SUMMARY

1. Gladiolic acid (4-methoxy-5-methyl-*o*-phthalaldehyde-3-carboxylic acid in the open-chain form) is the only fungistatic compound among the known metabolic products of *Penicillium gladioli* Machacek.

2. *o*-Phthalaldehyde shows fungistatic activity in the same concentration range as gladiolic acid and tests on a large number of derivatives of gladiolic acid and on simple model substances indicate that activity is connected with the presence of two formyl substituents in the ortho position on the aromatic nucleus. The lactol form of gladiolic acid (4-formyl-3-hydroxy-7-methoxy-6-methylphthalide) is believed to be inactive.

3. The percentage of dissociated molecules of gladiolic acid present at any given pH is in good agreement with the percentage of hydrated open-chain (dihydroxyphthalan) form calculated from the ultraviolet absorption. The undissociated lactol and hydrated open-chain anion make up 99% of the total gladiolic acid present at any given pH.

4. An attempt to explain the variation of the equieffective concentration of gladiolic acid with the external pH, on the basis that whereas the hydrated open-chain anion is the active species, only the undissociated lactol form penetrates into the spore, is not unsuccessful.

5. The variation with pH of the activity of acetylgladiolic acid and certain closely related neutral derivatives is not quite so satisfactorily explained on the hypothesis that the molecules penetrate unchanged but undergo internal enzymic hydrolysis to gladiolic acid.

6. The enhanced specificity of the fungistatic action of gladiolic acid compared with that of *o*-phthalaldehyde arises from the presence of the carboxyl substituent adjacent to the *o*-diformyl grouping.

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New Zealand Fish Oils

6. SEASONAL VARIATIONS IN THE COMPOSITION OF NEW ZEALAND GROPER (*POLYPRION OXYGENEIOS*) LIVER OIL

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The groper (*Polyprion oxygeneios*) is one of the larger and more important New Zealand edible fish. The fatty-acid composition of the head oil and of the liver oil has been described by Shorland (1938), while the vitamin A and vitamin D contents of the liver oils have been determined respectively by Shorland (1937, 1948, 1950), Cunningham (1935), Cunningham & Scott (1944) and Weeber (1945).

This paper describes the seasonal changes in size of liver, yield of oil, vitamin A, cholesterol, total unsaponifiable content, phospholipid and iodine value.

EXPERIMENTAL

Thirty-nine samples, each containing from 8 to 53 (average 24) livers, representative of a day's catch from one boat, were collected in the period 1938–43, at approximately equal intervals of time to minimize the possible differences between years. During the months of September and October, however, when there was invariably a marked increase in vitamin A potency, eleven samples were taken as

compared with five or six for the remaining 2-monthly periods. The fish were all from Cook Strait and the lipids were extracted as described by Oliver & Shorland (1948).

Vitamin A in the samples was determined from the $E^{1\%}_{1cm.}$ values in absolute ethanol at 328 mμ. using the conversion factor 1/16 for expressing it as a percentage vitamin A (cf. Carr & Jewell, 1933). No correction was made for irrelevant absorption but close agreement was obtained with the percentage vitamin A determined from the $SbCl_3$ blue colour, assuming that the pure vitamin has an $E^{1\%}_{1cm.}$ 620 mμ. value of 4800 (Baxter & Robeson, 1942).

In recent years (cf. Cama, Collins & Morton, 1951) higher values for the $E^{1\%}_{1cm.}$ absorption of vitamin A have been found using purer samples of this substance and the more exacting methods of analysis now available (Morton & Stubbs, 1946, 1948) require more absorption data than was obtained at the time of analysis. In any case the possible error involved is insufficient to affect the interpretation of the present results.

Iodine values were determined by the Wijs method, the percentage unsaponifiable matter by the Society of Public Analysts' Procedure (1933), and sterols by precipitation with digitonin (Dam, 1928).

RESULTS AND DISCUSSION

Seasonal variations in composition of liver and of pyloric-caecal oils

Liver. A comparison of the results (Figs. 1 and 2) for the period September–October with those for the remaining 2-monthly periods showed, apart from the percentage vitamin A and percentage unsaponifiable matter in January–February and the liver weight values in March–April, highly significant differences ($P \leqslant 0{\cdot}01$). During September–October, the marked increase in the percentage vitamin A, sterol, total unsaponifiable matter and lipid phosphorus of the liver oil, as well as the rise in iodine value, is accompanied by a reduction in oil content and in liver weight. The reduction in liver weight is greater than would be anticipated from the lowering in oil content indicating the possible loss of components other than oil.

Pyloric caeca. The changes in oil content of the pyloric caeca (Fig. 2) resemble generally those of the liver with a highly significant decrease ($P \leqslant 0{\cdot}01$) in September–October as compared with the remaining 2-monthly periods. The pyloric-caecal and liver oils both show a significant reduction ($P \leqslant 0{\cdot}05$) in vitamin A content during July–August as compared with the remaining 2-monthly periods. Following a reduction in oil content in July–August the vitamin A content of the pyloric-caecal oil during September–October is merely restored to the normal value observed during the remainder of the year. In the liver, by contrast, there is a spectacular increase in vitamin A content associated with the reduced oil content at this period.

Seasonal changes in the vitamin A content of fish-liver oils (cf. Drummond & Hilditch, 1930) have been attributed to the effects of spawning, whereby the oil (triglyceride) is removed preferentially as compared with vitamin A leaving the residual oil in the liver enriched in this constituent. During the months of May, June and July, the groper are heavy in roe, and in the middle of August the catches fall off markedly, but increase again towards the end of the month. This suggests that seasonal changes in the vitamin A content are associated with spawning which, to judge from the size of the gonads, imposes a severe strain on the fish. The present results show also that apart from vitamin A, other constituents, including total unsaponifiable matter, cholesterol and probably vitamin D (cf. Weeber, 1945), tend to remain in the liver during mobilization of triglycerides after spawning.

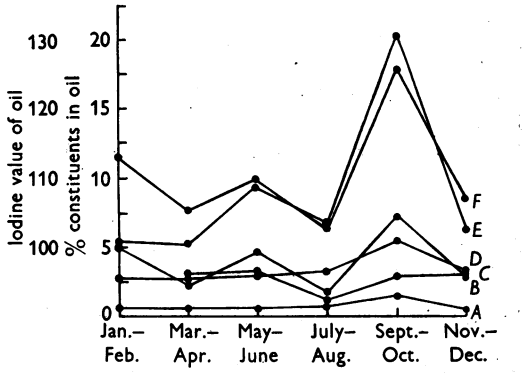

Fig. 1. Seasonal changes in: (*A*), % P in liver oil; (*B*), % vitamin A in liver oil; (*C*), % vitamin A in pyloric-caecal oil; (*D*), % cholesterol in liver oil; (*E*), iodine value in liver oil; and (*F*), % total unsaponifiable matter in liver oil.

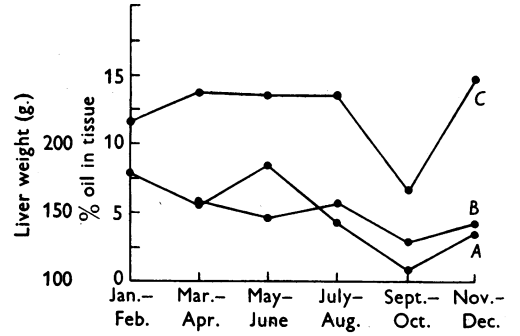

Fig. 2. Seasonal changes in: (*A*), liver weight; (*B*), % oil in pyloric caeca; and (*C*), % oil in livers.

Although the evidence of this work, as well as that of previous investigators (see Shorland, 1938), is consistent with the view that the increased vitamin A content of the liver oil is associated with a reduction in oil content following spawning, there is evidence in the present work that at other times

vitamin A may be preferentially mobilized or utilized while the proportion of triglycerides in the tissues remains unchanged. During the period July–August as compared with May–June, for example, there is significant decrease in vitamin A content of both the liver oil and of the pyloric-caecal oil without any significant change in the oil content of the tissues.

Investigations by Rapson & Schwartz (1944) on the South African stonebass (*Polyprion americanus*), which is closely related to the New Zealand groper, show that the liver of this species yields more oil (10–25 %) than the groper, and that this oil is generally richer in vitamin A (2·4–25·9 %) and in unsaponifiable matter (5·1–38·5 %). The vitamin A content of the stonebass is at its highest during the period September–December and remains at a relatively low level during the autumn and winter months when the oil content tends to increase. The changes in vitamin A and oil content of the stonebass are thus generally more gradual and less pronounced than in the case of the groper. The high phospholipid content (5·86–14·6 %) of stonebass-liver oil is paralleled in the present work on groper-liver oil where the mean 2-monthly values for the percentage phospholipid (calculated as percentage P × 100/3·9) range from 12·1 to 35·6, and generally indicate an inverse relationship between the yield of oil and its phospholipid content.

The present results accord with those of Rapson & Schwartz (1944) for stonebass-liver oil and with those of Haines & Drummond (1934) for halibut-liver oil in showing a more or less linear relationship between iodine value and vitamin A content.

During September, when the great majority of fish have spawned, two specimens about to spawn were selected for the purpose of studying the distribution of vitamin A and oil, as shown in

Table 1. *Distribution of oil and of vitamin A in the tissues of the groper*

	Male fish, total wt. 6852 g., caught 11. ix. 40					Female fish, total wt. 14975 g., caught 11. ix. 40				
Organ	Organ wt. (as % total weight of fish)	Oil (% of organ wt.)	Oil (as % total oil in fish)	Vitamin A (% of oil)	Vitamin A (as % of total vitamin A)	Organ wt. (as % total weight of fish)	Oil (% of organ wt.)	Oil (as % total oil in fish)	Vitamin A (% in oil)	Vitamin A (as % of total vitamin A)
Body (excluding backbone)	65·87	1·70	34·7	0·03	2·3	58·05	4·20	32·6	Trace	—
Head	23·92	6·93	51·4	Not found	—	22·10	14·90	43·9	Not found	—
Liver	1·34	6·45	2·8	14·1	88·9	2·25	8·19	2·4	11·1	93·5
Pyloric caeca	1·02	2·56	0·9	4·3	8·7	1·31	4·60	0·8	2·2	6·2
Intestines	0·77	1·58	0·3	0·2	0·1	0·89	2·73	0·3	0·3	0·3
Stomach	1·68	1·33	0·6	Not found	—	2·90	1·80	0·7	Not found	—
Milt (or roe)	1·18	1·92	0·6	0·03	Trace	9·27	11·34	14·0	Not found	—
Heart	0·19	1·41	Trace	Not found	—	0·19	4·28	—	Not found	—
Spleen	0·15	2·70	Trace	Trace	Trace	0·23	3·70	—	Trace	—
Backbone	3·88	7·10	8·7	Not found	—	2·81	14·34	5·3	Not found	—

Table 2. *Oil and vitamin A content in the livers as compared with those in pyloric caeca and intestines*

(For each material, five male and five female samples. All results as mean ± standard error of the mean.)

Organ	Weight of organ (g.)	Oil (%)	Vitamin A (%)
Male			
Livers	106·2 ± 13·6	9·08 ± 3·06	6·17 ± 1·73
Pyloric caeca	80·4 ± 7·7	4·76 ± 0·76	3·05 ± 0·83
Intestines	36·3 ± 8·1	5·10 ± 2·23	0·49 ± 0·22
Female			
Livers	162·7 ± 32·2	10·78 ± 2·61	3·33 ± 1·26
Pyloric caeca	104·5 ± 23·2	4·35 ± 0·69	1·97 ± 0·53
Intestines	49·0 ± 38·5	5·39 ± 1·00	0·21 ± 0·10

Table 1. The oil is widely distributed throughout the various tissues with the head, backbone, liver and roe forming the main depots. Most of the vitamin A is stored in the liver, but some is also found in the pyloric caeca, in addition to small proportions in the intestines; in the male specimen some vitamin A is also found in the body oil.

Table 2 shows results for the vitamin A and oil content of the livers, pyloric caeca and intestines from batches of fish sampled at intervals throughout the season. It will be seen that the oil content of the livers is approximately twice that of the pyloric caeca and intestines, while the vitamin A content of the liver oil is twice that of the pyloric-caecal oil and 12 to 13 times that of the intestinal oil.

Effect of size of liver on the percentage oil and its composition

The results of various investigators (cf. Drummond & Hilditch, 1930; Lovern, Edisbury & Morton, 1933; Pugsley, 1939) show that the vitamin A content of the liver oil increases with the size of the liver. In the case of the New Zealand ling (*Genypterus blacodes*) (Shorland, 1938) and the Cape ling (*G. capensis*) (Rapson, Schwartz & van Rensburg, 1944), however, there is a slight decrease in the vitamin A potency of the liver oil with size of liver, but owing to the probable increase in the proportion of liver in the larger fish, it is likely that the vitamin A reserves increase proportionately with the size of the fish.

Table 3 shows the results for small and large livers and the significance of the differences ascertained by pairing (Paterson, 1939) the large and small livers within each batch. It will be seen that the large livers, as compared with the small, contain approximately double the percentage oil, but the proportions of vitamin A, cholesterol, total unsaponifiable matter and phospholipid phosphorus in the oil are only about half those found in the case of the smaller livers. This means that the proportions of these constituents in the liver do not alter appreciably with the age of the fish, although the larger livers, as compared with the smaller, show a slightly increased content of total unsaponifiable matter and of cholesterol.

The livers from the male fish are smaller than those of the female, but the oil which was present in smaller proportions appears to contain more vitamin A, cholesterol and total unsaponifiable matter. The observations, however, were too few to establish any statistical significance in the differences observed.

SUMMARY

1. The percentages of vitamin A, cholesterol, total unsaponifiable matter and phospholipid, as well as the iodine value of the liver oil of the groper (*Polyprion oxygeneios*), have been shown to increase significantly immediately following spawning when the oil content of the liver is reduced.

2. The results are consistent with the view that the oil reserves of the liver are mobilized after spawning, while the reserves of vitamin A, cholesterol, total unsaponifiable matter and phospholipids are relatively unimpaired, leaving the remaining liver oil enriched in these constituents.

3. The pyloric caeca showed a similar reduction in oil content to that of the livers during September–October, but there was no spectacular rise in vitamin A content as observed in the case of the liver oils at this period.

4. In both the pyloric caeca and the liver there was a significant reduction in vitamin A content during

Table 3. *Effect of size of liver on the percentage oil and on the composition of the oil*

(All results as mean ± standard error of the mean. Significance of results is based on Student's technique (cf. Paterson, 1939) using nine pairs of samples, each pair representative of small and large livers from the same catch. HS ($P \leqslant 0{\cdot}01$), S ($P > 0{\cdot}01$ but $\geqslant 0{\cdot}05$).)

	Liver wt. (g.)	Oil (%)	Vitamin A	Cholesterol (as % of oil)	Total unsaponifiable material (as % of oil)	Phosphorus (as % of oil)
Small	108·0 ± 8·87	9·67 ± 1·21	3·09 ± 0·60	3·98 ± 0·48	9·79 ± 1·34	0·86 ± 0·30
Large	214·0 ± 19·3	18·61 ± 2·38	1·76 ± 0·27	2·52 ± 0·25	6·12 ± 0·38	0·38 ± 0·05
Significance of differences	HS	HS	S	S	HS	HS

Table 4. *Effect of sex on the percentage oil in the liver and on the composition of the liver oil*

(Five samples of male and five of female material containing each 7–30 livers. All results as mean ± standard error of the mean. All differences not significant $P > 0{\cdot}5$.)

	Liver wt. (g.)	Oil (%)	Vitamin A	Cholesterol (as % of oil)	Total unsaponifiable material (as % of oil)
Male	106·2 ± 13·6	8·11 ± 2·57	6·17 ± 1·74	4·75 ± 0·62	12·49 ± 3·01
Female	162·7 ± 39·5	11·23 ± 2·07	3·33 ± 1·13	3·79 ± 0·83	10·51 ± 2·91

July–August without any appreciable change in oil content suggesting the possibility of preferential removal of vitamin A as compared with the oil.

5. Studies on the distribution of vitamin A and of oil showed that whereas the oil was diffused more or less evenly throughout the fish, the vitamin A reserves are concentrated particularly in the liver, with somewhat smaller proportions in the pyloric caeca and in the body (male fish only).

6. It was found that although the percentage of oil increased with increasing size of liver, the contents of vitamin A, cholesterol, total unsaponifiable matter and lipid phosphorus in the liver were relatively unaffected.

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Efficiency of Oxidative Phosphorylation During the Oxidation of Pyruvate

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Krebs, Ruffo, Johnson, Eggleston & Hems (1953) have recently measured the value of the ratio

$$\frac{\text{equivalents of organic phosphate formed}}{\text{atoms of oxygen consumed}}$$

('phosphorylation quotient') for the reactions α-ketoglutarate → succinate and succinate → fumarate plus malate. The rate of formation of organic phosphate was determined by measuring the rate of incorporation of inorganic $^{32}PO_4$ into adenosinetriphosphate (ATP). The present paper is concerned with measurements, by the same procedure, of the phosphorylation quotient for the reaction pyruvate → acetate and for the complete oxidation of pyruvate.

EXPERIMENTAL

Preliminary experiments on liver and muscle suspensions prepared according to Krebs *et al.* (1953) showed that pyruvate, unlike the intermediates of the tricarboxylic acid cycle, did not maintain ATP in the suspensions. A more suitable material for the study of the oxidative phosphorylation associated with the oxidation of pyruvate was a suspension of washed subcellular particles of sheep kidney cortex, reinforced by cofactors, as previously described (Bartley, 1953). These were used in the present investigation. They were prepared from sheep kidneys within 3 hr. of collection and were used immediately. On keeping the tissue for 24 hr. at 0° in a mixture of frozen and liquid 0·9% (w/v) KCl the ability to carry out oxidative phosphorylation was reduced, although respiration remained unimpaired.

If the rate of incorporation of orthophosphate into ATP is to be maximal, ADP, as phosphate acceptor, must continuously be generated. The continuing formation of ADP from ATP can be brought about by the endogenous phosphatases, but, as shown by Potter & Recknagel (1951), the phosphatase activity of mitochondria separated in sucrose solutions is low. Active phosphatase may be liberated from the mitochondria by the action of KCl as shown by Berthet & de Duve (1951), or by the addition of nuclei as shown by Potter, Lyle & Schneider (1951). For these reasons the 'cyclophorase' type of preparation containing nuclei was