are reactive. The concentrations of glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, and glucose determine the extent and nature of sugar-amino reactions early post-mortem. Ribose and ribose 5-phosphate derive directly from nucleotide breakdown in muscle. In staling fish they are major components of the browning reaction complex (Burt and Jones, 1961; Jones, 1958).

While the pentoses occur at very much lower concentrations than hexoses (even maximally) in muscle post-mortem, they are of considerably greater reactivity in carbonyl-amino reactions (Tarr, 1954). Of particular interest in flesh foods is their combination with amino constituents not present in the more commonly studied model systems. In an early investigation on reactions in model dehydrated systems related to cod muscle, Jones (1959) showed that ribose was particularly reactive with taurine, anserine (β-alanyl-L-1-methylhistidine), and L-1-methylhistidine and that these were important nonprotein nitrogenous constituents in the flesh. Subsequently Macy *et al.* (1964) drew particular attention to losses of these compounds during the heating of lyophilized diffusate powders from beef to produce flavor.

NUCLEOTIDES AND CONTROL OF CONCENTRATIONS OF OTHER FLAVOR-PRECURSING COMPOUNDS

The tissues of living animals are in a state of continuous flux, with biosynthetic and biodegradative systems balanced. Frequently, the biosynthetic pathways are controlled by concentrations of nucleoside diphosphate addition compounds—see, for instance, Jones and Murray (1960) for cod muscle components—or of other nucleotides, the concentrations of which relate, in turn, to those of nucleoside triphosphates. ATP concentration is controlled by the status of oxidative phosphorylation and glycolysis in tissue, the systems being subject to complex feedback mechanisms.

With the slaughter of the animal, the bases of the regenerative systems fail. After a period dependent on species, and on pre-mortem nutritional and physiological status, concentrations fall. The rate of fall is species- and pH-dependent. Eventually concentrations of ATP can no longer support other biosynthetic mechanisms effectively. Degradative enzymes continue to function, however, and defense mechanisms fail. Consequently, concentrations of a number of amino compounds, etc., change rapidly. For instance, in cod muscles anserine disappears with formation of L-1-methylhistidine and β-alanine (Jones, 1955).

A considerable volume of literature exists on the free amino-compound composition of muscles and changes post-mortem (Bramstedt, 1962).

NUCLEOTIDE DEGRADATION AND QUALITY ASSESSMENT

Nucleotides and their breakdown products can affect taste and flavor in several ways. They possess tastes themselves at some concentrations in muscle, although these may be subject to external influences; they have flavor-enhancing properties; they are precursors of flesh food aroma; and their breakdown is a key factor in biochemical degradation producing other flavor-related compounds. Furthermore, concentrations of mononucleotides and free purine (and their organoleptic preception) relate usually both to autolytic activity during early shelf life and to the development of the spoilage microflora in incipiently spoiling material.

In most flesh foods we have to consider essentially the re-

action sequence (Jones, 1965b),

ATP (1)	IMP (2)	Ino (3)	Hy (4)	Xanthine, uric acid, ring cleavage
F, A	S, A	F or S, A or B	S, A or B	

F, fast; S, slow; A, autolytic; B, bacterial

In developing quality indices, work has been devoted mainly to estimations of IMP concentration, the dephosphorylation of the nucleotide, and Hy concentration. It is not proposed in this paper to consider the separate field of distinguishing between 2′-, 3′-, and 5′-monophosphates (Lento *et al.*, 1964), since this difficulty arises only in the flavor additive situation. For the natural flesh foods it is now accepted generally that an early suggestion (Tarr, 1958), that ribonucleic acid can be broken down (which would be relevant), has not been substantiated in subsequent work.

IMP concentration can be estimated with a high degree of precision by an ion exchange chromatography and less accurately by other chromatographic procedures (Bergkvist and Deutch, 1954; Fujita *et al.*, 1969; Jones and Murray, 1960; Lento *et al.*, 1964). However, such techniques are excessively lengthy for routine control purposes in the food industry and alternative rapid assays are being sought. On material in which deamination of the purine is almost complete, a good approximation to correct IMP concentrations can be obtained from an observation of ultraviolet absorption of tissue extracts before and after the removal of phosphorylated compounds by an ion exchange resin in batch systems in suspension (Jones and Murray, 1964).

It should be possible to develop a cheap, rapid, specific enzymic assay of IMP with currently available nucleotidases, phosphatases, ribosidase, and xanthine oxidase (Kalckar, 1947). However, the competing ionic requirements of different enzyme components have been a serious drawback to the ready use of such methodology.

That effective methods for the measurement of the extent of over-all nucleotide dephosphorylation would be useful, particularly for quality control purposes with fish muscle losing the sweet, meaty character associated with the sea-fresh condition, has been known for many years. Shewan and Jones (1957) separated the nucleotides of fish muscle from breakdown products by classical fractionation as the barium salts. Extent of dephosphorylation was determined from a colorimetric assay of ribose moiety in the fractions.

Saito *et al.* (1959) simplified a standard chromatographic procedure to measure percentage dephosphorylation. Both nucleotides and purine derivatives were placed on a column of an ion exchange resin at high pH. Ino and Hy were then eluted as a composite fraction independently of the mononucleotides. Comparison of 250-mμ absorption with that of the original extract gave a measure of dephosphorylation. The procedure assumed that the ultraviolet-absorbing compounds of the extracts were predominantly mononucleotide in origin and that the Ino-Hy fraction contained only these compounds. For many muscle extracts these assumptions are not altogether correct, but the test can provide a very useful index of quality, particularly for frozen fish.

Such procedures are time-consuming even when reduced to a single column manipulation, filtering out the nucleotides while allowing free passage of Ino and Hy (Jones and Murray, 1964; Spinelli and Kemp, 1966). However, further simplification to a batchwise operation with resin in suspension (Jones and Murray, 1964) yields very similar indices of dephosphorylation within a few minutes. The nucleotides are removed from diluted tissue extracts at pH 6.5 with 200- to 400-mesh Dowex 1 × 8 (formate) or (chloride).

From the time courses of nucleotide degradation as compared with flavor change to the point of rejection, it is clear that the monitoring of Hy concentration specifically is likely to yield more generally useful index of quality than dephosphorylation (Jones, 1960a; Kassemsarn *et al.*, 1963; Torry Research Station, 1959).

Hy can be determined readily by paper or thin-layer chromatography and by ion exchange chromatography (Jones, 1960a). In the latter methodology, difficulty resulting from the presence of Ino, which has a similar pK, elution characteristic can be eliminated by the addition of borate to the elution system (Arai and Saito, 1963; Jones, 1960b). As with the case of nucleotide separations, such determinations are too lengthy for routine quality control purposes; unfortunately, attempts to develop batch resin-shaking systems, similar to those described above for IMP and dephosphorylation estimations, have so far been unsuccessful.

Consequently, alternative rapid methods have been sought. Precipitation as the silver salt followed by double decomposition and ultraviolet spectrophotometry (Jones *et al.*, 1964) is effective except in situations (such as with extracts of the freshest fish) where small inaccuracies due to a finite solubility of the silver salt may be unacceptable. This is the method of choice if commercial xanthine oxidase is not readily available. Valencia *et al.* (1966) found it useful for certain southwest Atlantic species. Endo *et al.* (1963) suggested that direct differential spectrophotometry can be applied to such extracts to evaluate Hy concentrations. This lacks adequate specificity in the presence of small quantities of guanine which can introduce disproportionate errors (Jones, 1965b).

Specific analyses of nucleotide derivatives in biological materials, by specific enzyme reactions linked to spectrophotometry, were demonstrated by Kalckar as long ago as 1947. With the increasing commercial availability of enzyme preparations of adequate specificity, such procedures are now very attractive for routine quality appraisal. Following a preliminary comparison of methods (Torry Research Station, 1959, 1960, 1961, 1962) Jones *et al.* (1964) reported a modification of the manual Kalckar procedure, eliminating inaccuracy arising from varying orthophosphate concentration such as occurs in muscle post-mortem. Spinelli *et al.* (1964) developed an essentially similar manual method. Hy in extracts is oxidized to uric acid by xanthine oxidase. Concentrations are then evaluated from 290-mμ absorption after subtraction of appropriate blanks. Care is taken to avoid substrate-inhibition effects.

Subsequently, Jones *et al.* (1965) described an automated procedure for enzymic analysis with xanthine oxidase using the Technicon AutoAnalyser system. Good correlation was obtained with the results of the manual method.

In a further study, aimed at the development of a simple field test that would eliminate the necessity for expensive spectrophotometric equipment, Burt *et al.* (1967) linked the xanthine oxidase reaction with the redox dye, 2,6-dichlorophenol indophenol. Fish were graded according to the ability of extracts to decolorize arbitrarily predetermined reaction systems. Recently this method has been automated in its essentials (Burt *et al.*, 1968). The dye concentration is fixed at an excess level. Reaction is carried out and monitored as a fall in concentration, in a conventional AutoAnalyser system. This procedure has the considerable advantage over uric acid monitoring in that in practice it eliminates the necessity for blank determinations. It has the disadvantage, however, that for the freshest material, of low hypoxanthine concentration, the operator is attempting to

measure small falls in absorbance against a high background. Comparison of the performance of the two automated methods indicates that the dye procedure may introduce an unacceptable degree of error in such material for some purposes.

Currently, this laboratory is attempting to develop a dye-linked system yielding absorbances that increase with concentration of hypoxanthine in extracts. Some success has been obtained with a system in which the peroxide formed in xanthine oxidase reaction is linked with peroxidase and redox dyes in a secondary reaction.

While in meat the evidence is not so conclusive as for fish (Batzer *et al.*, 1960; Dannert and Pearson, 1967a, b; Doty *et al.*, 1961; Fujita *et al.*, 1959; Howard *et al.*, 1960; Kazeniac, 1961; Rhodes, 1965; Solov'eva, 1952; Terasaki *et al.*, 1965) on balance it would appear that measurements of nucleotide and hypoxanthine will frequently be of value in quality assessment for processing industries.

For fish muscle the situation is more soundly established over a range of storage and processing conditions. With the great majority of species examined to date, the monitoring of dephosphorylation or of hypoxanthine concentration yields very useful information on the state of freshness and potential shelf life. The estimations correlate well also with assessments of flavor quality by taste panel (Burt *et al.*, 1967; Connell and Howgate, 1968; Fraser *et al.*, 1968; Hughes and Jones, 1966; Jones, 1967a, b; Kassemsarn *et al.*, 1963; Saito *et al.*, 1959; Spinelli, 1967a, b; Spinelli *et al.*, 1964, 1967). The three species for which no (or poor) correlation has been observed between flavor change and nucleotide decomposition have been fatty or semifatty. There is a requirement for further research in this respect, particularly on interrelated masking effects. However, progress to date shows that these tests have generally sound, rapidly measurable indices of quality superior to the others available.

However, the situation remains (Jones, 1967a) that "patterns of spoilage can vary widely even within a species. In a situation where flavor is derived from a multiplicity of compounds, it is most unlikely that the analysis of any single compound would be an adequate cover for all eventualities."

LITERATURE CITED

Arai, K., *Bull. Fac. Fisheries Hokkaido Univ.*, **11,** 67, 255 (1960).
Arai, K., Saito, T., *Bull. Japan Soc. Sci. Fisheries* **29,** 168 (1963).
Batzer, O. F., Santoro, A. T., Landmann, W. A., Tan, M. C., Schwiegert, B. S., J. AGR. FOOD CHEM. **8,** 498 (1960).
Bendall, J. R., Davey, C. R., *Biochim, Biophys. Acta* **26,** 93 (1957).
Bergkvist, R., Deutch, A., *Acta Chem. Scand.* **8,** 1877 (1954).
Bramstedt, F., *Fette Seifen Anstrich.* **64,** 820 (1962).
Brant, A. W., Hanson, H. L., Proceedings of 12th World Poultry Congress, Sydneys', p. 487, 1962.
Burt, J. R., Jones, N. R., *J. Sci. Food Agr.* **12,** 344 (1961).
Burt, J. R., Murray, J., Stroud, G. D., *J. Food Technol.* **3,** 165 (1968).
Burt, J. R., Stroud, G. D., Jones, N. R., FAO Symposium on Freezing and Irradiation of Fish, Madrid, 1967 (in press, Fishing News, London).
Caul, J. F., Raymond, S. A., *Food Technol.* **18,** 353 (1964).
Connell, J. J., Howgate, P. F., *J. Sci. Food Agr.* **19,** 342 (1968).
Dannert, R. D., Pearson, A. M., *Food Technol.* **21,** 777 (1967a).
Dannert, R. D., Pearson, A. M., *J. Food Sci.* **32,** 49 (1967b).
Doty, D. M., Batzer, A. F., Landmann, W. A., Santoro, A. T., Proceedings of Flavor Chemistry Symposium, p. 7, Campbell Soup Co., Camden, N. J., 1961.
Dyer, W. J., Fraser, D. I., Lohnes, D. P., *J. Fisheries Res. Board Can.* **23,** 1821 (1966).
Endo, K., Hujita, M., Simidu, W., *Bull. Japan Soc. Sci. Fisheries* **29,** 366 (1963).
Fraser, D. I., Pitts, D. P., Dyer, W. J., *J. Fisheries Res. Board Can.* **25,** 817 (1968).
Fujita, T., Hashimoto, Y., Mori, T., *Bull. Japan Soc. Sci. Fisheries* **25,** 147 (1959).
Groniger, H. S., Spinelli, J., J. AGR. FOOD CHEM. **16,** 97 (1968).
Hashimoto, Y., FAO Fish Technologists Meeting, Germany, Paper **WP/II/6** (1964).
Hornstein, I., "Chemistry and Physiology of Flavors," H. W. Schultz, E. A. Day, L. M. Libbey, Eds., p. 228, Avi, Westport, Conn., 1967.
Howard, A., Lee, C. A., Webster, H. L., C.S.I.R. Aust. Division on Food Preservation, Tech, Paper, **21,** (1960).
Hughes, R. B., Jones, N. R., *J. Sci. Food Agr.* **17,** 434 (1966).
Jones, N. R., *Analyst* **85,** 111 (1960a).
Jones, N. R., *Biochem. J.* **60,** 81 (1955).
Jones, N. R., "Chemistry and Physiology of Flavors," H. W. Schultz, E. A. Day, L. M. Libbey, Eds., p. 267, Avi, Westport, Conn., 1967a.
Jones, N. R., 1st International Congress of Food Science and Technology, London, 1963 in press.
Jones, N. R., *J. Sci. Food Agr.* **9,** 672 (1958).
Jones, N. R., *J. Sci. Food Agr.* **10,** 615 (1959).
Jones, N. R., *New Scientist* **7,** 783 (1960b).
Jones, N. R., *Nutr. Bromatol. Toxicol.* **6,** 41 (1967b).
Jones, N. R., Proceedings of Flavor Chemistry Symposium, p. 61, Campbell Soup Co., Camden, N.J., 1961.
Jones, N. R., Proceedings of 2nd International Congress of Food Science and Technology, Warsaw, D. J. Tilgner, A. Borys, Eds., p. 109, 1966.
Jones, N. R., Proceedings of XI International Congress on Refrigeration, Munich, 1963, p. 917, Pergamon Press, London, 1965a.
Jones, N. R., "Technology of Fish Utilization," R. Kreuzer, Ed., p. 179, Fishing News Books, London, 1965b.
Jones, N. R., Murray, J., *Biochem J.* **77,** 567 (1960).
Jones, N. R., Murray, J., *Bull. Japan Soc. Sci. Fisheries* **32,** 197 (1966).
Jones, N. R., Murray, J., *J. Sci. Food Agr.* **13,** 475 (1962).
Jones, N. R., Murray, J., *J. Sci. Food Agr.* **15,** 684 (1964).
Jones, N. R., Murray, J., *Z. Vergleich. Physiol.* **44,** 174 (1961).
Jones, N. R., Murray, J., Burt, J. R., *J. Food Sci.* **30,** 791 (1965).
Jones, N. R., Murray, J., Murray, C. K., Livingston, E. I., *J. Sci. Food Agr.* **15,** 763 (1964).
Kalckar, H. M., *J. Biol. Chem.* **167,** 429, (1947).
Kassemsarn, B. O., Sanz Perez, B., Murray, J., Jones, N. R., *J. Food Sci.* **28,** 28 (1963).
Kazeniac, S. J., Proceedings of Flavor Chemistry Symposium, p. 37, Campbell Soup Co., Camden, N.J., 1961.
Kobayashi, K., *Bull. Japan Soc. Sci. Fisheries* **32,** 227 (1966).
Kodama, S., *J. Tokyo Chem. Soc.* **34,** 489 (1913).
Komata, Y., quoted by Hashimoto, Y., FAO Fish Technology Conference, Germany, Paper **WP/II/6** (1964).
Kuninaka, A., "Chemistry and Physiology of Flavors," H. W. Schultz, E. A. Day, L. M. Libbey, Eds., p. 515, Avi, Westport, Conn., 1967.
Lento, H. G., Ford, J. A., Denton, A. E., *J. Food Sci.* **29,** 435 (1964).
Macy, R. L., Naumann H. D., Bailey, M. E., *J. Food Sci.* **29,** 136, 142 (1964).
Moncrief, R. W., "The Chemical Senses," Leonard Hill, London, 1951.
Pippen, E. L., "Chemistry and Physiology of Flavor," H. W. Schultz, E. A. Day, L. H. Libbey, Eds , p. 251, Avi, Westport, 1967.
Rhodes, D. N., *J. Sci. Food Agr.* **16,** 447 (1965).
Saito, T., *Kagaku (Kyoto)* **15** 101 (1960).
Saito, T., Arai, K., Matsuyoshi, M., *Bull. Japan Soc. Sci. Fisheries* **24,** 749 (1959).
Shewan, J. M., Jones, N. R., *J. Sci. Food Agr.* **8,** 491 (1957).
Solov'eva, V. I., *Myasn. Ind. SSSR* **23,** (2), 43 (1952).
Spinelli, J., *Comm. Fisheries Rev.* **29,** (11), 69 (1967a).
Spinelli, J., *J. Food Sci.* **30,** 1063 (1966).
Spinelli, J., *J. Food Sci.* **32,** 38 (1967b).
Spinelli, J., Eklund, M., Miyauchi, D., *J. Food Sci.* **29,** 710 (1964).
Spinelli, J., Kemp, B., J. AGR. FOOD CHEM. **14,** 176 (1966).
Spinelli, J., Pelroy, G., Miyauchi, D., FAO Symposium on Freezing and Irradiation of Fish, Madrid, 1967, (in press).
Tarr, H. L. A., *Ann. Rev. Biochem.* **27,** 223 (1958).
Tarr, H. L. A., *Food Technol.* **8,** 15 (1954).
Tarr, H. L. A., *J. Food Sci.* **31,** 846 (1966).
Terasaki, M., Kajikawa, M., Fukita, E., Ibsii, K., *Agr. Biol. Chem.* **29,** 208 (1965).
Toi, B., Ikeda, S., Matsuno, T., October Meeting, Japan Home Economics Association, 1961.
Torry Research Station (Dept. of Scientific and Industrial Research), Ann. Reports, H. M. Stationery Office, London, p. 26, 1959; p. 31, 1960; p. 8, 1961; p 6, 1962.
Udo, S., Sato, T., *J. Agr. Chem. Soc. Japan* **36,** 838 (1962).
Valencia, M., Casavola, H., Sanahuja, J. C., VII Congreso Panamericano de Farmacia y Bioquímica, Buenos Aires, 1966.
Wasserman, A. E., Gray, N., *J. Food Sci.* **30,** 801 (1965).
Wood, T., *J. Sci. Food Agr.* **12,** 61 (1961).

Received for review October 1968. Accepted April 1, 1968. Division of Agricultural and Food Chemistry, 156th Meeting, ACS, Atlantic City, N. J., September 1968.