

THE EFFECT OF HYPOXIA AND ANAEMIA ON THE SWIMMING PERFORMANCE OF RAINBOW TROUT (*SALMO GAIIRDNERI*)

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INTRODUCTION

Many studies have been made on the respiratory metabolism of fish, although the majority have been directed at determining the minimum resting rate or maximum sustained rate of metabolism (Winberg, 1956, 1961; Fry, 1957). Intermediate levels of metabolic rate have also been investigated (Job, 1957; Beamish & Mookherjee, 1964; Beamish, 1964*a*; Brett, 1962, 1964; Smit, 1965). The effects of temperature, size, environmental oxygen and carbon dioxide concentrations, salinity and photoperiod on active, standard and routine levels of metabolism have been reported (Job, 1955; Basu, 1959; Hickman, 1959; Saunders, 1962; Evans, Purdie & Hickman, 1962; Brett, 1964, 1965; Beamish & Mookherjee, 1964; Beamish, 1964*a, b, c*; Rao, 1968; Farmer & Beamish, 1969).

Despite this large volume of literature it is still difficult to make an accurate prediction of what factors may be limiting the maximum performance of a fish. Davis *et al.* (1963) and Brett (1964) have discussed factors that may limit the swimming speeds and oxygen uptake rates of salmon. Brett (1964) concluded that above 15 °C the environmental oxygen concentration could be a limiting factor since one population of salmon showed a significantly increased oxygen uptake at 20 °C in the presence of elevated environmental oxygen tensions. However, Dahlberg, Shumway & Doudoroff (1968) found that above a certain level the maximum swimming speed of both largemouth bass and coho salmon was independent of environmental oxygen tension. Reduction in environmental oxygen tension below air-saturation usually result in a fall in the maximum sustained swimming speeds of coho salmon, chinook salmon and largemouth bass (Davis *et al.* 1963; Dahlberg *et al.* 1968). These results might be taken to imply that the ability of the fish to present sufficient oxygen to the gills is a factor limiting maximum performance. There are at least two ways of accounting for this conclusion, either that the relatively small muscle mass is unable to perform more than a certain amount of ventilatory work (Cameron & Cech, 1970), or that the cost to the metabolism of the work done in breathing is unreasonably high (van Dam, 1938; Schumann & Piiper, 1966), although the latter supposition has been recently challenged (Alexander, 1967; Cameron & Cech, 1970). Furthermore, during swimming, fish may make use of 'ram ventilation' which may reduce the energy cost of gill ventilation (Brown & Muir, 1970).

Dahlberg *et al.* (1968) also concluded that the intensity of sustained activity may

be limited by the rate of delivery of oxygen to the tissues under some conditions, particularly the presence of moderate environmental tensions of carbon dioxide which reduces the ability of the blood to transport oxygen (Root, 1931; Ferguson & Black, 1941; Black, Kirkpatrick & Tucker, 1966*a, b*; Black, Tucker & Kirkpatrick, 1966*a, b*). However, at the carbon dioxide tensions found in arterial blood of moderately active trout (Stevens & Randall, 1967) the so-called Root effect is negligible (Cameron, 1971). At any given level of oxygen uptake a reduction in the oxygen capacity of the blood will mean that the heart must do more work; the question is whether in normal animals the cost to the metabolism of cardiac work limits maximum performance. Garey (1970) calculated that 5.2 % of the energy production in resting carp supports cardiac work and the cost is likely to increase during maximum activity. Furthermore severely anaemic pinfish and trout are incapable of sustained swimming activity (Cameron & Wohlschlag, 1969; Cameron & Davis, 1970).

On the basis of this kind of evidence Jones (1971) presented a theoretical analysis in which it was considered that if a salmonid fish came into a steady state at all levels of activity then the oxygen consumed could be regarded as supplying energy for the tissues, heart and branchial musculature. Under the conditions chosen (15 °C) it was predicted that the scope for activity would be 8–10 times the basal oxygen uptake and at this level of performance the costs of presenting oxygen to the gas exchanger and of transporting oxygen to the tissues would both be limiting. Since the relationship between swimming speed and oxygen consumption has been established for some salmonids (e.g. Brett, 1964) it was also possible to predict the maximum swimming performance of anaemic (half normal oxygen capacity of the blood) and hypoxic (half normal environmental oxygen tension) fish. It was predicted that under these conditions salmonids should be able to achieve at least half their maximum performance. It proved difficult to extend this analysis to lower or higher temperatures with any confidence owing to the magnitude of the assumptions involved. Consequently the present series of experiments was designed to test the predictions of the theoretical analysis regarding maximum swimming speeds during hypoxia and anaemia. It was also felt desirable to delimit the effect of temperature on the response and for this reason the experiments were carried out at two temperatures.

METHODS

The experiments were performed on 164 hatchery-reared rainbow trout (*Salmo gairdneri*). At the start of the experiments the fish were 20 months old. The animals were divided into two stocks and acclimated to temperature for 2 months in round culture tanks, remaining there during the experiments. The acclimation temperatures of the two stocks were 8–10 °C and 21–23 °C. The water in the culture tanks was kept in circular motion by pressure water jets installed vertically in the tanks, and the fish swam constantly against this water current. The animals were fed a diet of pellet feed but were starved for 20–30 h before each experiment.

The fish were exercised in a respirometer similar to that described by Brett (1964) at the temperature to which they were acclimated. Basically the respirometer is a recirculating water-tunnel incorporating a Plexiglass fish chamber connected to a pump through fibreglass expansion and contraction cones. The total volume of the

respirometer was 24 l. A laminar flow of water occurred over the velocities at which the fish were forced to swim, the flow pattern being checked periodically by introduction of dye at the upstream end of the fish chamber. The water velocity within the animal chamber at any given pump speed was determined before the experiments by means of a small current meter. During an experiment temperature was controlled within ± 0.5 °C by continuous flow of refrigerant through a chilling jacket, counter-balanced by a heater and relay.

The water in the system was renewed continuously at a rate of 1 l/min. The oxygen tension of the water flushed out of the respirometer was monitored using a Radiometer or Beckman oxygen electrode and remained sensibly constant during any one experiment. Oxygen tensions of half air-saturation were achieved by introducing nitrogen at the bottom of a vertical Polythene column filled with glass marbles through which the water flowed downward before entering the respirometer. Careful adjustment of the flows of nitrogen and water allowed maintenance of the desired oxygen tension in the respirometer.

Intraperitoneal injections of phenylhydrazine hydrochloride in 10% ethanol were given to assure haemolytic anaemia. A dose of 15 µg/g wet body weight which usually caused a reduction in haematocrit of between 50–70% was determined from tests on 32 fish. The animals were worked on 2–3 days after the injection, this period being deemed necessary for effectiveness of the drug and to allow complete recovery from the trauma of handling and anaesthetization. The fish were anaesthetized for the injection with MS 222 (Sandoz). Two groups of animals, one from each acclimation temperature, were injected with a blank volume of 10% ethanol to delimit the effects of the injection procedure on performance (controls).

A total of 39 fish were used in preliminary experiments in order to practice the techniques and establish a protocol which would give meaningful results. The following information was derived from these test runs:

(a) Contrary to Brett (1964) a cover area at the front of the fish chamber was not required since identical performances were achieved with or without the cover area in position.

(b) Fish examined alone or in groups of up to three exhibited no difference in maximum performance.

(c) Significantly higher maximum swimming speeds were obtained when an electrified grid was placed at the downstream end of the fish chamber. The grid consisted of a series of vertical wires embedded in a Plexiglass ring and it was connected to a stimulator which gave a biphasic pulse of 3–5 V/sec.

(d) No detectable difference in the maximum swimming speed was found with exposures of 20–40 min at each velocity increment.

(e) Velocity increments of approximately one-sixth to one-ninth of the maximum swimming speed appeared to have no effect on the final performance of fish, both at air-saturation and during varying degrees of hypoxia.

(f) Acclimation to the respirometer from 2 to 20 h appeared not to affect maximum performance. Consequently in the experiments either regime was imposed as proved convenient. Similarly hypoxic fish showed no statistically significant differences in performance whether they were acclimated to hypoxia for the shorter (2 h) or longer (20 h) period.

Fish were selected at random from the culture tanks and introduced into the animal chamber by means of the water overflow port in the top of the chamber. The fish chamber was 50 cm long and 9 cm diameter and the fish were usually examined in pairs. The respirometer pump was set to deliver a velocity of 2–4 cm/sec for the conditioning period of 2–20 h. At this velocity the fish remained upright in the chamber and maintained their position with minimum expenditure of energy. Following this introductory phase the fish were subjected to 20 min periods of swimming at fixed velocities. The velocity increment was approximately 9 cm/sec, which was between one-seventh and one-eighth of the maximum swimming speed in air-saturated water, measured in lengths/sec. When a fish became fatigued it was removed carefully with forceps from the electrified grid through the water overflow port, which was sited just in front of the grid. A fish was deemed to have become fatigued when it could not remove itself from the grid even in response to a doubling of the applied voltage. After removal the fish was killed, weighed, measured from the tip of the snout to a line joining the tips of the tail, and the tail sectioned to provide blood for determination of the haematocrit. Micro-haematocrits were determined in triplicate using commercially prepared heparinized capillary tubes.

Since the fish almost always became fatigued during the last velocity increment in a time interval less than the desired time period (20 min), a measure of critical swimming speed was provided. This was obtained empirically, as has been described by Brett (1964). Even when three fish were in the animal chamber their total areas did not amount to more than 5 % of the cross-section of the chamber, so no adjustment for swimming-speed error from this source was applied (Brett, 1964).

In the results all values are given \pm S.E. of the mean. Results were analysed statistically by Fischer's *t* test, and 5 % was considered as the fiducial limit of significance.

Table 1. *The effect of the temperature of acclimation on the maximum swimming speed of rainbow trout*

Temperature (°C)	No. of animals	Weight (g)	Length (cm)	Water oxygen tension (mmHg)	Haemato- crit (%)	Condition factor (100 $\times W/L^3$)	Maximum velocity (L/sec)
11.9 \pm 0.09	15	13.2 \pm 1.17	10.9 \pm 0.35	150 \pm 1.37	36.2 \pm 1.4	0.99 \pm 0.02	6.05 \pm 0.2
22.6 \pm 0.1	14	21 \pm 1.6	12.5 \pm 0.35	153.9 \pm 0.5	34.4 \pm 1.8	1.06 \pm 0.02	6.34 \pm 0.22

RESULTS

(a) *The effect of the temperature of acclimation on the maximum swimming speeds of rainbow trout*

There was no significant difference between the maximum swimming speeds of trout at 11.9 \pm 0.1 and 22.6 \pm 0.1 °C (Table 1). On average, the group acclimated to the higher temperature swam somewhat faster but there was also more variability in this population. The range of 4.97–8.1 l/sec exceeded that for the lower-temperature group (range 4.32–7.12 l/sec). It is unfortunate that the two groups of fish examined were not of comparable sizes, the high-temperature group was significantly longer and heavier than the low-temperature group. The condition factor, an index relating weight (g) to length (cm) has been used to provide a measure of well-being in a fish

(Carlander, 1950). The condition factors for both groups of fish were not significantly different and approximated to 1.0 (Table 1), which is in the range of values found in naturally occurring stocks of trout (Fessler & Wagner, 1969; J. N. Cameron, unpublished observations). It is difficult to relate physical fitness to the condition factor but, as Brett (1964) has pointed out, the implication exists. From the present data it was determined that of fish with a condition factor in excess of 0.975 only 4 out of 23 failed to achieve a maximum swimming speed above 6 l/sec, whereas no animal with a condition factor of less than 0.975 achieved 6 l/sec. It was not possible to establish any relationship between haematocrit and maximum swimming speed.

Table 2. *The effect of hypoxia on the maximum swimming speed of rainbow trout at two temperatures*

Water oxygen tension (mmHg)	Temperature (°C)	No. of animals	Weight (g)	Length (cm)	Haematocrit (%)	Condition factor (100 $\times W/L^3$)	Maximum velocity (L/sec)
77.8 \pm 1.04	14.1 \pm 0.14	18	23.4 \pm 1.3	12.5 \pm 0.24	32.4 \pm 1.07	1.18 \pm 0.02	3.47 \pm 0.13
68.3 \pm 0.75	22.4 \pm 0.01	15	18.1 \pm 1.4	11.8 \pm 0.25	40.1 \pm 1.14	1.08 \pm 0.02	4.43 \pm 0.13

(b) *The effect of hypoxia on the maximum swimming speeds of rainbow trout at two temperatures*

Exposure to an environmental oxygen tension of half air-saturation resulted in a reduction in the maximum swimming speeds of fish acclimated both to high and to low temperatures (Table 2). For the low-temperature group the maximum swimming speed was reduced by 43 % compared with normal fish, whereas for the high-temperature group the reduction was only by 30 % (Tables 1, 2). The final swimming speeds under hypoxic conditions were significantly different at the two temperatures. It was surprising in the light of Brett's (1964) observation that the fish acclimated to high temperature achieved better performance even though they were subjected to a greater degree of hypoxia (Table 2). In this series of experiments there was no significant difference between the weight, length or condition factor of the two groups, but the high-temperature group had a significantly higher haematocrit than the low-temperature group. This factor may obviously be important, especially during hypoxia, and will be discussed later.

(c) *The effect of anemia on the maximum swimming speed of rainbow trout at two temperatures*

The handling and injection procedure appeared to have a slight retarding effect on the maximum swimming performance of the fish (Table 3) but neither the high-temperature nor the low-temperature group was significantly different from normal animals (Tables 1, 3). In the case of the low-temperature group the haematocrit was reduced to about half of control, whereas for the high-temperature group the haematocrit was reduced to one-third of control although the final haematocrits themselves were not significantly different. Both groups of fish achieved about the same final swimming speed, representing a reduction of 34 % of control at low temperatures and 40 % at high temperatures (Table 3). Although the performance of individual

fish could often be accounted for on the basis of haematocrit, owing to the paucity of data it was not possible to derive any strict relationship between these two factors for either high-temperature or low-temperature animals. There was no significant difference in weight, length or condition factor between the two groups of fish in this series of experiments.

Table 3. *The effect of anaemia on the maximum swimming speed of rainbow trout at two temperatures*

Haemato- crit (%)	Water oxygen tension (mmHg)	Tempera- ture (°C)	No. of animals	Weight (g)	Length (cm)	Condition factor (100 × W/L^3)	Maximum velocity (L/sec)
15.67 ± 1.16	142.6 ± 0.9	11.73 ± 0.24	12	23.03 ± 2.98	13.16 ± 0.57	0.95 ± 0.01	3.53 ± 0.23
29.3 ± 0.6	145 ± 2.4	12.86 ± 0.68	5	16.7 ± 3.3	11.76 ± 0.6	0.98 ± 0.04	5.37 ± 0.34
10.9 ± 2.25	157.98 ± 0.95	21.6 ± 0.28	6	23 ± 4.17	12.7 ± 0.8	1.04 ± 0.025	3.615 ± 0.24
32.85 ± 1.11	156.5 ± 0.56	22.15 ± 0.14	8	20.8 ± 2.67	12.3 ± 0.6	1.06 ± 0.021	5.97 ± 0.21

DISCUSSION

One of the problems with this type of study is to decide whether the fish were in a steady state at all levels of activity before the velocity at which they became fatigued. There is certainly some controversy in the literature over this point. Brett (1967) suggests that unrealistic levels of critical swimming speed may result from a series of short exposure times, and recommends a test period of 60 min, although the results he obtained using a 45 min period (1967) do not appear to differ markedly from values obtained earlier in which the test period was 75 min (Brett, 1964). In increasing-velocity tests Davis *et al.* (1963) found that doubling the length of the time interval did not result in an appreciable decrease in final swimming speeds of coho salmon, and settled for a 10 min interval. Dahlberg *et al.* (1968) also used a 10 min interval allied to small velocity increments and provided evidence that a test period of 30–50 min was reasonable for obtaining an estimate of sustained speed as opposed to 5 h suggested by Brett (1964). In preliminary experiments it was determined that a time interval of 40 min did not give significantly lower results than the 20 min interval ultimately adopted in the present experiments. Nevertheless doubt must remain whether the swimming speeds obtained in these experiments prior to fatigue speeds could have been sustained indefinitely.

Brett (1964), utilizing data of Bainbridge (1960, 1962), was able to establish a fatigue curve with three zones, the two transition points lying between 12–24 sec and at 300 min. It is the later transition which terminates the fatigue curve and represents sustained activity, the period between 25 sec and 300 min representing steady performance in which the maximum achieved velocity is dependent upon the length of exposure. Exposures of 20 min as opposed to 300 min would only be expected to cause an elevation in final velocity of some 15 %.

Another way in which an attempt can be made to solve this problem of the steady state is to compare values of swimming speed obtained in the present experiments with values previously reported in the literature; however, corrections for differences in temperature and body length must be made. It is probably safe to ignore temperature effect over the range of 10 to 20 °C, since Brett (1967) has shown that the effect on

final swimming speed in increasing-velocity tests is slight. The problem of differences in body length can also be overcome if the assumption is made that velocity (V cm/sec) is proportional to the square root of body length (L cm) (Brett, 1964; Bainbridge, 1962; Fry & Cox, 1970). In other words, $V/\sqrt{L} = K$. This constant (K) has been calculated for speeds reported in the literature as being sustained for periods in excess of 5 min. For sockeye salmon (Brett, 1964), coho salmon (Dahlberg *et al.* 1968), underyearling wild coho (Davis *et al.* 1963), brown trout (Reimers, 1956) and rainbow trout (Reimers, 1956, and present experiments) the constant lies in the range of 18–22, whereas for trout studied by Bainbridge (1962), king salmon (Kerr, 1953), and underyearling coho (Brett, Hollande & Alderdice, 1958), the constant lies in the range of 9.5–12.5. The constants imply no specific differences between trout or salmon and furthermore support the suggestion that there is no real difference in critical swimming speeds as determined in water tunnels (Davis *et al.* 1963; Brett, 1964; Dahlberg *et al.* 1968; present experiments) within the range of time intervals over which experiments have been performed. It is interesting to try to explain the low constants obtained by Kerr (1953), Brett *et al.* (1958) and Bainbridge (1962). There appears to be no relation between the constants and condition factor of the animals tested; the condition factor of Bainbridge's (1962) trout was approaching 1.4, whereas in the present series of experiments the condition factor was about 1. Similarly the condition factor of Dahlberg *et al.*'s (1968) salmon was higher than that of Brett's (1964) salmon. Of course it is possible that the relationship $V = K\sqrt{L}$ does not hold for very short or very long fish. Certainly the low constants were associated with the shortest and the longest fish. Alternatively it may be a matter of technique in that two of the low constants were found in experiments performed in 'fish wheels', whereas four of the five higher constants were found in experiments performed in 'water tunnels'.

After considering the accumulated information it seems reasonable to assume that at speeds below the critical velocity the fish were in a reasonably steady state, satisfying one of the conditions of the theoretical analysis (Jones, 1971). At the highest speeds several factors may be limiting performance, such as: inability to supply enough oxygen to the gills, inability to deliver enough oxygen to the tissues, inability to remove metabolic products or to provide adequate substrate or to activate enzymic processes. Brett (1967) has pointed out that since much higher burst speeds can be achieved than the maximum sustained speeds (Bainbridge, 1962), energy liberation at the muscle site is not likely to be the failing mechanism. Brett (1964) also suggested that performance which can be maintained for 2–200 min probably relates to the supply of metabolites and the ability of the liver to provide adequate supplies of glycogen. The ability to mobilize glycogen reserves and withstand an oxygen debt depends on preconditioning of the fish (Hochachka, 1961), and since the present fish were trained it would be advantageous to presume this presented no problem to them. Of course the increased capacity to withstand an oxygen debt is possibly a complicating factor in that the final period of swimming may be achieved after the breakdown of metabolite supply. However, this is unlikely to produce very large errors for, at burst speeds at least, within 2 min muscle glycogen is reduced to one-half of resting levels with rapid elevation of pyruvate and lactate (Black, Robertson & Parker, 1962).

The implication of these factors is that during steady activity the limiting factors are the supply and removal of oxygen and carbon dioxide at the gills and tissues. Collapse ensues shortly after supply fails to match demand. One possibility is that the cardiac and branchial muscles are unable to meet the demands placed on them, but the other and certainly more attractive possibility is that their own oxygen requirement becomes so great as to limit oxygen supply to other active tissues. The latter assumption was made in the theoretical analysis (Jones, 1971) and it was predicted that at 15 °C maximum performance would be limited almost equally by the oxygen demands of the cardiac and branchial pumps. This conclusion seemed reasonable since maximum performance implies that all systems should be at their maxima rather than one factor being dominant over the others.

Both anaemia and hypoxia considerably reduced the maximum swimming speeds of the fish tested at both temperatures, the simplest interpretation of these results being that the metabolic cost of both cardiac and branchial pumps tends to place a restraint upon the maximum performance of normal fish. However, interpretation of the results in this manner is probably a gross oversimplification. For instance, the reduction in haematocrit and consequently haemoglobin concentration of the blood as well as increasing cardiac work due to the reduction in oxygen-carrying capacity will also considerably reduce the buffering capacity of the blood. This latter factor may have an important effect on performance during anaemia.

Similarly at the levels of hypoxia used there will undoubtedly be an increase in cardiac work due to the reduction in arterial oxygen saturation from normal levels. At both temperatures the work done by the branchial pump will be approximately doubled at any given level of activity whereas how much cardiac work will be increased is a matter of some speculation. Judging from oxygen-dissociation curves of trout blood published by Cameron (1971), the values of arterial carbon dioxide tensions found during activity (Stevens & Randall, 1967), and arterial oxygen tensions recorded under hypoxia (Holeton & Randall, 1967), it might be concluded that for high-temperature fish the reduction in oxygen-carrying capacity of the blood would be some 30–40%. For low-temperature fish the reduction would be much less than this and can probably be ignored. Nevertheless the high-temperature fish swam significantly faster than the low-temperature fish.

The inability to establish a relationship between swimming performance and haematocrit further complicates the reasoning behind any explanation of the performance of the high-temperature fish. There would appear to be at least two suggestions that could be made on the basis of the present evidence: (1) that the metabolic cost of the branchial pump in high-temperature fish is insignificant; or (2) that the reduction in oxygen content of the blood is nowhere near as great in the swimming hypoxic fish as would be predicted from data in the literature. Certainly in some mammals changes in oxygen affinity of the haemoglobin may be brought about by alterations in 2–3 diphosphoglycerate levels within the red cells (Bromberg, 1970) and there is some evidence that ATP may act in a similar manner in teleost fish (Gillen & Riggs, 1971). There is also some evidence in the literature for the branchial pump limiting performance at high temperatures. For instance, Davis *et al.* (1963) obtained reductions in maximum performance even with very small reductions in environmental oxygen tensions, before effects on the oxygen content of the blood could have been significant.

Also Brett (1964) obtained an increase in oxygen consumption during maximum performance of one population of salmon at 20 °C in the presence of elevated environmental oxygen tensions.

There was yet another complicating factor in this series of experiments in that the haematocrit of high-temperature fish was some 25 % greater than that of low-temperature fish. However, it seems likely that this increased oxygen-carrying capacity of the blood (Holeton & Randall, 1967) would have been offset somewhat by the greater degree of hypoxia to which the high-temperature fish were exposed.

In order to delimit precisely the role of the branchial and cardiac pumps in limiting maximum performance a far more sophisticated series of experiments is required. At the moment it seems safe to conclude that at low temperatures both hypoxia and anaemia affect the maximum swimming performance, and increased metabolic costs of both cardiac and branchial pumps may be two of the components by which this effect is exerted. At high temperatures the situation is more complicated, and it is not presently possible to separate conclusively the effects of these two components. Even so the results obtained are not incompatible with the results of a theoretical analysis presented previously (Jones, 1971).

SUMMARY

1. A series of increasing water-velocity tests in a water tunnel has been used to investigate the maximum swimming performance of two groups of rainbow trout, one acclimated to high temperature (21–23 °C) and the other to low temperature (8–10 °C).
2. At temperatures close to their acclimation temperatures there was no significant difference between the maximum swimming speeds of the two groups of trout.
3. Exposure to an environmental oxygen tension of half the air-saturation value resulted in a 43 % reduction in maximum swimming performance at low temperature and a 30 % reduction at high temperature compared with normal animals.
4. Reduction in haematocrit to one-half or one-third normal resulted in a 34 % reduction in maximum swimming speed at low temperature and a 40 % reduction at high temperature compared with control animals (blank injected).
5. The results are discussed in terms of whether fish can be assumed to be in a steady state at all velocities below the critical velocity and whether it is possible to attribute the differences in performance, during anaemia and hypoxia, to increased metabolic cost of the cardiac and branchial pumps.

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