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Aspects carbohydrate metabolism in the plerocercoid of Schistocephalus solidus.

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ASPECTS OF ~~GLUCOSE~~ Carbohydrate METABOLISM IN THE PLEROCERCOID OF *Schistocephalus solidus*.

BY

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ABSTRACT

Three spined sticklebacks infected with Schistocephalus solidus plerocercoids were used for *in-vivo* experiments to determine the relative distribution of ^{14}C-labelled sugars (D-glucose and 3-O-M D-glucose) in fish and worm tissues.

A maximum of 23.6% labelled material from D-glucose was found in plerocercoids after 4.5 hours and a maximum of 2.8% labelled material from 3-O-M glucose after 24 hours. Comparison of distribution levels between the two sugars and between infected and uninfected fish tissues did not show significant differences except in a few cases.

In-vitro studies with plerocercoids demonstrate D-glucose uptake to be sodium dependent, saturable with an apparent Kt of 0.66mM, inversely related to dry weight and absorbed against a concentration gradient. Uptake is also affected by temperature with high rates at 20° and 40°C and lower rates at 10° and 30°C.

Uptake is inhibited (non-competitively) by phlorizin and to a lesser extent by ouabain and DNP. The nature of inhibition caused by the latter two compounds was not determined.

The effect of 3-O-M glucose on D-glucose uptake was inconclusive. Similarly the effect of D-galactose was inconclusive and also inconsistent, causing both stimulation and inhibition of D-glucose uptake.

A gradient of glucose absorption is present along the length of the plerocercoid; the lowest rate being found at the anterior section and the highest at the posterior end.

Plerocercoids incubated under aerobic conditions in ^{14}C-D-glucose produced insignificant amounts of labelled CO_2.

Under aerobic conditions lactate is produced by plerocercoids at the rate of 0.13mg/ml/hour but no detectable amounts of pyruvate were found under similar conditions.

Glycogen extracts from plerocercoids incubated *in-vivo* contain increasing levels of ^{14}C-labelled material with increased incubation periods.

Host metabolism is significantly affected by the presence of plerocercoids. Lower levels of glycogen are found in the liver tissue of infected fish compared to uninfected fish. Also the rate of D-glucose uptake by fish stomach and intestinal tissues is considerably reduced in infected fish.

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Introduction and Aims

A significant proportion of the research carried out in recent years on tapeworm physiology has been concerned with nutrient absorption and the mechanisms involved in this process.

Much of the work has been performed with species readily cultured and maintained in the laboratory, e.g. Hymenolepis diminuta (Phifer, 1960a,b,c; Read, 1961,1967; Read, Rothman and Simmons, 1963; Podesta and Mettrick, 1974), Taenia taeniaeformis (von Brand and Bowman, 1961; von Brand and Gibbs, 1966) and Taenia crassiceps (Murrell, 1968; Pappas and Read, 1972).

Schistocephalus solidus has been the subject of many investigations, due in part to its relatively widespread occurrence in stickleback populations, and although much has been discovered concerning its physiology and life-cycle, apart from the observations of Hopkins (1952) little is known of nutrient uptake by plerocercoids of this species. Morris and Finnegan (1968, 1969) have shown that the structure of the plerocercoid tegument is basically similar to that described for other tapeworms with microtrichs on the outer surface and basal invaginations near the basal lamina; structures which are often associated with tissues capable of transporting ions against a concentration gradient. Considering the relatively high glycogen content of Schistocephalus plerocercoids and the importance of glucose in growth and development of Hymenolepis species, it was considered necessary to obtain information concerning the role of glucose in plerocercoid nutrition.

The use of radioactive tracers has proved an invaluable aid in the accurate measurement of metabolic processes involved in the absorption and assimilation of nutrients, ^{14}C compounds were

therefore used in this study in conjunction with <u>in-vitro</u> and <u>in-vivo</u> experimental methods.

Although many in-vitro techniques are successful in providing valuable experimental data concerning the physiology of cells and organisms, they still serve only as an indication of possible <u>in-vivo</u> mechanisms and reactions. Certain questions concerning the distribution of 'labelled' nutrients between host and parasite tissues would have proved difficult to investigate by <u>in-vitro</u> methods. Similarly mechanisms involving glucose transport in and CO_2 production by plerocercoids would have proved difficult to investigate by <u>in-vivo</u> methods. Therefore a combination of both methods was important to provide a wider and more comprehensive survey on this aspect of plerocercoid physiology.

Schistocephalus solidus

Schistocephalus solidus Muller, 1776 is a pseudophyllidean tapeworm belonging to the Family Diphyllobothriidae, Sub-Family, Ligulinae. The species has a widespread distribution throughout Europe and North America (Chappell and Owen, 1969; Hanek and Threlfall, 1970; Lester, 1971, 1974; Vik, 1954).

The adult worm is typically found in the intestinal lumen of piscivorous birds. The procercoid occurs in Cyclops species and the plerocercoid in three-spined sticklebacks. As in all Diphyllobothrid tapeworms a free-living ciliated coracidium is released from an operculate egg. This stage infects a number of Cyclops species, the copepodid stage apparently being most susceptible (Clarke, 1954). Within the haemocoele of Cyclops growth and development of the procercoid occurs. Infected copepods, when eaten by a stickleback allow the procercoid to penetrate the fish gut, subsequently developing to the plerocercoid. Finally when eaten by a warm-blooded host (piscivorous bird) the plerocercoid is transformed into the adult.

Information on the life cycle was reviewed by Hopkins and Smyth (1951), Clarke (1954) and Dubinina (1959) with the following general conclusions. The time interval for release of coracidia from a single batch of eggs varies from three weeks to six months. As S. solidus has no distinct dormant stage in the life cycle, variations in the period of egg hatching allow some degree of overwintering to occur(Clarke, 1954).

Nybelin (1919) first demonstrated that Cyclops species acted as hosts for S. solidus larvae and infected Cyclops serratulus and Cyclops bicuspidatus experimentally. Subsequent work by Callot and Desportes (1934), Thomas (1947) and Clarke (1954) has shown that

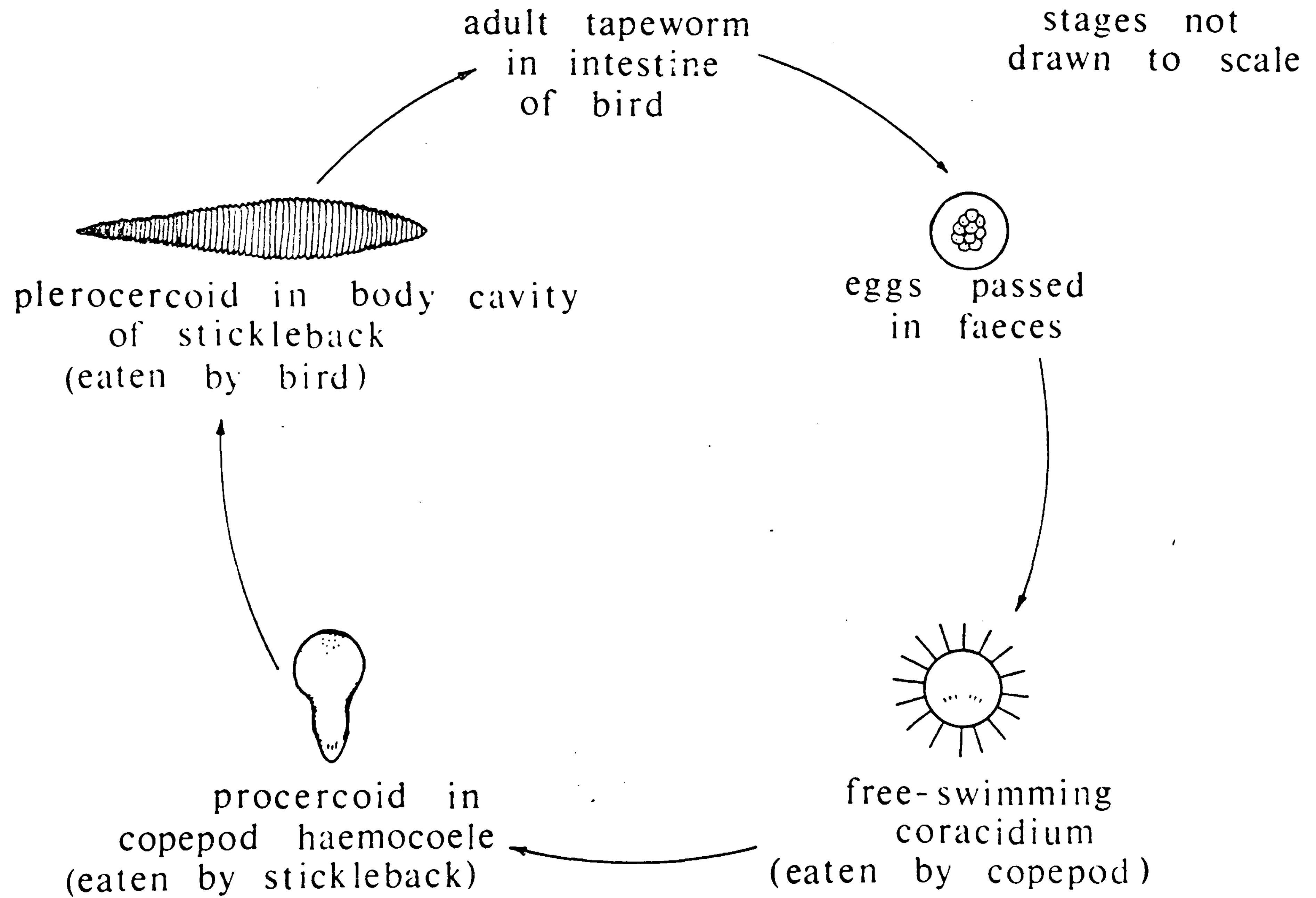


fig. 1

other species; Cyclops viridis and Cyclops leukarti may be infected.

Within the body cavity of the invertebrate host the procercoid develops a cercomer with hooks. Forms capable of infecting fish grow within 3 - 4 weeks and can remain viable in the crustacean host for up to three months (Clarke, 1954).

Following ingestion by a stickleback the procercoid is freed from the copepod and penetrates the fish gut to reach the coelom. This process can take as little as two hours (Clarke, 1954), compared to twelve hours in Diphyllobothrium species (Janicki and Rosen, 1917).

The plerocercoid of Schistocephalus is usually very specific with regard to its fish host. Although there are many records of this stage being found in a variety of fish species, Vik (1954) considers these accounts as cases of pseudoparasitism.

Procercoids can infect the closely related ten-spined stickleback, Pungitius pungitius, but development rarely proceeds beyond two weeks which implies a rejection response (Orr, Hopkins and Charles, 1969).

Three phases of growth are described for the plerocercoid (Hopkins and McCaig, 1963). A 3 - 6 mg phase at which the plerocercoid becomes segmented into 60 - 90 proglottids, a 10 - 20 mg phase when the genital primordia become easily recognisable in stained sections and a 50 - 70 mg phase when the rate of change in chemical composition alters with deceleration of the growth rate.

Formation of proglottids is unusual in the larval stage of pseudophyllidean tapeworms but a necessary corollary of the short adult life span, enabling rapid development to occur within the final host. Contrary to earlier observations (Clarke, 1954) there is a significant increase in proglottid number after initial formation

(Orr and Hopkins, 1969).

Infection rates in fish vary in different localities and from season to season, rates of up to 100% being reported in some populations (Smyth, 1946). Arme and Owen (1967) found that numbers of S. solidus tended to decrease with increasing stickleback weight and stated that it is unusual for the incidence of infection to exceed 50%. Chappell(1969a) found 1 - 55% infected in a Yorkshire pond , whereas Smyth (1946) found 100% infection rate in a Yorkshire lake. There is clearly a wide variation depending on the availability of host species and environmental conditions.

The burden of infection in individual fish is also a very variable factor with single and multiple infections commonly occurring. The number of plerocercoids in fish is usually low, being in the region of 1 - 4 worms per fish, although cases where as many as 130 worms in a single fish have been recorded (Arme and Owen, 1967).

Maturation of the plerocercoid into the adult is dependent on a critical temperature threshold of 35°C (Smyth, 1954). This can be induced in-vitro when plerocercoids are incubated in a suitable medium. A minimum critical weight of 10 mg (wet weight) must be reached before maturation can be successfully achieved (Hopkins and McCaig, 1963).

In contrast to the plerocercoid the host range of the adult is extensive, with up to forty two definitive host species having been reported. Maturation has been demonstrated in pigeons and even in mice (Clarke, 1953).

Maturation to the adult form is a rapid process; spent eggs have been shown to pass out of the bird host 44 hours after infection (Hopkins, 1950). The adult rarely lives longer than six or seven days. Since there are no functional organs for attachment of the

adult worm, its presence in the gut is presumably maintained by muscular movements to counteract gut peristalsis. Proglottids remain attached until the worm dies, eggs being passed out through the uterine pore.

The effects of parasitism by plerocercoids upon three-spined sticklebacks has been examined by Arme and Owen (1967), Meakins and Walkey (1975), Lester (1971) and Pennycuick (1971d).

Investigations of plerocercoid growth have been made by Hopkins and McCaig (1963), McCaig and Hopkins (1965), Sinha and Hopkins (1967), Orr and Hopkins (1969), Meakins and Walkey (1973).

A reduction in worm growth rates with increase in worm size and number was demonstrated *in-vivo*, (Meakins and Walkey,1973). It would seem from this finding that a growth capacity exists in the fish body cavity with characteristics similar to the 'crowding effect' of *Hymenolepis* species.

In terms of host-parasite energy budgets Walkey and Meakins (1970) demonstrated that the calorific conversion efficiency of the plerocercoid/stickleback relationship was greater than that of unparasitised fish. This however resulted in a greater depletion of host food reserves; infected fish surviving periods of starvation less successfully than unparasitised fish.

Despite the apparent restrictions imposed by host size and competition for nutrients among plerocercoids in multiple infections, *S. solidus* nevertheless achieves a highly successful relationship with the three-spined stickleback, as indicated generally by its close specificity, its enormous size increase and its advanced state of development; namely proglottid formation and maturation.

Gasterosteus aculeatus

The three-spined stickleback *Gasterosteus aculeatus* L., is a small fish, 4 - 6 cm in length, characteristically armed with three dorsal spines (modified pelvic fins) all with a flexible base allowing them to be raised for defence. The species has a world wide distribution and is able to tolerate a wide range of marine and freshwater environments, and has also been the subject of much research. *Gasterosteus* belongs to the Order,Mesichthyes; Super-Order, Teleostei; Class, Actinopterygii. Sub-species were described by Heuts (1947) on the basis of lateral plate numbers. Forms with large numbers,(i.e. greater than 15) extending from pectoral fins to the tail being designated as *G. aculeatus trachurus* while those with only a few plates near the pectoral fins being designated *G. aculeatus-leiurus*. Hybrids are produced by interbreeding between these two sub-species yet electrophoretic separation of muscle extract shows distinct bands for both. Hagen (1967) concluded that the North American *leiurus* and *trachurus* forms were reproductively isolated with well developed isolating mechanisms and exhibiting genetic divergence. In certain areas of North America he classed them as distinct species or sub-species, ecological factors acting to separate them.

The reproductive behaviour which involves courtship, nest building and parental care was extensively studied by Hoogland et al (1956), Nelson (1965), Symons (1965) and Tinbergen and Iersel (1947). A detailed study of feeding habits by Hynes (1950) showed that the diet of this fish consisted largely of crustaceans and insects with slight differences depending on fish age. Small fish,under one year, fed mainly on copepods, cladocerans and chironomid larvae whereas larger fish, over one year, fed predominantly on insect larvae. Fish also fed on algae when no animal food was available. However Clarke (1954)

found very few copepod species in the stomach contents of sticklebacks during a twelve month survey.

In both marine and freshwater forms up to thirty-four parasite species are recorded (Lester, 1974). Other studies, (Chappell and Owen, 1969) and a review by Chubb (1970a) indicate the wide variety of parasites which infect this fish; a reflection on the wide-spread distribution of the species.

The pathological effects of S.solidus plerocercoids upon sticklebacks (this being one of the commonest parasites of the fish) were studied by Arme and Owen (1967), Pennycuick (1971) and Meakins and Walkey (1975). Lester (1971) examined the behavioural response of infected sticklebacks in relation to oxygen requirements and suggested that the higher frequency of infected fish in the surface areas of ponds and lakes, was a direct result of increased oxygen demand due to the presence of plerocercoids. At routine and maximum activity levels, infected fish appear to have a greater oxygen consumption than uninfected fish, (Meakins and Walkey, 1975).

Other pathological effects upon sticklebacks attributed to Schistocephalus plerocercoids are, reduction of liver weight, reduction in packed cell volume of erythrocytes and delay or prevention of oogenesis (Arme and Owen, 1967). Pennycuick (1971d) also observed delay and prevention of spermatogenesis.

Carbohydrate absorption by cestodes

Absence of a distinct digestive system in cestodes suggests that the absorption of nutrients is a process taking place through the worm tegument. Evidence for this structure acting as a nutrient absorption site has been presented by Read (1955), Kent (1957), Rosario (1962), Threadgold (1962), Rothman (1963) and reviewed by Lee (1972) and Lumsden (1975). From detailed studies of electron microscope preparations microtrichs were shown to be present on the surface of nearly all tapeworms investigated,(Read, 1955; Rothman, 1959; 1960; Threadgold, 1962). The tegument is shown as coenocytic in nature with mitochondria present in the distal region (Read, 1955; Lumsden, 1966). This implies that the region is a metabolically active one. Initially evidence for an absorptive function of the structure was suggested by the presence of phosphatases which are indirectly associated with certain absorption phenomena in _Taenia pisiformis_ and _Monieza expansa_ (Erasmus,1957ab) _Hymenolepis diminuta_ (Phifer, 1960c) larval and adult _Ligula intestinalis_ (Arme, 1966).

The role of phosphatases in sugar uptake has been questioned however since inhibitors of the phosphatase systems do not appear to affect sugar absorption by helminths (Lumsden, 1975). There is also no evidence for a coupled phosphate-sugar transport system (Kaback, 1970, 1972) nor has an ATP-ase mediated ion pump of the type suggested for hexose transport in mammalian intestinal mucosa been demonstrated.

Other enzyme systems which may play an important role in carbohydrate assimilation by tapeworms have been shown to influence the digestion of polysaccharides. Taylor and Thomas (1968) demonstrated the enhancement of starch hydrolysis by extrinsic α-amylase in the presence of living tapeworms. Read (1973)

investigating possible contact digestion mechanisms concluded that there was an adsorption of pancreatic α-amylase on the tapeworm tegument, since maximum enzyme activity was noted at low enzyme concentrations and could be reversed by washing.

The mechanism of sugar and amino-acid uptake has been the subject of extensive investigation, (Phifer, 1960b,c; Read, 1961; Read and Simmons, 1962; Read, Rothman and Simmons, 1963; von Brand, McMahon, Gibbs and Higgins, 1964). Read (1966) considered that at least five processes were involved in nutrient uptake;

(1) Mass flow through pores.

(2) Diffusion.

(3) Facilitated diffusion involving a specific structural relationship between the membrane and the substrate.

(4) Active transport involving a specific structural relationship between the membrane and the substrate and an energy requiring source from metabolism.

(5) Pinocytosis.

Evidence for pinocytosis in Schistocephalus plerocercoids is presented by Threadgold (1977, personal communication) although most evidence for other tapeworm species indicates that a predominantly active uptake mechanism is involved with features very similar to those for vertebrate intestinal cells.

The presence of sodium ions appears to be essential in most cases for the movement of glucose molecules across intestinal cell membranes. The role of sodium ions in carbohydrate absorption by tapeworms has been investigated by von Brand and Gibbs (1966), Fisher and Read (1971), Dike and Read (1971b), Pappas et al (1973), Pappas and Freeman (1975) and Read et al (1974). It was generally shown that sodium ions are an important component in the process. Influxes of sodium coupled with glucose uptake were demonstrated

in Hymenolepis diminuta (Read et al, 1974) and Calliobothrium verticillatum (Pappas and Read, 1972).

The most important feature of active uptake is the selective movement of solute molecules against a concentration gradient. This has been shown for a number of tapeworms including Hymenolepis microstoma (Pappas and Freeman, 1975), Taenia crassiceps larvae (Pappas, Uglem and Read, 1973) and H. diminuta (Phifer, 1960b).

Michaelis-Menten equations designed to resolve enzyme kinetics can be applied to interpret the uptake process, indicating that enzyme complexes are possibly involved.

Although solute transfer across cell membranes is still not fully understood, certain theories have been put forward in an attempt to explain the process. Two of these are the 'Sodium carrier hypothesis' (Crane, 1960) and the 'Association induction hypothesis' (Ling, Miller and Oschenfeld, 1973). The main difference between these two is that the latter theory does not require the presence of a carrier molecule complex; only the presence of specific absorption sites.

Carbohydrates are now generally regarded as essential requirements for the normal development of most adult tapeworms. Variations in the amount of carbohydrate available to worms living in the intestine can considerably alter growth rates, glycogen levels and worm size. H. diminuta adults fail to grow or establish themselves when the host diet is deficient in carbohydrate (Read, 1959; Read and Rothman,1957ab; Read and Simmons, 1963). This effect varies depending on the quality of carbohydrate fed to the host. Calorifically and quantitatively identical amounts of simple sugars such as galactose, glucose, maltose and others such as sucrose and dextrin when fed to rats infected with H. diminuta resulted in a decrease in worm dry weight. In contrast to this, when hosts were fed with polysaccharides

an increase in worm weight was shown. A diet containing potato starch increased worm weight by 22% and one consisting of corn starch increased worm weight by 30%. The explanation offered for this is that digestion of polysaccharides in the host diet allows, in total, a greater amount of carbohydrate to become available for the tapeworms, due to the slower breakdown and assimilation rate of polysaccharide in comparison to simpler sugars (Dunkley and Mettrick, 1969).

Carbohydrates are found in the tissues of tapeworms in a variety of forms from simple monosaccharides to the glycogen, glycolipid and glycoprotein complexes. Hydatigera taeniaeformis larvae were found to contain 1.2% sugar dry weight and 43% glycogen dry weight (Hopkins, 1960).

In most tapeworms the metabolism of carbohydrate is essentially by means of the Embden-Meyerhof pathway of glycolysis, with organic acids as the end product. According to the species of tapeworm, environment in which it lives and substrate metabolised, end products can vary considerably.

Aerobic and anaerobic fermentations are characteristic of most parasitic helminths (von Brand, 1966). Most will also take up oxygen when available as has been found for H. diminuta (Read, 1956) larval and adult Taenia taeniaeformis (von Brand and Bowman, 1961), larval Taenia crassiceps (Taylor et al, 1966), and Diphyllobothrium dendriticum plerocercoids (Reuter,1967). Schistocephalus solidus plerocercoids were calculated to take up oxygen at a lower rate than for free-living poikilotherms (Davies and Walkey,1966).

Although a number of'Krebs cycle' enzymes have been identified in helminths, very few tapeworms apart from Echinococcus granulosus scoleces appear to have a fully functional Citric acid cycle. The general lack of effect that oxygen has on glycogenesis and glucose

uptake corresponds with the theory that oxygen is energetically unimportant.

The role of carbon-dioxide in glycogenesis and glycolysis seems to be more important than that of oxygen. Carbon-dioxide fixation has been demonstrated in H. nana (Prescott and Campbell, 1965), E. granulosus (Agosin and Repetto, 1965) and Monieza expansa (Bryant, 1972). Read (1967) investigated the effect of carbon-dioxide on glucose absorption in H. diminuta and Hymenolepis citelli and found a stimulation of both glycogenesis and glucose uptake by 5% CO_2. This effect was reduced at 20% CO_2 both under aerobic and anaerobic conditions. Prescott and Campbell (1965) however, found a reduction in the amount of labelled CO_2 incorporated into polysaccharide under anaerobic conditions.

Adult H. diminuta produce aspartic, malic, lactic, succinic and fumaric acids (Overturf, 1966). The larvae and adults of H. taeniaeformis produce fumaric, lactic, succinic, pyruvic and acetic acids in addition to ethanol as products of fermentation, (von Brand and Bowman, 1961). Larval scoleces of E. granulosus excrete pyruvic, lactic, succinic and acetic acids although under anaerobic conditions no pyruvate is formed (Agosin, 1957).

The range of sugars utilised by tapeworms is limited compared to the number utilised by other endo-parasites. Most cestodes can utilise glucose and galactose but vary in their ability to use other sugars. Glucose and galactose were absorbed by eight species of tapeworm from elasmobranchs. Six of these absorbed glucose and galactose exclusively, one exception being Phyllobothrium foliatum which absorbed maltose at a rate ten times greater than that of glucose (Laurie, 1961). The explanation given for this ability is that the position of Phyllobothrium in the host gut (being in the

last quarter of the spiral valve) means that only the more complex sugars reach this point.

Experiments with Diphyllobothrium latum and Ligula intestinalis plerocercoids have conclusively shown that these worms are capable of absorbing glucose (Markov, 1939). Experiments on the carbohydrates metabolised by H. diminuta and H. citelli revealed that they were unable to utilise maltose, lactose, mannose or sucrose; glucose and galactose however were utilised (Read and Rothman, 1958).

Schistocephalus solidus plerocercoids cultivated in glucose media conserve more of their glycogen reserves than those cultured in non-glucose media (Hopkins, 1952). A corresponding decrease of glucose in culture media indicated that absorption was taking place. This was found to be the case only when glucose was present in the medium above a minimal absorption threshold value. The rate of glycogen depletion for plerocercoids incubated in glucose-containing media was essentially similar to the rate for worms matured in-vivo. Non-glucose containing media failed to conserve carbohydrate reserves in plerocercoids, indicating the importance of exogenous glucose in nutrition.

Hopkins (1950) pointed out the possible significance of high glycogen levels in pseudophyllidean larvae. He suggested that a direct correlation exists between the amount of glycogen present in tissues and the level of maturation reached in larval development, especially of the reproductive system. Diphyllobothrium larvae attain a relatively low level of development and a glycogen content of 17.9% (Markov, 1939). Ligula intestinalis plerocercoids have a higher level of morphological development and a glycogen content of 33.7% whereas Schistocephalus plerocercoids have even more highly developed genital structures than either of the other two species and a glycogen content of 51% (Hopkins, 1950; 1952).

The role of an energy-requiring process, such as an active transport mechanism involved in glucose uptake is supported by Phifer (1960c)in experiments using H. diminuta. Starved worms which were incubated in a metabolisable sugar; glucose or galactose, subsequently increased their glucose uptake rate over that of starved worms which had been pre-incubated in fructose, a sugar not utilised by this species. Worms which had not been starved showed no increase in glucose uptake rate after pre-incubation in glucose or galactose. The significance of this was interpreted as the absorptive mechanism having been supplied with an energy source during the pre-incubation period thus accounting for the increase in sugar uptake by starved worms when incubated in glucose or galactose containing media.

The active absorption of glucose can be affected by the presence of isomers such as galactose or mannose which are capable of 'competitively' inhibiting glucose uptake in some tapeworms.

In experiments using H. diminuta, Phifer (1960c)concluded that glucose uptake was apparently very stereospecific since there was no inhibition of uptake by structurally related sugars. This conclusion was disputed by Read (1961) who found that galactose and certain other monosaccharides competitively inhibited glucose influx in short term incubations. The difference between these results can possibly be explained by the fact that Phifer did not use the sugars investigated in large enough concentrations for any possible inhibitions to be demonstrated.

In experiments using Calliobothrium verticillatum, Fisher and Read (1971) found that both galactose and maltose inhibited glucose uptake in two minute incubations although no inhibition was evident with fructose, mannose or 3-O-Methyl glucose.

Non-competitive inhibition of glucose absorption is often

caused by compounds which appear to interfere with the actual transport mechanism. B-methyl glucoside inhibits glucose uptake in H. microstoma but has no effect on H. diminuta (Read, 1961), or C. verticillatum (Fisher and Read, 1971). 6-deoxygalactose inhibits glucose uptake in T. crassiceps larvae but has no effect on glucose transport in H. microstoma. Phlorizin inhibition of glucose uptake has been recorded in H. diminuta (Laurie, 1957; 1961; von Brand et al, 1964; Fisher and Read, 1971; Pappas and Read, 1972; Pappas et al, 1973; Phifer, 1960). Inhibition was also recorded for seven out of eight tapeworm species from elasmobranchs, the exception being Onchobothrium pseudo-uncinatum in which glucose absorption was actually stimulated by phlorizin (Laurie, 1961).

The nature of this inhibition has been suggested as a surface active mechanism with phlorizin competing for the sodium-dependent component and forming secondary bonds with other membrane molecules (Caspary et al, 1969). The view that the inhibition is a surface reaction is supported by the fact that washing reverses the effect (Laurie, 1957;). The amount of phlorizin actually entering the worm was measured and described as insignificant (Read, 1966).
The competitive or non-competitive nature of phlorizin action has also been investigated. Phifer (1960a)could find no conclusive evidence for either type of inhibition in H. diminuta although McCracken and Lumsden (1974) decided that the effect was a competitive one in this species and suggested that Phifer was unable to demonstrate this due to excessive amounts of inhibitor being used. Laurie (1957) concluded that phlorizin was a non-competitive inhibitor of glucose fermentation in H. diminuta. Fisher and Read (1971) working on C. verticillatum showed phlorizin to be a competitive inhibitor of glucose uptake.
In H. microstoma the effect was regarded as a partial competition of glucose absorption (Pappas and Freeman, 1975).

Inhibition by ouabain is thought to be a result of its action upon the sodium-dependent ATP-ase mechanism. The effect is only apparent when applied to the inner side of transporting epithelia (Bonting and Canady, 1964).

Most of the evidence for carbohydrate transport in tapeworms points to a predominantly active mechanism, essentially similar to that found in vertebrate intestinal cells. Carbohydrates also appear to be an obligatory nutritional requirement for growth and development of most tapeworms, in contrast to other animals. The nature and quantity of carbohydrate utilised varies with species, environment and uptake mechanism and sometimes even between the larval and adult stages of the same species.

Collection of Material

Gasterosteus aculeatus is a fish common in many ponds, lakes and reservoirs in Britain. The species therefore provides a readily obtainable source of material for scientific purposes.

Seventeen ponds of varying size and characteristics in the Epping Forest area were surveyed together with a section of the Regents canal, Victoria Park, London E1 and a section of the river Lee adjacent to Lockwood reservoir, Walthamstow.

Three-spined sticklebacks were collected in samples of sixty (examined) from thirteen of the ponds, the river Lee and Regents canal. Of these only five of the ponds , the river and canal actually contained fish infected with *Schistocephalus*. Of these sites only three (two ponds and the river Lee) had sufficient numbers of infected fish for use in experimental work. These were:

Fairmead pond	Grid ref.		TQ 409967
Walthamstow pond	"	"	TQ 393898
River Lee	"	"	TQ 350893

Sampling was carried out by means of a beam trawl, approximately 1.5m wide and 1m deep. Hand nets were used for collecting during the warmer months when infected fish were easier to detect and most abundant paricularly in surface waters of ponds.

Fish were transported to the laboratory in either large polythene bins or small plastic containers, depending on the numbers obtained. They were then transferred into a galvanised metal tank (150cm. 70cm. 95cm) with constant running water. Infected fish were later placed into perspex tanks, constantly aerated and maintained at room temperature. Dried and live *Daphnia* or live *Tubifex* were supplied as food at 3-4 day intervals.

All fish used for *in-vivo* experiments were of the sub-species *leiurus* with a mean lateral plate number of 3.7 ± 0.5.

Additional supplies of infected sticklebacks were obtained on two occasions from Queen Mary Reservoir, N.W. London. These fish differed in size and lateral plate number (11.5 ± 0.6) from the fish collected in other areas. Schistocephalus from this additional supply of fish were used only for in-vitro experiments.

Peak periods of plerocercoid infection in fish collected from the ponds were June and July; approximate values of 20% and 23% infection rates respectively being recorded in 1974. This level fell to approximately 4% during December 1974 and for one pond during January 1975, to zero.

Endo-parasites other than Schistocephalus found in fish were larval Diphyllobothrium species, larval Triaenophorus sp., and also metacercaria of Diplostomum species. Fish containing Diphyllobothrium and/or Triaenophorus in addition to Schistocephalus were not used for in-vivo experiments as it was considered that the presence of these other parasites would yield inconsistent results when used with those containing only Schistocephalus.

Ecto-parasites found were, Gyrodactylus sp., Trichodina sp., Glugea sp., Ichthyophthyrius sp., Argulus sp. and Hemiclepsis species.

In-vivo techniques

Infected and uninfected fish were allowed at least three days for acclimatisation to laboratory conditions before being used in experiments.

Fish were anaesthetised in a solution of MS 222 1mg/10ml in tap water until all opercular movement had stopped.

Five microlitres of experimental, labelled hexose solution was injected into the fish stomach through the mouth by means of a plastic cannula connected to a 10µl syringe (Hamilton).

To facilitate this injection technique, fish were held between two pieces of plastic foam attached to a small clamp. In case the cannula should accidentally emerge through a gill opening instead of entering the stomach, fish were placed ventral side uppermost to allow a better view of the injection procedure.

After injection fish were placed into 150ml of tap water until they showed signs of recovery (normally after 1-2 minutes). They were then washed in a fresh solution of water before being placed in the incubation beakers (250ml) containing 100ml distilled water. These were located in a constant temperature water bath (20°C) and periodically aerated as fish became too disturbed when aeration was continuous.

After the incubation period fish were pithed and washed briefly in fresh tap water. The body was opened from the anus to the pectoral region. Schistocephalus plerocercoids were removed, washed briefly in ringer and gently dried on filter paper before placing in 70% ethyl alcohol for extraction. The fish was dissected into six main parts which were extracted separately in ethanol. These were, Stomach, Intestine, Liver and spleen, Head, Tail, Trunk. The larger parts were cut into smaller sections to facilitate the

extraction process. All tubes containing plerocercoids and fish tissues in alcohol were double sealed with Parafilm "M" (Marathon Products), to prevent evaporation. Test-tubes were left in racks for at least 24 hours and shaken occasionally.

Samples of extract were counted for radioactivity and tissues dried to constant weight at 90°C after washing for 3-4 hours in 70% ethanol. Dried fish tissues were then digested by means of the 'nitric acid technique' to obtain the remaining labelled material. Dried plerocercoids were digested by the KOH method (p.26).

Since large differences in fish size may alter the rate of glucose absorption and incorporation, fish of approximately similar size were chosen for each series of experiments.

The injection technique also suffered from the disadvantage that fish sometimes regurgitated stomach contents on recovery from anaesthesia. Fish were therefore transferred to fresh water during recovery. This reduced the chances of low levels (labelled sugar) being gradually swallowed or absorbed through the gills.

<u>Technique for measurement of ^{14}C glycogen content of plerocercoids</u>

Plerocercoids from fish used in the 0.13, 0.5, 2.5, 10 and 18 hour *in-vivo* experiments were analysed for their glycogen content by the method of Roe and Dailey (1966) and samples measured for radioactivity.

In-vitro Techniques

General Incubation methods

Infected fish were maintained in the laboratory at ambient room temperature; approximately 20°C, for a minimum of three days.

Plerocercoids were pre-incubated for 15 minutes then placed in experimental media (2-5ml) in 10ml beakers or 15ml test-tubes. It was necessary to alter the volume of medium used to compensate for differences in larval size.

Ringer solution (incubation media) consisted of $\frac{3}{4}$ Locke solution buffered to pH 7.2 with Tris-maleate.

Locke solution: NaCl, 9g; KCl, 0.42g; $CaCl_2$, 0.24g; $NaHCO_3$, 0.2g; distilled water to 1l.

$\frac{3}{4}$ Locke buffered with Tris (hydroxymethyl) methylamine 0.25M and Maleic acid 0.25M.

Labelled sugars; D- (U-^{14}C) glucose and 3-O-Methyl-(D-glucose-(U-^{14}C) (The Radiochemical Centre, Amersham) were used with varying concentrations of unlabelled sugars to provide appropriate molarity.

Smyth (1946) found $\frac{3}{4}$ Locke solution to be the medium in which plerocercoids survived longest amongst incubation media he investigated.

For this study plerocercoids were shown to survive for at least 14 days in the buffered ringer solution.

All incubation containers were kept open to atmospheric conditions but maintained at a constant temperature of 20°C $\pm$ 2 except during experiments on the effect of varying temperature levels.

The standard incubation period was 30 minutes, after which plerocercoids were washed twice in fresh ringer and then dried on filter paper before extracting in 70% ethanol for at least 24 hours. Worms were then dried to constant weight at 90°C and digested in KOH.

Samples of extracts and digests were assayed for radioactivity by liquid scintillation techniques (p. 24).

General Analytical Procedures

Introduction

Certain analytical techniques were considered necessary to measure the levels of radioactivity in fish and worm tissues, therefore all radioactivity assays were carried out using a basic liquid scintillation counting technique for both ethanol extract and tissue digest samples.

In addition to ethanol extraction of 'free-pool' labelled compounds it was also thought necessary to digest tissue samples and determine the levels of radioactive material incorporated into them. Three methods of solubilising tissues were initially tested for this purpose.

In order to identify some of the by-products of glucose metabolism and the proportion of labelled 'free' glucose present, chromatographic techniques and certain biochemical tests were employed.

Liquid Scintillation Technique

The technique of liquid scintillation counting involves the transfer of energy from radioactive disintegrations to solvent molecules and onto fluorescent solute molecules. Energy pulses are then emitted from these and detected by the scintillation counter.

When a radioactive sample is highly coloured or reacts chemically with the scintillation mixture, a reduction in counting efficiency known as quenching can occur. Correction for this effect can be carried out by using external standard techniques involving an external gamma source and counting in two channels to obtain a ratio value. This value varies according to the degree of quenching, and with the help of calibration graphs can be measured.

Internal standard methods involve the addition of small

quantities of 'labelled' material, ideally with low or non-quenching characteristics and pre-determined activity levels. By simple calculation the difference between calculated and actual recorded activity levels will give the quenching value.

A detailed account of both the theoretical and practical aspects of scintillation counting is given by Birks (1964).

Procedures for solubilisation of biological materials to be used in liquid scintillation assays are described by Hansen and Bush (1967) and Rapkin (1967).

A more recent description of basic scintillation techniques and methods of sample preparation is presented by Neame and Homewood (1974). Descriptions of solvent systems and sample preparation are also given by Birks (1969) and Kobayashi and Maudsley (1969).

Scintillation materials used

Scintillation fluid: 59% toluene (scintillation grade); 5g/l of PPO (2,5-Diphenyloxazole), Koch-Light; 30% Triton X-100, Beckman; 11% ethanol abs, B.P.

Vials, 20ml glass with polyethylene seal screw caps, Beckman.

Pipettes, 10 and 50 microlitre, Eppendorf, (for samples). Zipette automatic dispenser (for scintillation fluid).

Extraction and Digest Techniques

Two methods were used for the tissue digests; potassium hydroxide method and the nitric acid method. A third method was used initially but rejected because it failed to digest tissues sufficiently.

Potassium hydroxide method

Tissue samples were hydrolysed in 4ml of 30% KOH at 70°C. 1ml was removed and neutralised with HCL (1-2ml). 1ml of this was then placed into 15ml of scintillation fluid and counted.

Nitric acid method (O'Brien, 1964)

Tissues were dissolved in 1-2ml concentrated nitric acid at 70°C. The solutions were then diluted to four times the original volume with distilled water and neutralised with equal volumes of 3M Tris (hydroxymethyl) methylamine. 1ml aliquots were counted in 15ml scintillation fluid.

Glycogen determination

Anthrone Reagent method (Roe and Dailey, 1966).

Reagents used: 1N sodium hydroxide, 1N perchloric acid, 95% ethanol containing 0.1% lithium chloride. Anthrone reagent; a solution containing 0.05% anthrone, 3% thiourea and 72% by volume sulphuric acid(conc. acid/distilled water ratio 2.57:1).

Tissue samples were dissolved in 3ml 1N sodium hydroxide in test-tubes placed in a boiling water bath. Deproteinisation of the solution was carried out by adding 6ml 1N perchloric acid. After dilution and filtering , glycogen was precipitated by the addition of 95% ethyl alcohol containing 0.1% lithium chloride. Samples were washed and centrifuged three times before dissolving in 2ml distilled water and 10ml anthrone reagent. Colorimetric determination of glycogen levels was assessed by means of a Unicam spectrophotometer set at 620 mμ

Biochemical Tests

Introduction

Helminths characteristically produce a number of acidic end products from glucose metabolism. Two of the most common products of glycolysis are lactic and pyruvic acid, with the levels varying according to whether conditions of incubation are aerobic or anaerobic. Although many other by-products are known to be excreted by helminths it was decided to investigate production of two of the generally more important products, lactic and pyruvic acid in the form of their salts, lactate and pyruvate.

General procedure

Plerocercoids were incubated individually in 15ml test-tubes containing 2-4ml ringer with 5mM D-glucose. Incubation period was up to 1 hour. Samples (0.1-0.5ml) were removed at specified intervals and replaced with equivalent amounts of ringer, containing glucose, to maintain the original volume. The samples were placed immediately into 5ml vials and frozen at -10°C for analysis on completion of the experiment.

Analytical procedures

Boehringer Mannheim Biochemica (Test) kits were used for lactic acid, pyruvic acid and D-glucose analysis.

G O D Perid method for glucose:

Samples (0.1ml) of standard (9.1mg D-glucose/100ml) and experimental solutions were incubated with 5ml solution 2 consisting of 100mM phosphate buffer, pH 7.0; POD = 0.8 U/ml; GOD = 10 U/ml; 1mg ABTS/ml. Incubation was carried out at 20°C for 30 minutes. Optical densities were measured at 620nm by means of a Unicam spectrophotometer.

Lactate method

Aliquots (1ml) of (0.5 glycine buffer, pH 9.0; 0.4M hydrazine), 0.1ml (27mM NAD) and 0.01ml (LDH = 650 U/ml) were added to 0.01ml sample of experimental solution. All samples were then incubated at 25°C for 1 hour before optical densities were measured at 340nm.

Pyruvate method

Samples (0.5ml) of experimental solution were incubated for 10 minutes at 25°C with 0.05ml (2.5mM NADH) and 5ul (LDH = 900 U/ml). Measurements of optical density were at 366nm.

Chromatographic analyses

Paper chromatography techniques were employed to analyse the alcohol extracts from plerocercoids used for in-vitro experiments, in an attempt to determine the quantity of free ^{14}C-D-glucose recovered after a 30 minute incubation period.

Although this method is probably less sensitive than T.L.C. it was chosen mainly because of the ease with which sections of chromatogram could be prepared for elution of labelled material.

Method

Plerocercoids were incubated for periods of 2, 5, 12, 20, and 30 minutes in media containing ^{14}C-D-glucose (0.5 µCi/ml) with 2.5mM D-glucose carrier. After washing and drying they were homogenised in 2ml absolute ethyl alcohol by means of a glass manual homogeniser. Homogenates were centrifuged, the supernatant removed and allowed to evaporate till dry. 0.05 ml distilled water was then added to this and samples spotted onto chromatography paper. The descending method was used and chromatograms removed once the solvent front had reached a pre-determined distance from the origin.

One centimetre strips cut across the width of the paper, from the origin to the solvent front were removed. These were cut into smaller sections and eluted in 1ml distilled water. Samples of eluate (0.5ml) were counted for radioactivity.

Materials used included: Shandon Unikit Descending Chromatography Tanks, Whatman No. 1 Paper (10 by 30 cm), Solvents (n-Butanol:glacial acetic acid: distilled water, 120: 30: 50.). (Ethanol: ammonia: distilled water, 160: 10: 30). Reagents were, bromocresol purple, bromocresol green for organic acids and benzidine for glucose. To determine Rf values of reference standards analar grade D-glucose together with lactic, pyruvic and succinic acids was used.

Results

After initial investigations with unlabelled reference compounds, the ethanol: ammonium solvent was found to give better separation of lactic and pyruvic acids than did the butanol: acetic acid solvent. The former solvent was therefore used for all chromatograms.

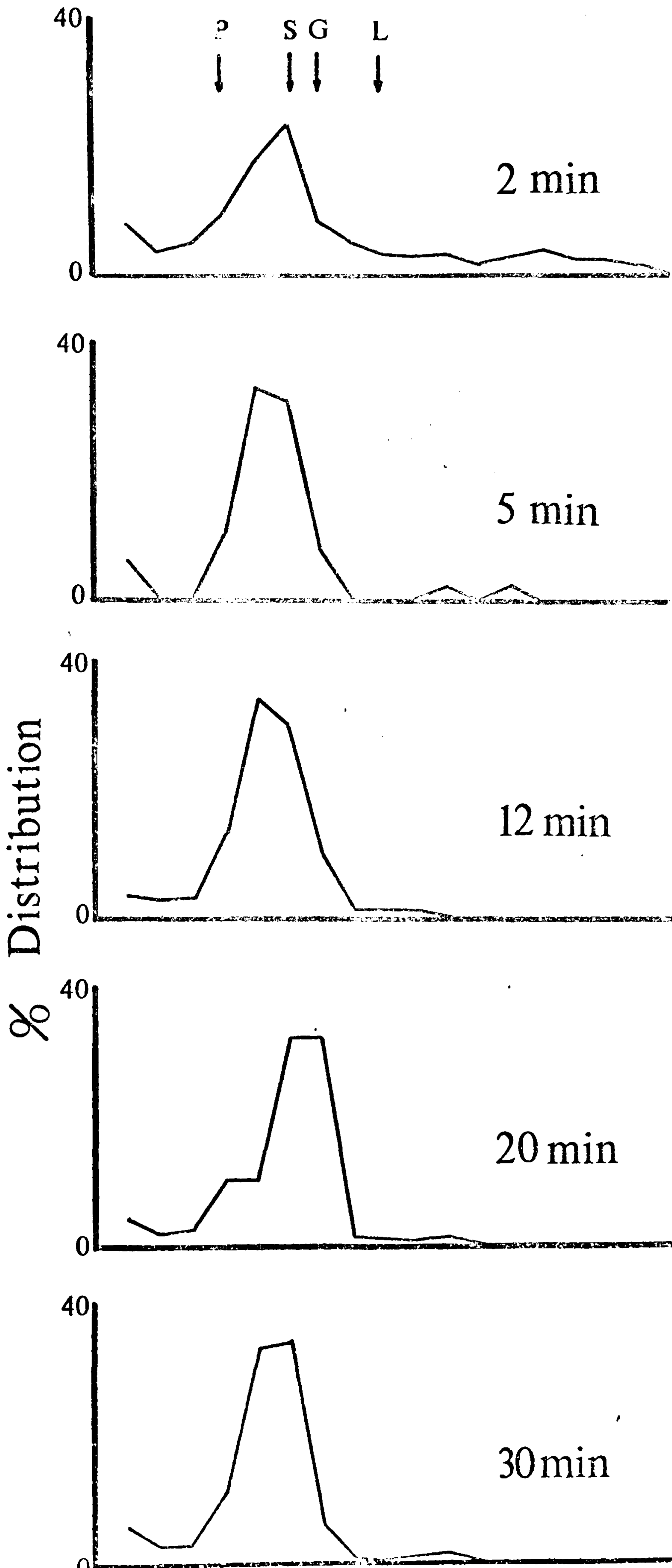
Although there were slight variations in the distribution of labelled material, a relatively stable pattern appears to have been reached after 30 minutes (Fig. 2). This apparently shows a peak of succinic acid with a lower production of D-glucose and insignificant levels of pyruvic and lactic acid.

Graphs show the percentage distribution of total radioactivity present in chromatograms from alcohol extracts. (Fig. 2).

Times indicate the incubation period for each plerocercoid.

Rf values of unlabelled reference standards run as co-chromatograms are indicated.

D-glucose (in vitro) chromatogram



G. d-glucose

P. pyruvic acid

S. succinic ..

L. lactic ..

fig. 2

In-vivo experiments

D-glucose with infected fish

Incubation period (hours)	Glucose Quantity (µl)	concentration (mM)	specific activity (µCi/ml)	No. of fish
0.13	10	100	9.0	7
0.5	10	"	"	5
1.0	10	"	"	6
1.5	5	"	"	6
2.5	5	"	"	6
4.5	10	"	"	5
7.0	5	"	"	3
10.0	5	"	"	5
18.0	5	"	"	5
24.0	10	"	"	5
D-glucose with uninfected fish				
1.0	5	100	9.0	5
2.5	5	"	"	6
10.0	5	"	"	6
24.0	5	"	"	10
3-O-M glucose with infected fish				
0.13	5	100	9.0	6
0.5	5	"	"	3
1.0	5	"	"	4
1.5	5	"	"	6
2.5	5	"	"	6
4.5	5	"	"	2
18.0	5	"	"	4
24.0	5	"	"	4
3-O-M glucose with uninfected fish				
1.0	5	100	9.0	4
2.5	5	"	"	4
10.0	5	"	"	4
24.0	5	"	"	4

TABLE 1.

In-vitro Experiments

Experiments were designed to examine the effects of a number of environmental and physiological factors influencing glucose uptake by plerocercoids *in-vitro*. These were arranged into the following sections

(1) Environmental and physical.

(2) Chemical inhibition.

(3) End products.

(4) Pathology (host).

Standard incubation and sample preparation techniques (p. 23;24) were used except where stated otherwise.

Environmental and physical

(i) D-glucose uptake with differing temperature, time and substrate concentration.

Temperature

Incubations of plerocercoids were carried out at temperatures of 10, 20, 30 and 40°C. Three individuals were used for each temperature. Concentration of glucose was 1mM with specific activity 0.5µCi/ml.

Time

Incubation times were, 0.5, 2.0, 5.0 and 7.0 hours. Solutions were changed every 0.5 hours with fresh media until the incubation time had been completed. Glucose concentration was 0.8mM with s.a. 1µCi/ml. Three plerocercoids were used for each time period.

Glucose concentration

Plerocercoids were incubated in four different glucose concentrations; 0.8, 1.6, 5.0, and 10mM. The ratio

between labelled and unlabelled glucose was constant being 0.08, 0.16, 0.5, and 1.0µCi/ml respectively. Five to six plerocercoids were used for each concentration.

Glucose concentration difference

Three plerocercoids were incubated separately in media containing unlabelled glucose (2mM). Samples of media were removed at intervals of 2, 12, 22, 40 and 60 minutes and analysed by means of the G O D-Perid method (p.27) for glucose concentration. Plerocercoids were analysed for glucose content by extracting in ethanol. Control worms incubated in non-glucose media were extracted in a similar manner.

(ii) Uptake of D-glucose and 3-0-M glucose in relation to dry weight of plerocercoid

Eleven larvae all differing in weight and size were used for each sugar.

For D-glucose experiment sugar concentration was 1.0mM with s.a. 0.25µCi/ml.

For 3-0-M glucose experiment sugar concentration was 1.0mM with s.a. 0.25µCi/ml.

(iii) Uptake rates of D-glucose along the strobila

Four plerocercoids were incubated in media containing D-glucose 1.0mM with s.a. 0.05 µCi/ml. After washing and drying they were cut transversely into four regions of approximately equal length; anterior, mid-anterior, mid-posterior and posterior. Each region was then extracted separately in ethanol and the proportion of labelled material in each determined.

Chemical inhibition

(i) Effect of 3-0-M glucose

Plerocercoids were incubated in the following solutions;

ringer media with D-glucose	0.1mM	and	0.002 µCi/ml
" " " "	0.5mM	"	0.012 "
" " " "	2.5mM	"	0.062 "
" " " "	5.0mM	"	0.125 "

Unlabelled 3-0-M glucose was added in concentrations of 0.1, 0.5, 2.5 and 5.0 mM to D-glucose media with the same concentration. Three larvae were used for each concentration.

(ii) Effect of D-galactose

(a) Plerocercoids were incubated in the following solutions;

ringer media with D-glucose	1.0mM	and	0.0125 µCi/ml
" " " "	5.0mM	"	0.0625 "
" " " "	8.0mM	"	0.1 "
" " " "	10.0mM	"	0.125 "

Unlabelled D-galactose was added in concentrations of 1.0, 5.0, 8.0 and 10mM to D-glucose media with the same concentration. Three to four larvae were used in each.

(b) Plerocercoids were incubated in the following media

ringer with (D-glucose 1mM and 0.25 µCi/ml) and D-galactose 10mM
" " " " " " " 20mM
" " " " " (no galactose)

Controls for the above experiments (i) and (ii) contained only D-glucose.

(iii) Effect of metabolic inhibitors

Plerocercoids were incubated in media containing D-glucose 5mM with 0.25 µCi/ml except where stated otherwise. Three to four larvae were incubated in each inhibitor concentration. Controls contained no inhibitor.

(a) Ouabain

Three concentrations were used; 0.1, 0.5, 1.0 mM. with D-glucose (5mM; s.a. 0.03µCi/ml)

(b) 2-4-Dinitrophenol

Three concentrations used were, 0.1, 0.5, 1.0 mM.

(c) Phlorizin dihydrate

Concentrations used were, (0.1, 1.0, 10, 100) x 10^{-3}

(d) Phlorizin with varying glucose concentrations

Phlorizin concentration was constant, 10^{-3}mM.
Glucose concentrations were, 0.1, 0.5, 1.0, 2.0 mM with s.a. of 0.012, 0.06, 0.125, 0.25 µCi/ml respectively.

(iv) Sodium dependence

Experimental media: Ringer with equimolar concentrations of LiCl or KCl replacing the NaCl component. Controls contained NaCl. Three larvae were used in each experiment.

(a) Li^+ medium and control medium contained D-glucose 1mM with 0.04 µCi/ml.

(b) K^+ medium and control medium contained D-glucose 1mM with 0.24 µCi/ml.

End products

(i) Lactate and pyruvate production

Three plerocercoids were incubated separately in ringer with unlabelled D-glucose 5mM for 1 hour. Samples of media, removed at specified intervals were analysed by means of Boehringer Biochemica tests for lactate and pyruvate (p.28)

(ii) Carbon-dioxide from glucose

Incubation containers were sterilised with boiling water and washed in absolute alcohol. Ringer medium was sterilised by boiling for 20 minutes. After cooling to 20°C D-glucose was added, 5mM with 0.25 µCi/ml. Plerocercoids, which had been removed from fish washed in 90% ethanol after pithing, were

incubated separately in sealed containers through which (CO_2- free) air was slowly bubbled, (Fig.3)
Carbon-dioxide produced was collected in 1N NaOH solution. Samples were neutralised with 1N HCl and counted for radioactivity.

Pathology

(i) Glycogen content of fish liver

Infected and uninfected fish of approximately similar size were killed and their livers removed. Livers were placed immediately into 100% ethanol for 4 hours, to inactivate enzymes. After washing for 10 minutes in diethyl ether to remove lipid, samples were weighed and analysed for glycogen content by the Anthrone method (p.26).

(ii) Glucose uptake by fish gut

Infected and uninfected fish were killed, the stomach with intestine removed intact, from the pharynx to the anus and all viscera attached to these removed. The stomach and intestine were then split lengthwise , washed free of food with ringer and incubated in media containing glucose 1mM and 0.08 µCi/ml. Standard procedures were used for incubation and sample preparation except that a constant flow of air was slowly bubbled through the medium.

Apparatus for CO_2 experiment

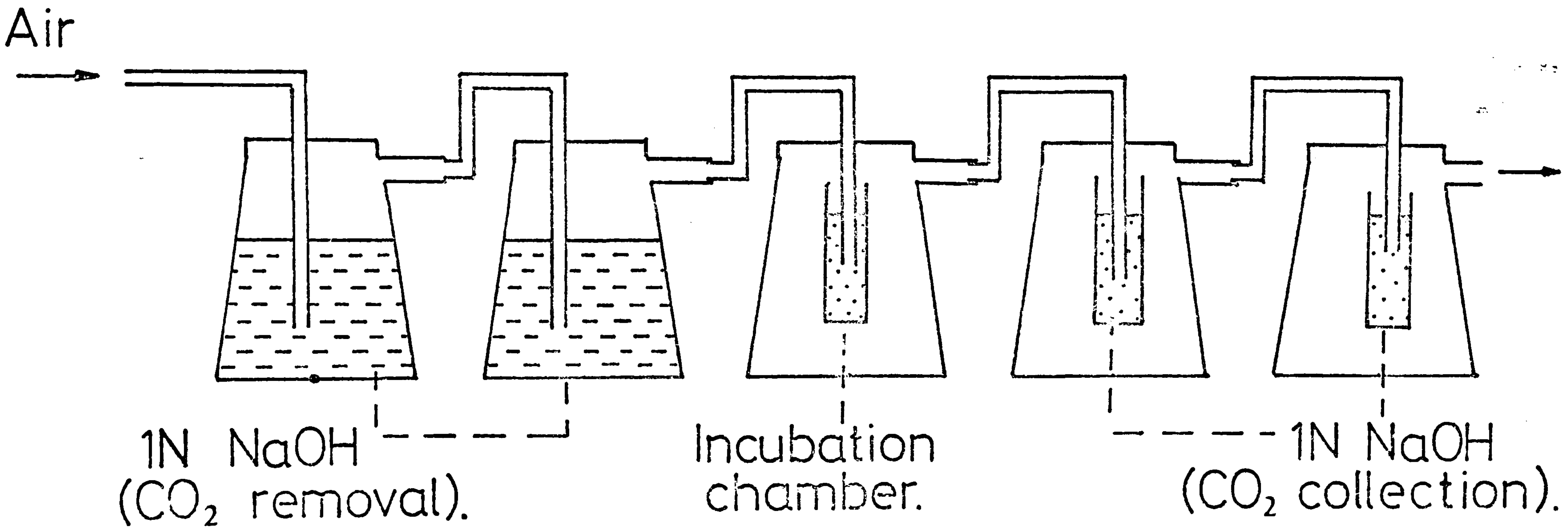


fig. 3

In-vivo Results

(1) Presentation

(2) Tissues showing significant differences between D-glucose and 3-O-M glucose from experiments with infected fish.

(3) Percentage uptake of 3-O-M glucose by stomach and parasite tissues. (Fig.3)

(4) Percentage uptake of D-glucose by stomach and parasite tissues. (Fig.4)

(5) ^{14}C-glycogen levels found in plerocercoids.

(1) Presentation

Results from all the in-vivo experiments are expressed as percentage of CPM/mg dry weight of tissue plotted against incubation time. This figure is obtained by comparing total counts from individual samples of fish tissue and parasites against total counts for each fish and its parasites. To simplify the investigation of gross distribution between host and parasite tissues, plerocercoids from individual fish were grouped and counted as parasite biomass irrespective of whether the infection was single or multiple.

No compensation was made in the results for loss of radioactivity by fish respiratory mechanisms although an attempt was made to measure loss of $^{14}CO_2$ through fish respiration. Results however were inconclusive and difficult to relate to plerocercoid metabolism. and therefore not included.

Results are also presented in such a manner as to indicate any significant differences in the distribution of labelled material between results for D-glucose and 3-O-M glucose.

(2) Tissues showing significant differences.

Comparison of D-glucose and 3-O-M glucose results for

infected fish showed only a few cases of significant differences for the percentage uptake of labelled material between D-glucose and 3-O-M glucose, most showing no significant difference.

Those tissues for which differences are significant at the 0.05 level:

Head (1.0, 1.5, and 18.0 hours).

Trunk (18.0 hours).

Stomach (24.0 hours).

Plerocercoid (18.0, 24.0 hours).

Only those results considered important are included in this section, additional <u>in-vivo</u> results being located in the addenda.

3-O-M glucose in-vivo (infected fish). Fig. 4

Stomach

These values are initially arratic with a maximum of 52.7% (2.5 hours). The level then falls dramatically to 28% (18.0 hours), rising slightly to 31.5% (24.0 hours).

Parasite

The concentration of labelled material found in plerocercoids after 10 minutes is very low; 0.08%. This increases only very slowly to reach a maximum of 2.8% (24.0 hours).

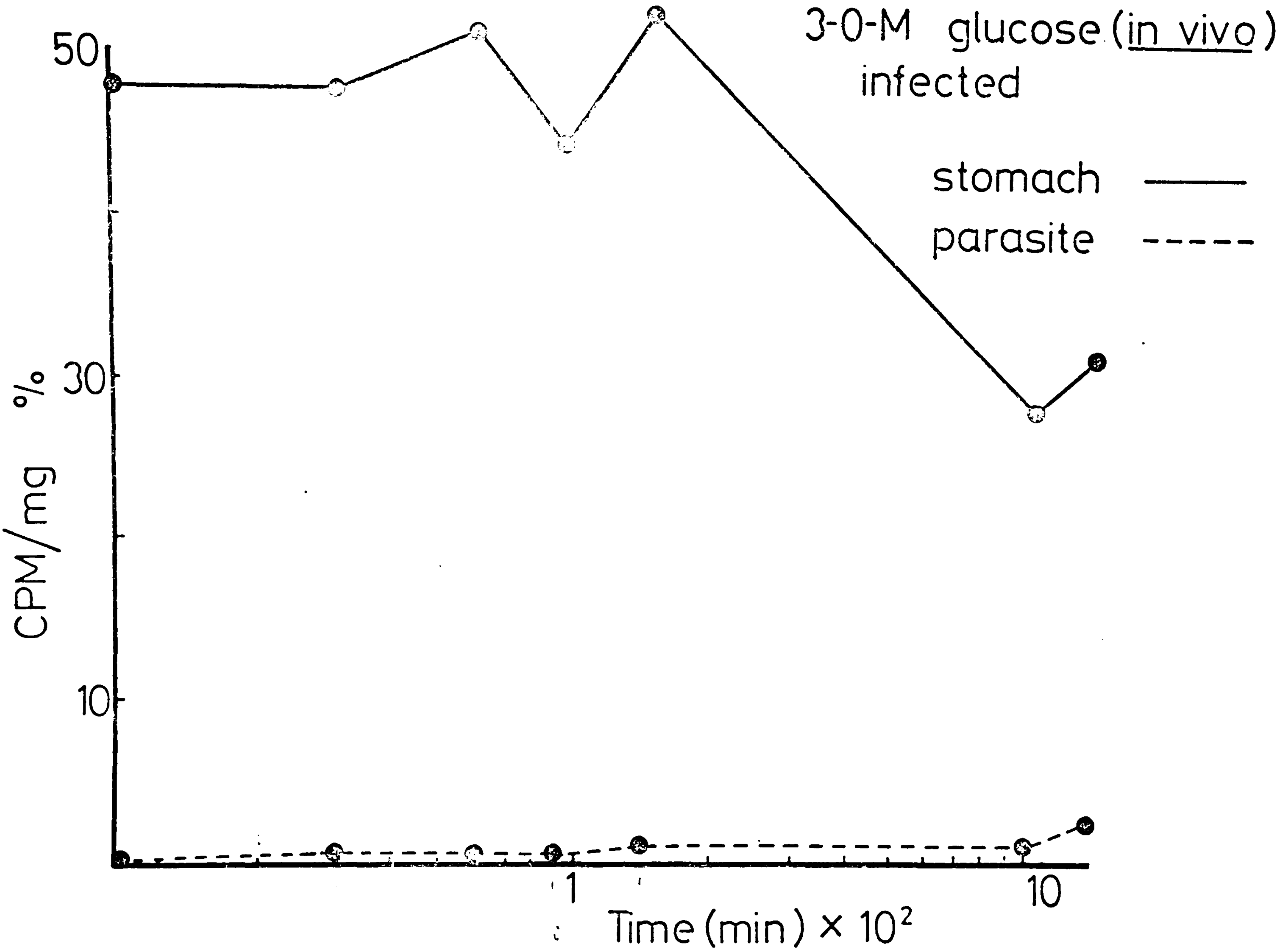


fig. 4

D-glucose <u>in-vivo</u> (infected fish) Fig. 5

<u>Stomach</u>

From an initial value of 67% (10 minutes) the levels for the stomach fall almost consistently to a final point of 13.0% (24 hours).

<u>Parasite</u>

Starting with a level of 1.9% (10 minutes) the figures remain low until a maximum is reached of 23.6% (4.5 hours). This is followed by a fall to 4.2% (10 hours) and a subsequent increase to 13% (24 hours).

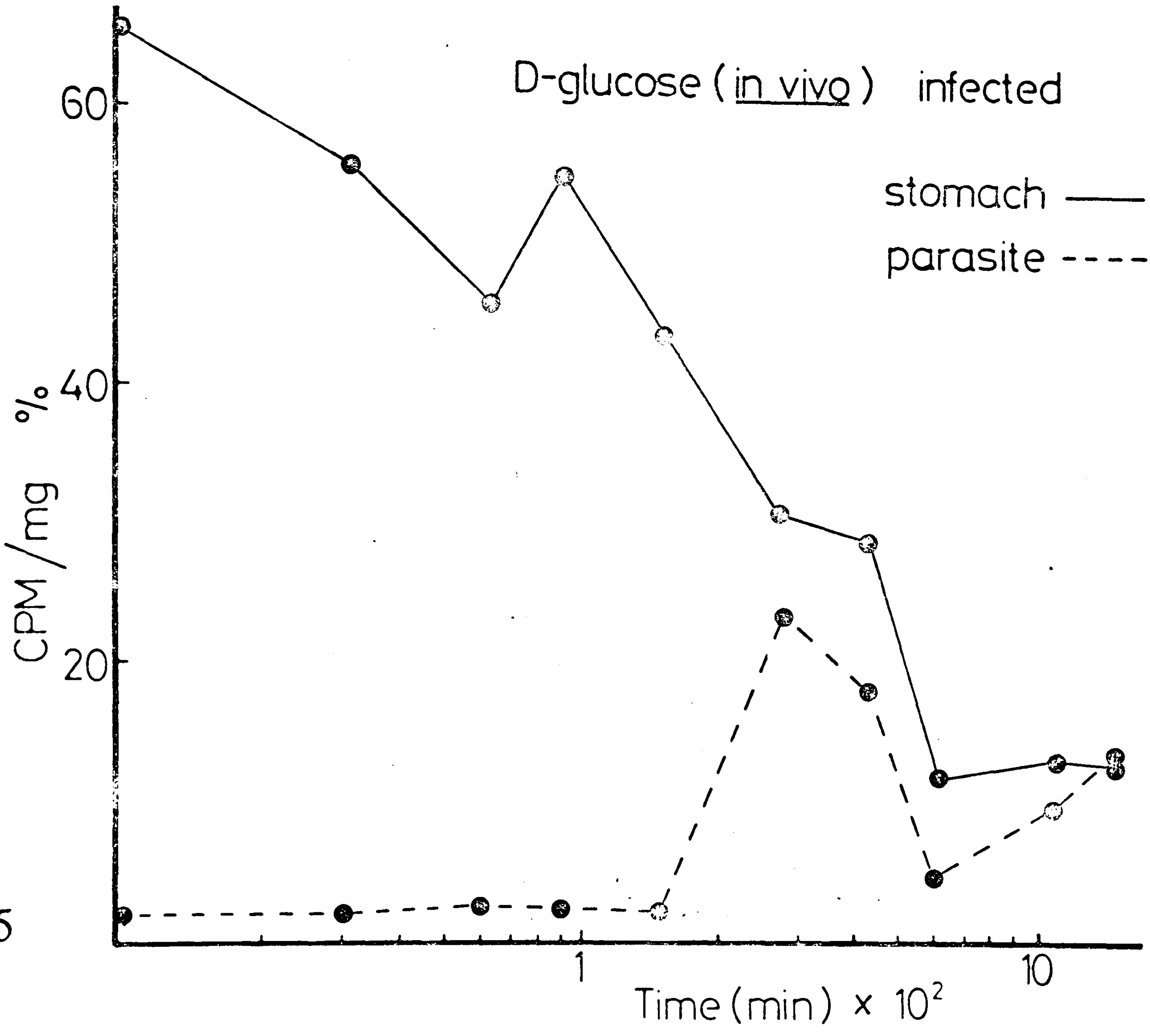


fig.5

TABLE 2

^{14}C-glycogen levels found in plerocercoids *in-vivo*

Incubation period	% of total ^{14}C in glycogen	No. of plerocercoids
0.5 hours	6.65	4
1.0 "	3.73	6
2.5 "	6.2	3
10.0 "	18.2	4
18.0	25.9	4

Figures are means of the percentage total ^{14}C present in glycogen extracts from plerocercoids *in-vivo*.

Glycogen extraction was by means of the method of Roe and Dailey (1966)

D-glucose uptake with temperature   Fig. 6

A sharp increase in uptake rate is apparent between 10°C and 20°C. This falls at 30°C before increasing to a maximum at 40°C.

Points are means for three plerocercoids $\pm$ s. error.

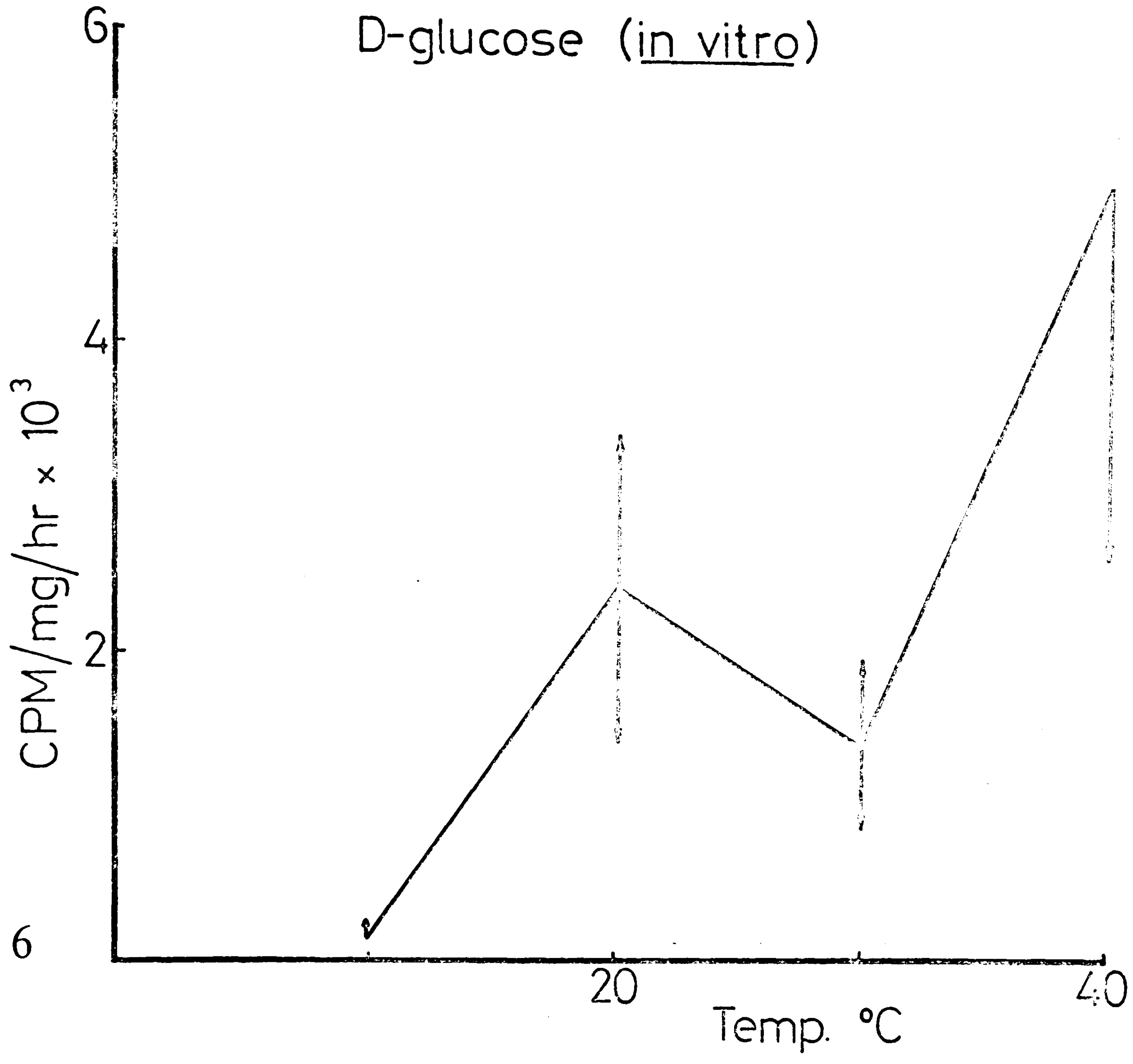


fig. 6

D-glucose uptake with time   Fig.7

Uptake rates increase progressively from 0.5-7.0 hours.

Points are means for 5-6 plerocercoids $\pm$ s. error.

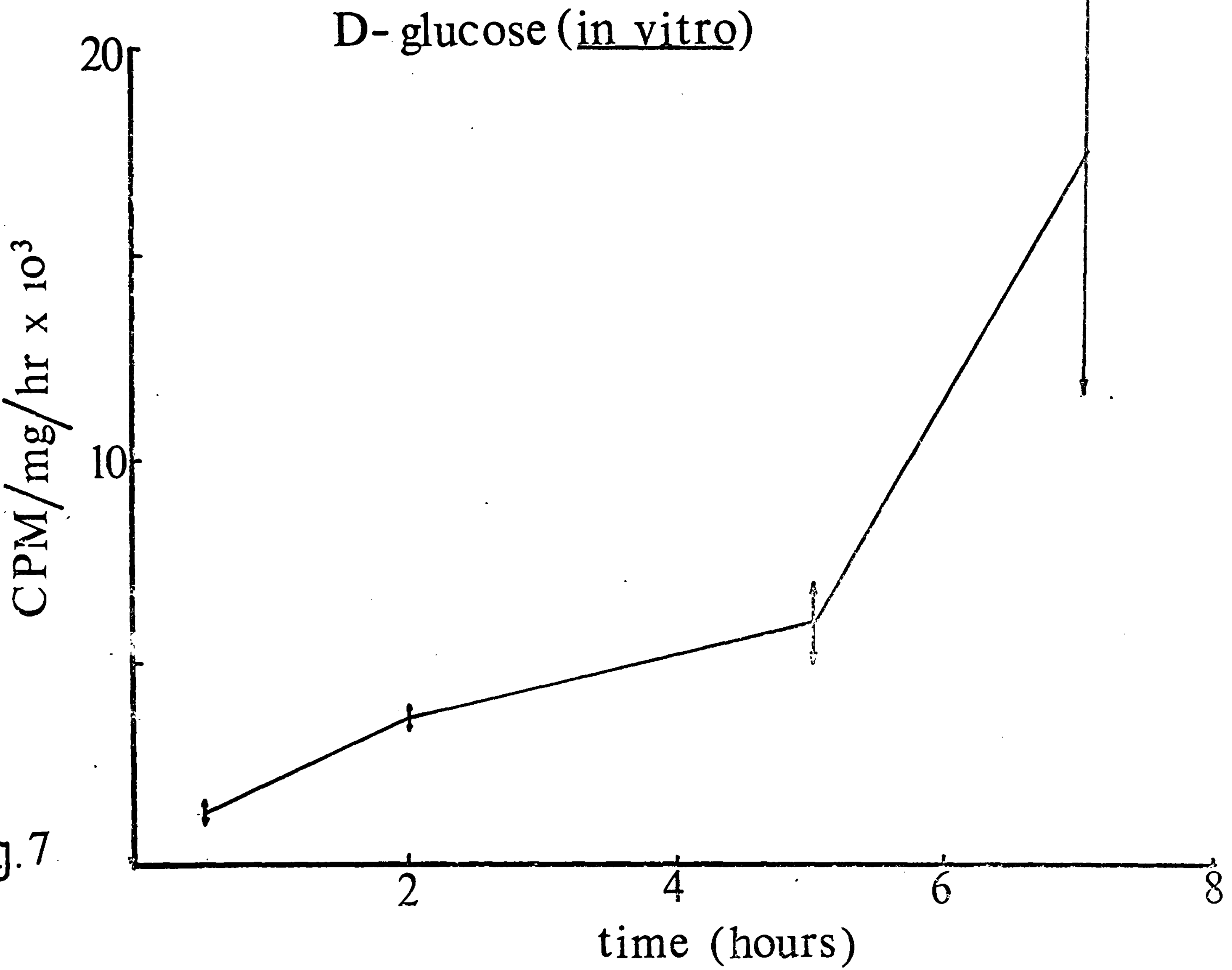


fig.7

D-glucose uptake with different substrate concentrations

A maximum level of 400 CPM is reached between substrate levels of 2-5 mM (Fig. 8).

Regression line from Lineweaver-Burk reciprocal plot (Fig. 9). $y = 2.29 + 0.013x$

V = glucose uptake rate

S = substrate concentration.

Kt for glucose = 0.66mM = ½ maximal attainable rate

u = reaction rate at any substrate concentration (S)

Michaelis - Menten equation

$$u = \frac{V.S}{Kt + S}$$

reciprocal plot (Lineweaver-Burk)

$$\frac{1}{u} = \frac{Kt}{V.S} + \frac{1}{V}$$

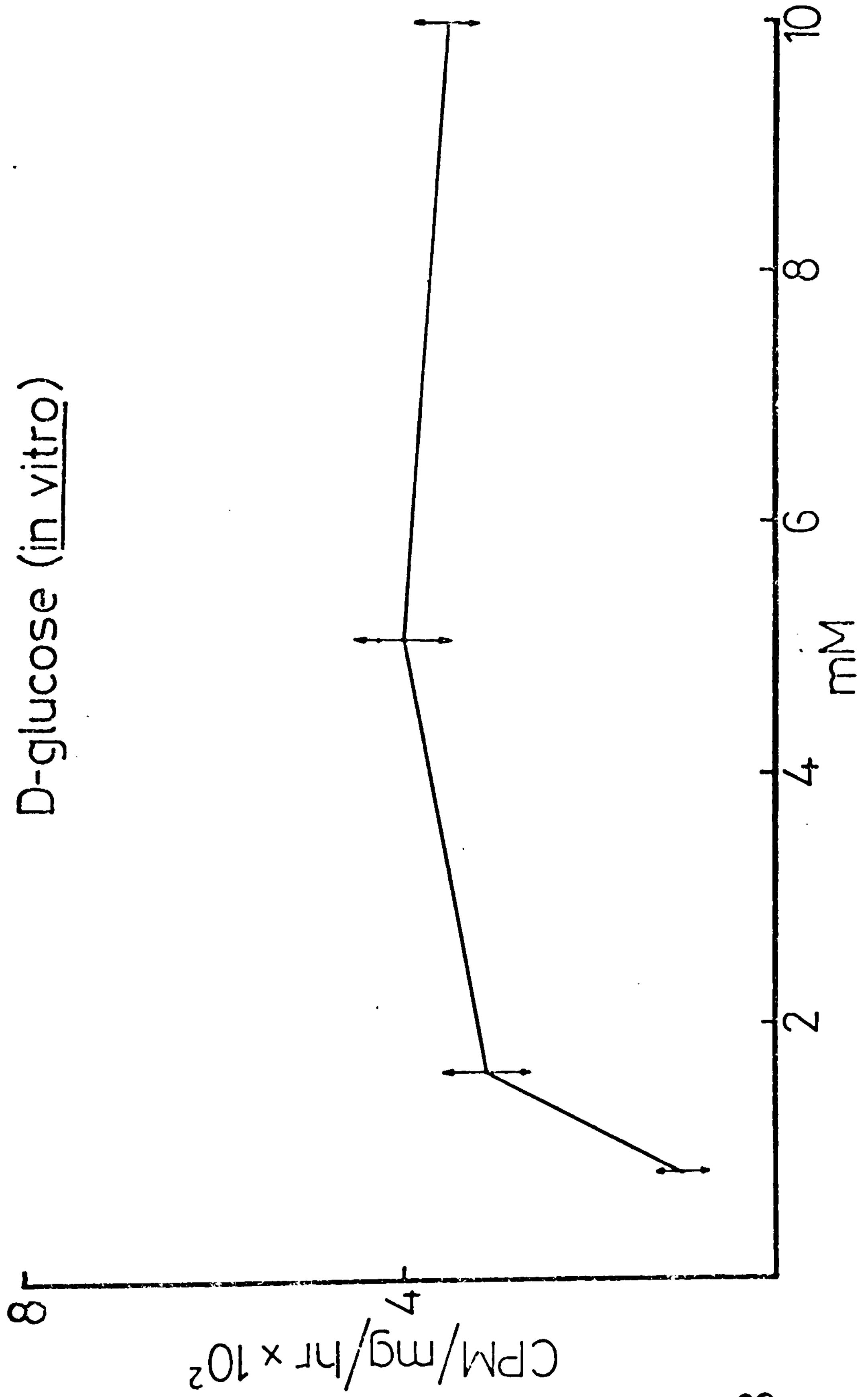


fig. 8

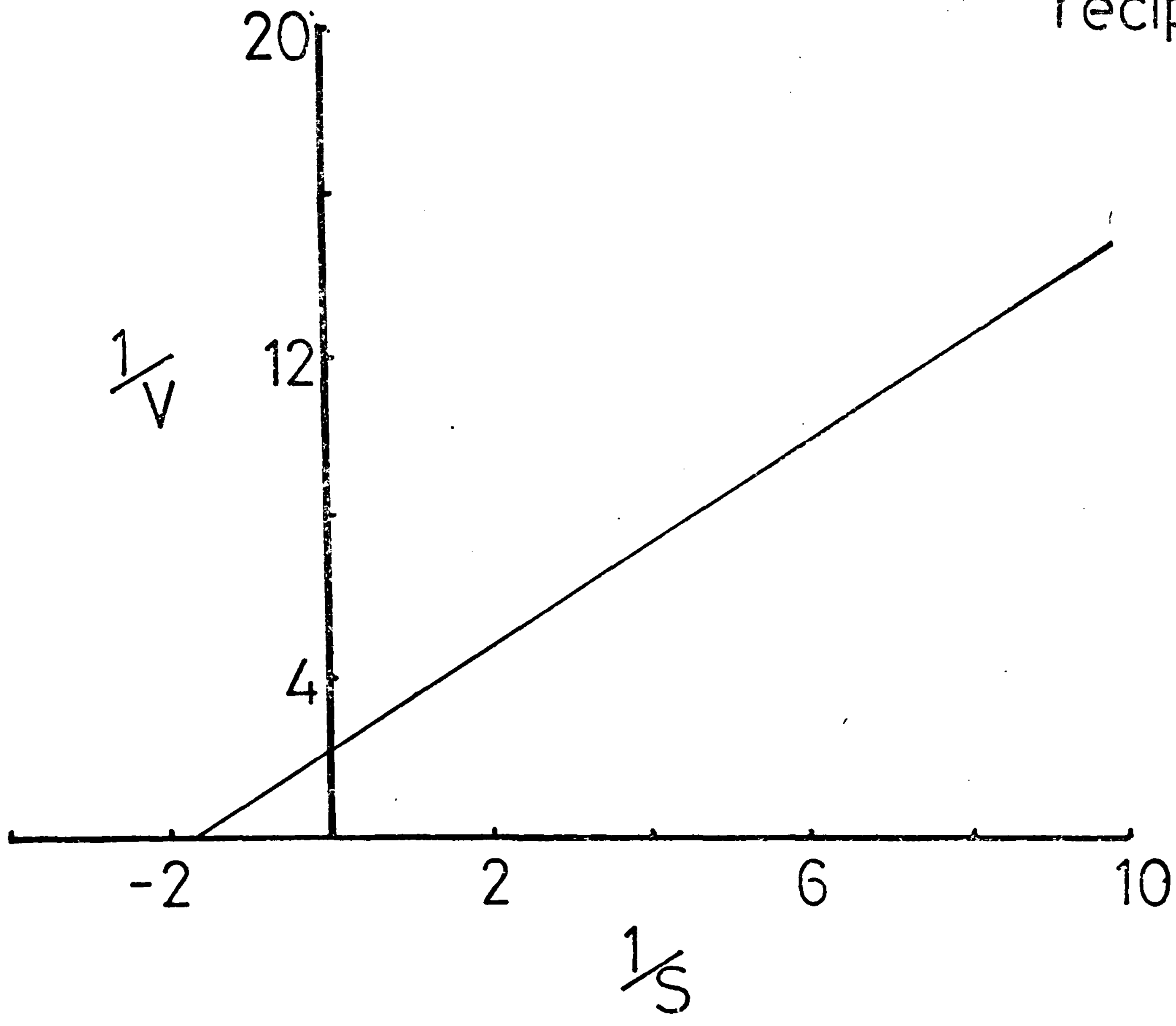


fig. 9 D-glucose(*in vitro*) Lineweaver- Burk reciprocal plot for fig. 8

TABLE 3

D-glucose uptake against a concentration difference

Incubation period	mean glucose concentration of incubation media
2 min.	100 mg/100ml
12 "	81 "
22 "	82 "
40 "	86 "
60 "	60 "

Mean 'free glucose' content of worms after 60 minute incubation in glucose medium was 226mg/100ml.

Mean 'free glucose' content of controls was 77mg/100ml.

All glucose values are means for three worms.

Uptake of D-glucose in relation to plerocercoid dry weight (Fig 10)

A regression line is drawn for uptake rate (y) on plerocercoid weight (x) plotted on a log/log scale.

$$y = 2.553 - 1.54x$$

A significant negative correlation coefficient of - 0.619 is shown.

Points represent individual plerocercoids.

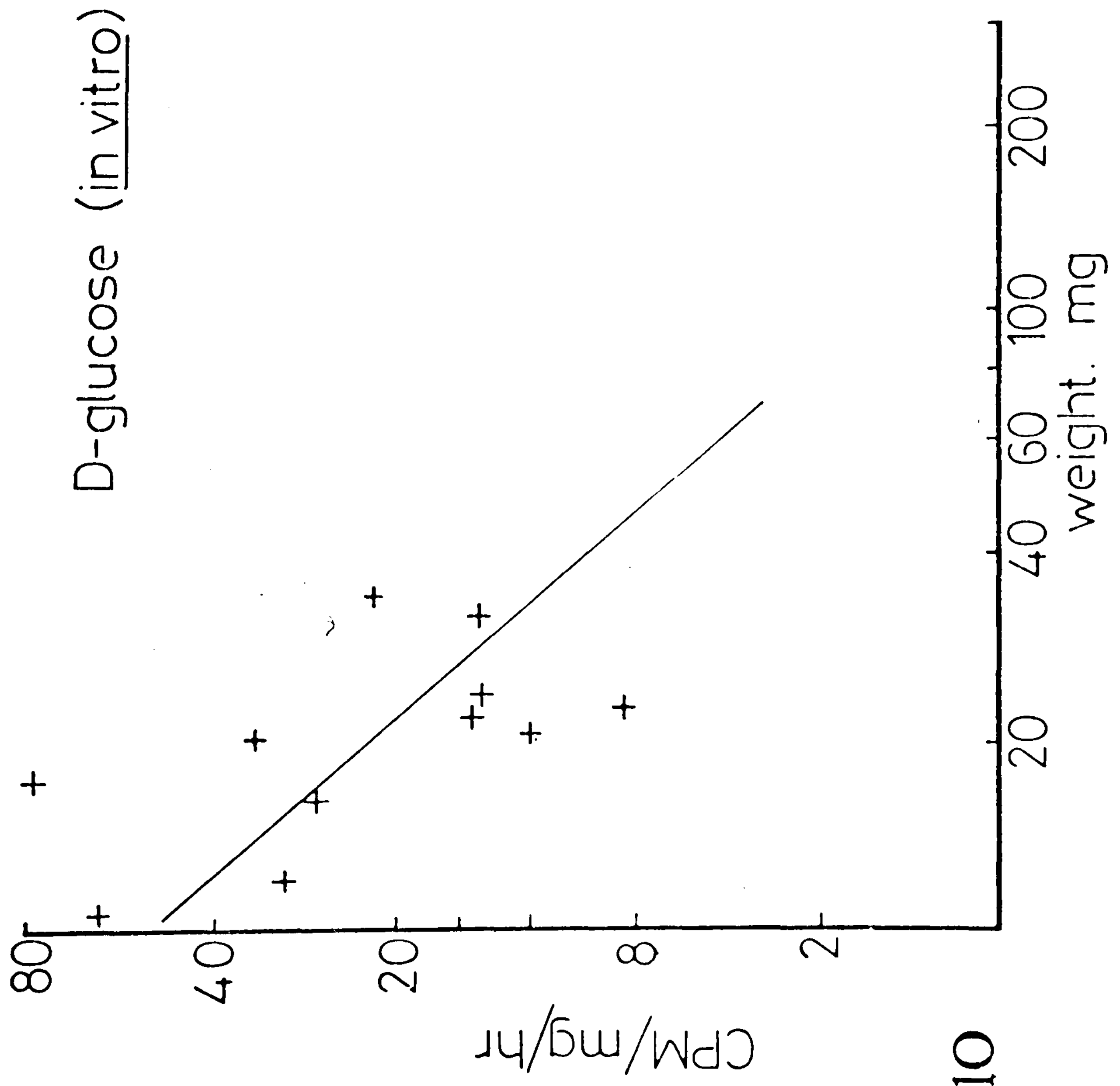


fig.10

Uptake of 3-O-M glucose in relation to plerocercoid dry weight

(Fig. 11)

No significant correlation could be derived for this data.

Points represent individual plerocercoids.

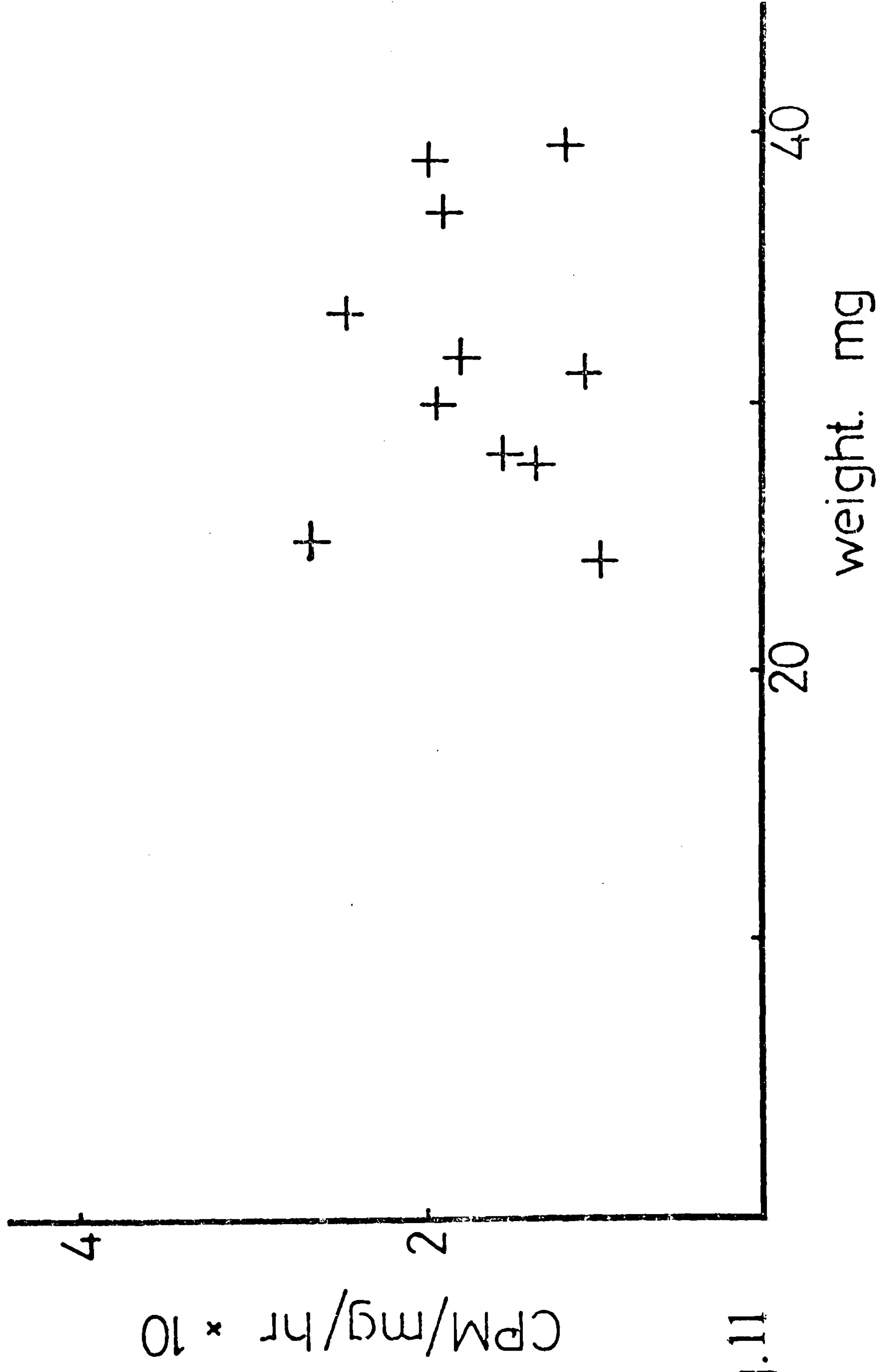


fig.11

TABLE 4

Uptake rates of D-glucose along the strobila

% of individual worm totals
in each section (CPM/mg/hour).

Worm No.	Section A	B	C	D
1	19	18	30	33
2	22	21	29	28
3	22	22	27	29
4	17	29	23	31
means	80	90	109	121

A = anterior of plerocercoid

B = mid-anterior " "

C = mid-posterior " "

D = posterior " "

Analysis of variance shows means to be significantly different at the 0.01 level.

Effect of 3-0-M glucose on D-glucose uptake (Fig. 12)

Results for the first three concentrations; 0.1, 0.5 and 2.5mM are significantly different at the 0.1, 0.05 and 0.1 probability levels respectively. Figures for the 5.0mM concentration are not significantly different.

Points are means ± s. errors for 3-4 plerocercoids.

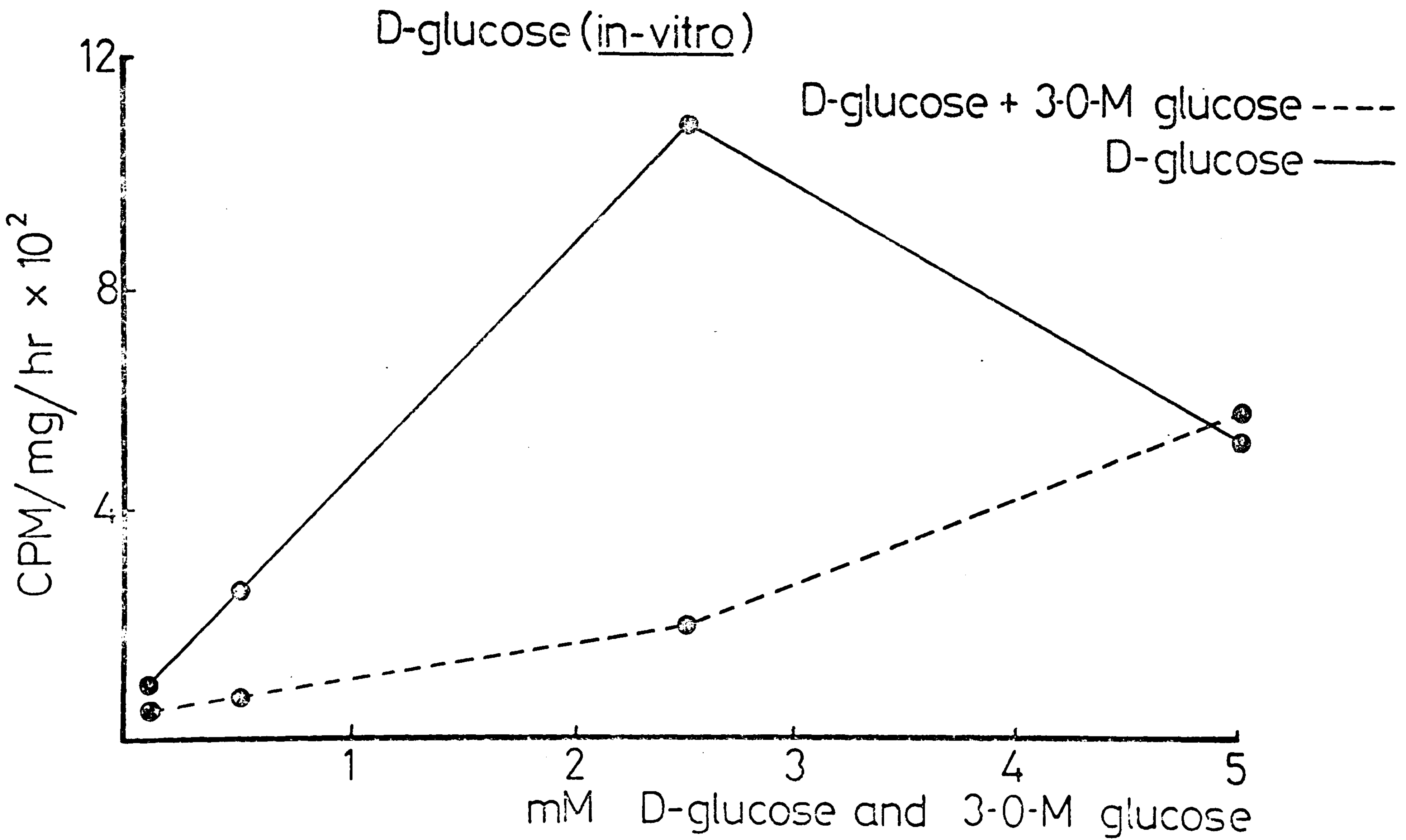


fig. 12

Effect of D-galactose on D-glucose uptake (Fig. 13)

Regression lines are drawn for uptake rate (y) on substrate concentration (x).

All experimental results are significantly different from controls at the 0.05 level.

Points are means ± s. error for 3-4 plerocercoids.

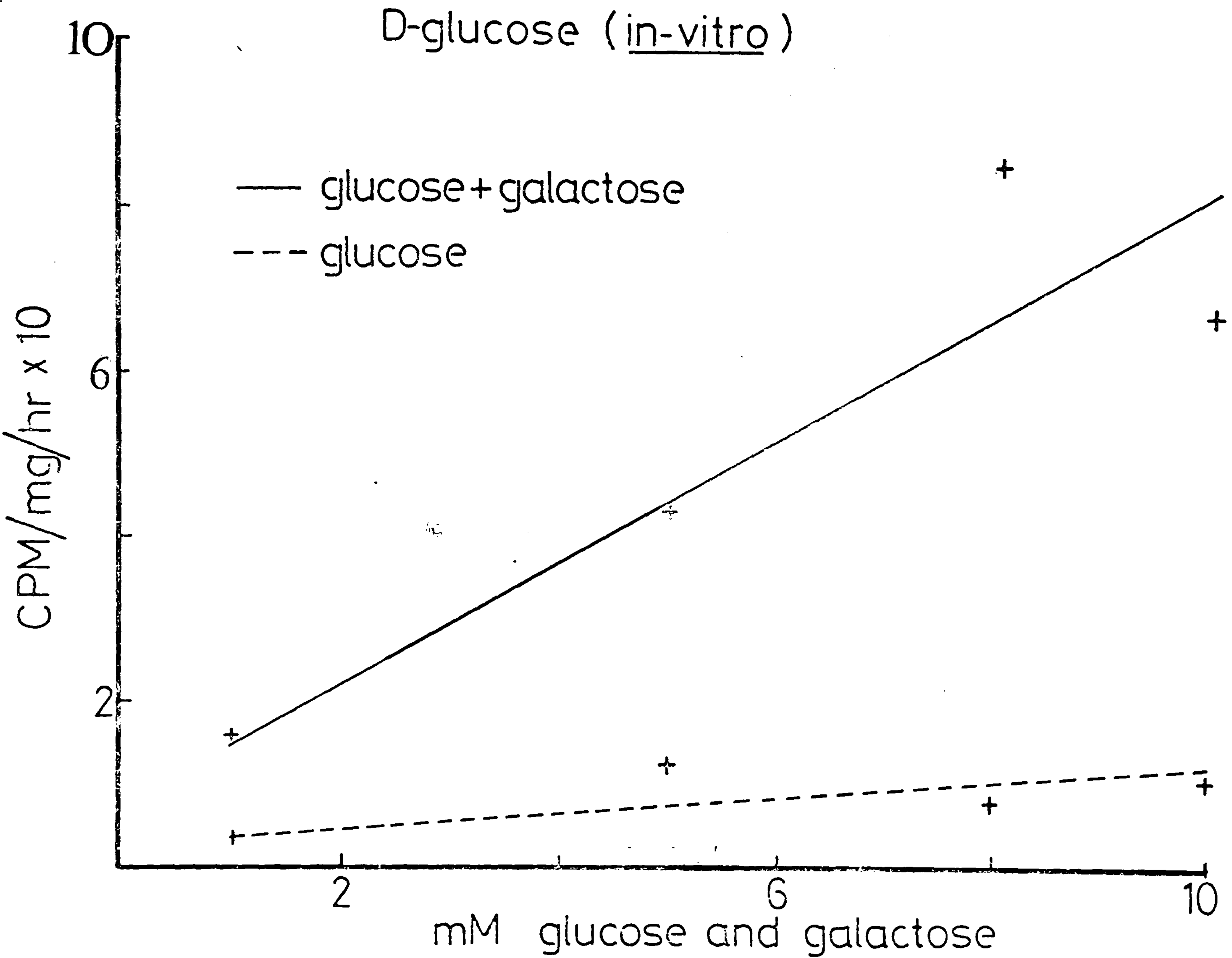


fig. 13

TABLE 5

D-glucose uptake with higher D-galactose concentrations

	CPM/mg/hour
(A)D-glucose 1.0mM with 0.25µCi/ml	1972 ± 680
(B)D-glucose 1.0mM with 0.25µCi/ml and D-galactose 10.0mM.	1387 ± 520
(C)D-glucose 1.0mM with 0.25µCi/ml and D-galactose 20.0mM.	1852 ± 456

(A) and (B) are significantly different at the 0.05 level but (A) and (C) are not.

Results are means ± s. errors for three plerocercoids.

Effect of ouabain (Fig. 14)

A general fall in glucose uptake is shown over the range of inhibitor concentration examined. There is no significant difference between (5mM and 10mM) x 10^{-1} ouabain.

Points are means ± s. error for 3-4 plerocercoids.

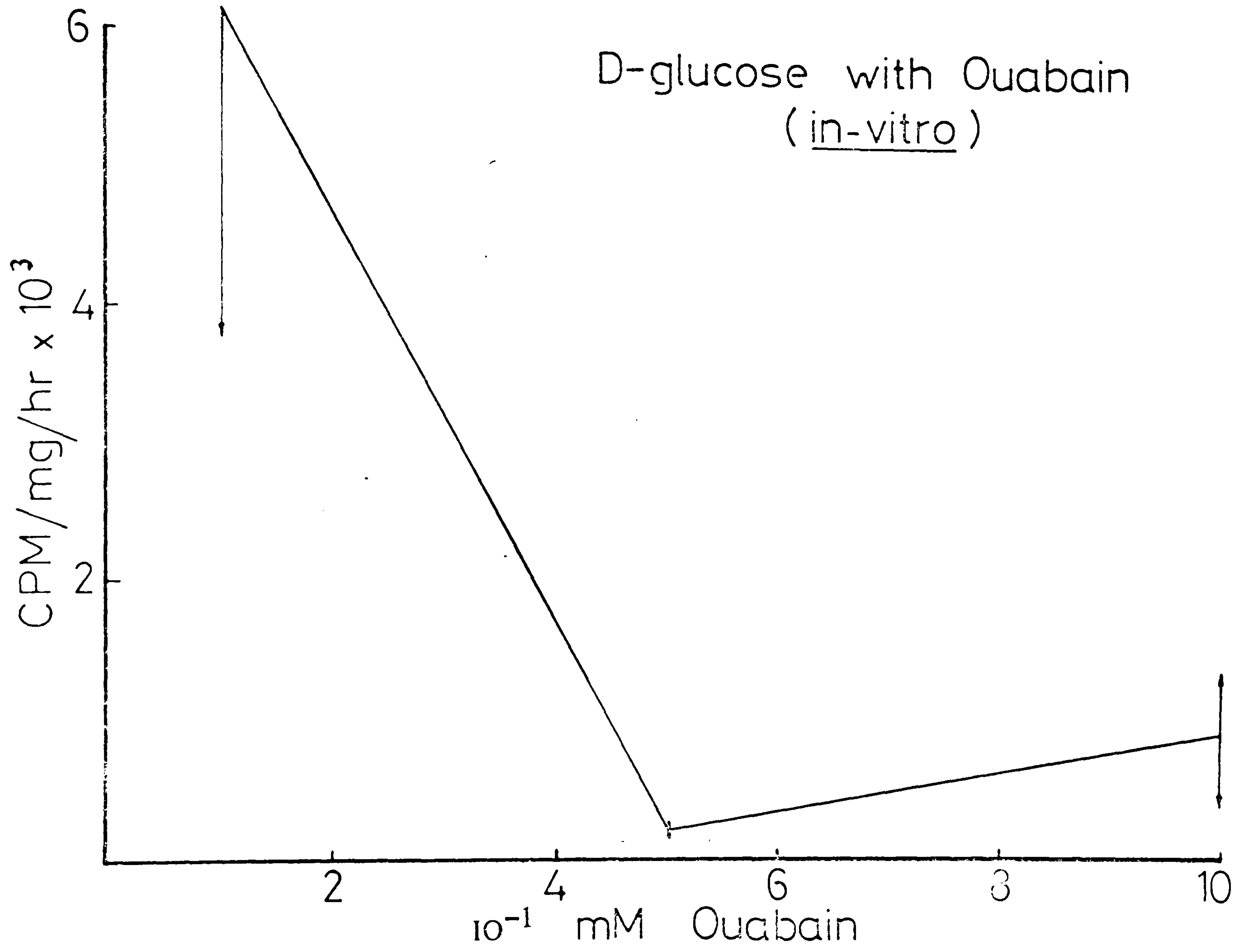


fig.14

<u>Effect of Dinitrophenol</u> (Fig. 15)

A large reduction in glucose uptake is apparent between 0.1mM and 0.5mM DNP, with the level rising slightly at 1.0mM.

Points are means $\pm$ s. error for 3-4 plerocercoids.

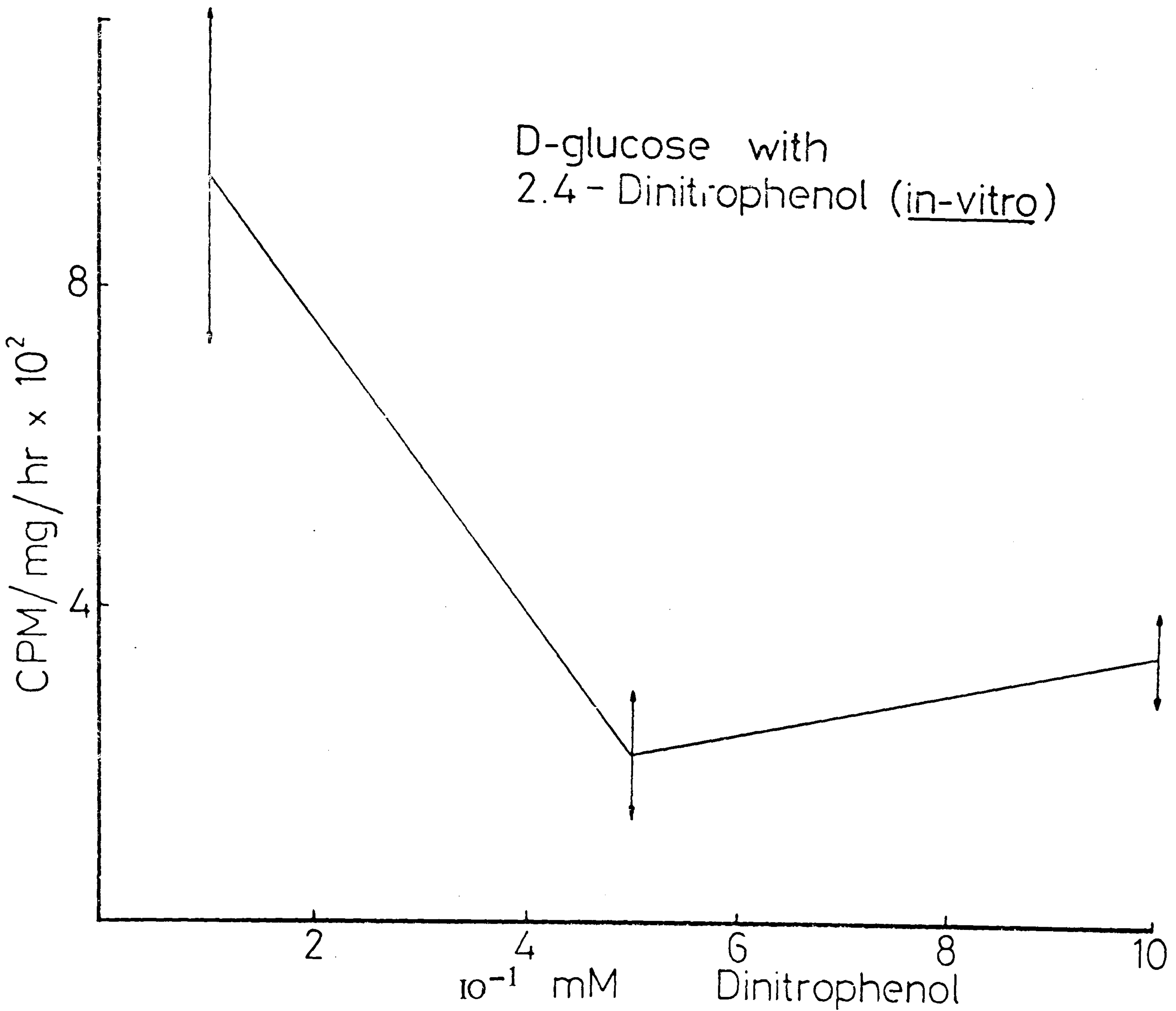


fig. 15

Effect of phlorizin (Fig. 16)

A continuous decline in glucose uptake is shown to occur with $(0.1 - 100.0) \times 10^{-3}$ mM phlorizin.

Points are means $\pm$ s. error for 3-4 plerocercoids.

fig.16

Effect of phlorizin with varying D-glucose concentrations

D-glucose uptake is significantly reduced with all concentrations of phlorizin (Fig. 17).

Regression lines are drawn from Lineweaver-Burk reciprocal plots (Fig. 18).

V = glucose uptake rate.

S = substrate concentration.

Kt for glucose (control) = 0.62 mM.

Kt for glucose with phlorizin = 0.07 mM.

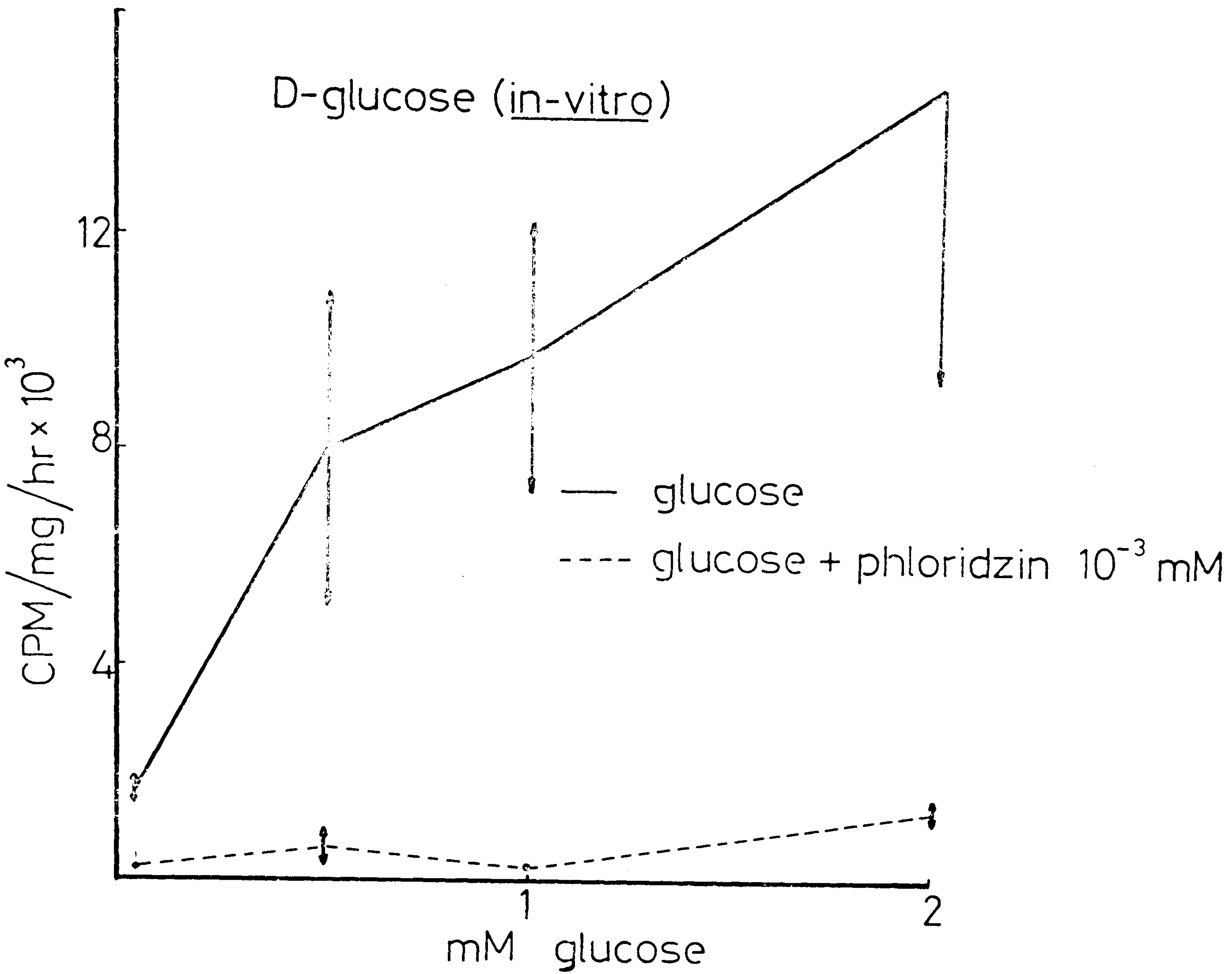
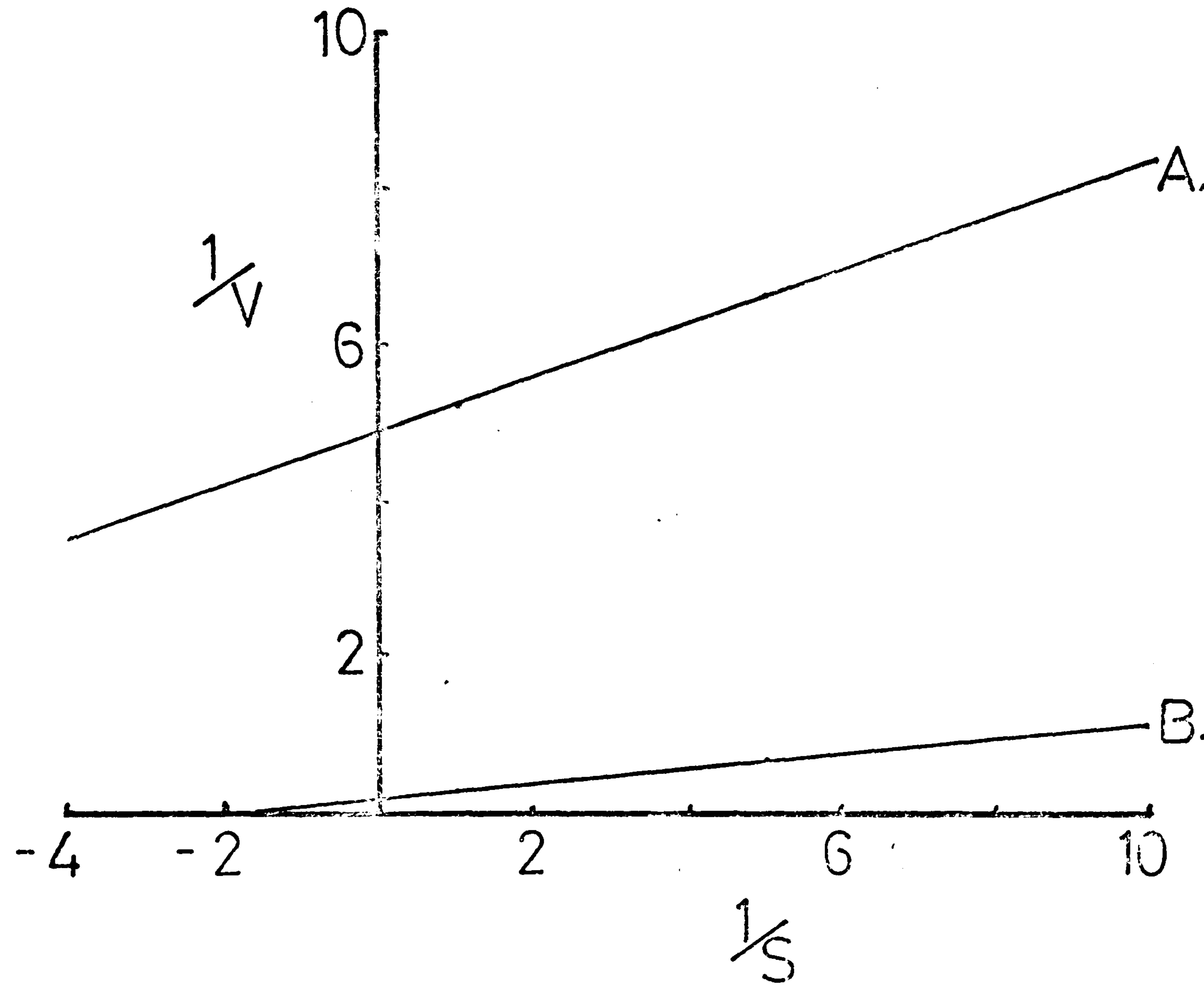


fig. 17

D-glucose (<u>in-vitro</u>) Lineweaver-Burk reciprocal plot



A- glucose + phlorizin

B- glucose

S- mM glucose

V- CPM/mg/hr × 10^3

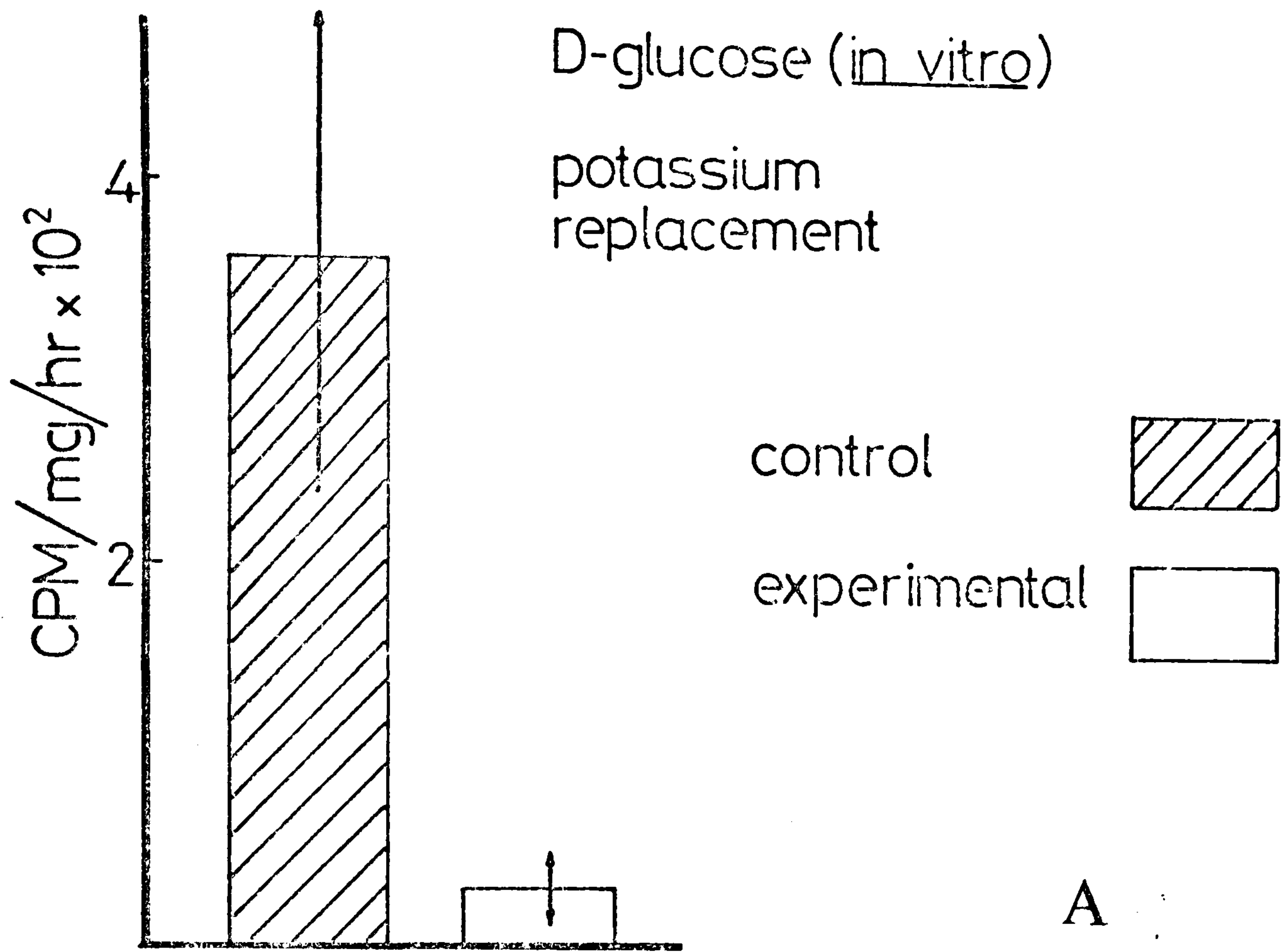
fig. 18

Effect of sodium on D-glucose uptake

The uptake of glucose in ringer (with sodium replaced by potassium). Experimental and control results are significantly different at the 0.001 level (Fig. 19A).

The uptake of glucose in ringer(with sodium replaced by lithium). Experimental and control results are significantly different at the 0.01 level (Fig. 19B).

Each histogram is the mean ± s. error for three worms.



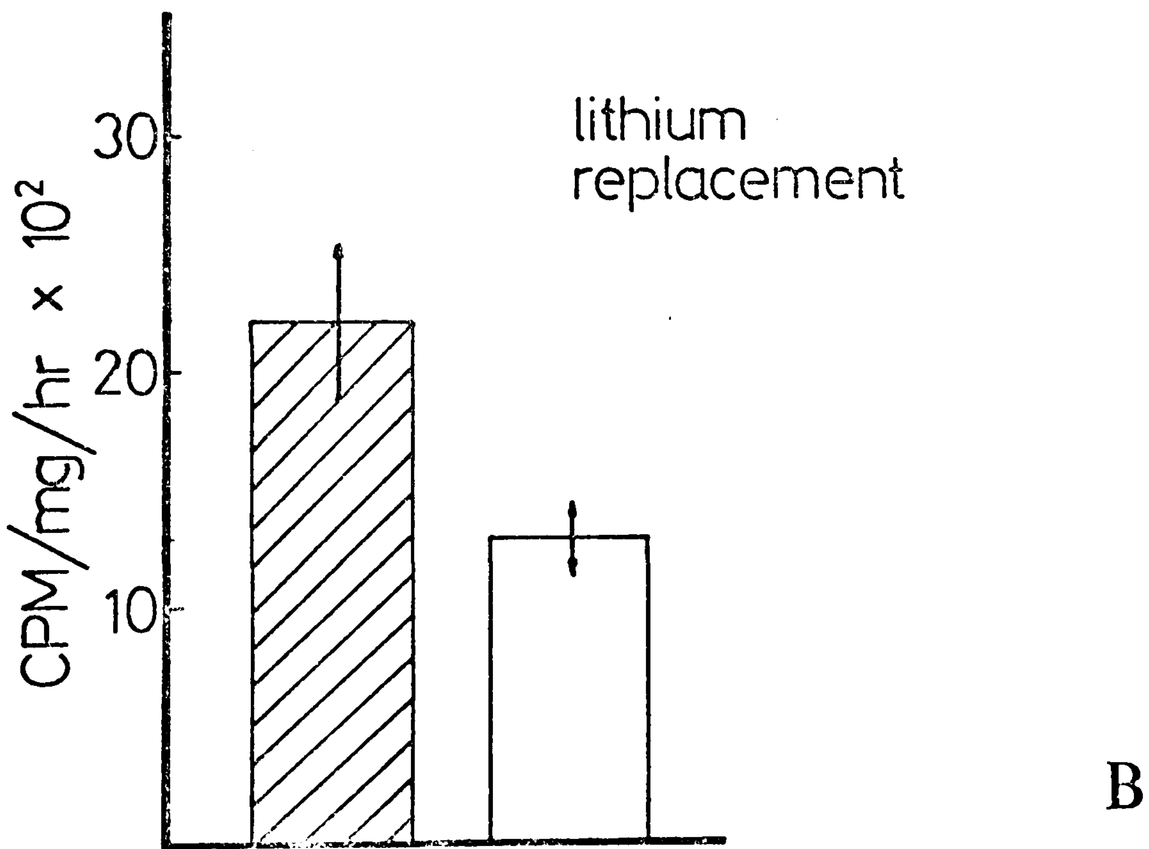


fig. 19

TABLE 6

Lactate and pyruvate production

Incubation period (minutes)	Lactate mg/100ml	Pyruvate mg/100ml
2	11.7	0
12	11.0	0
22	12.3	0
40	14.1	0
60	13.4	0

Figures are means ± s. errors for three plerocercoids.

$^{14}CO_2$ production from ^{14}C-D-glucose

The production of $^{14}CO_2$ was assessed. Unfortunately the means of three determinations (49 CPM) was not significantly higher than that of controls.

TABLE 7

Glycogen content of fish liver

	mg glycogen/100g dry weight liver	
	Infected fish	Uninfected fish
	7160	5808
	1179	2810
	3237	11884
	1650	41255
	950	8908
	1796	24065
	5461	4510
	6002	33214
	1967	8928
mean	3290	15600

Means are significantly different at the 0.02 level.

TABLE 8

D-glucose uptake by fish gut

CPM/mg/dry weight

	Infected fish	Uninfected fish
	754	204
	58	322
	299	676
	262	484
	372	314
	245	606
	142	869
	325	752
	112	561
means	285 ± 64	532 ± 69

Means are significantly different at the 0.02 level.

Discussion

Planning of Experiments

The main problem concerning planning of all experiments proved to be the unpredictable variation in condition and supply of material. Although initially a single site provided adequate numbers of infected fish for experimental purposes, this source eventually became exhausted and samples therefore had to be collected from a variety of other sources. This did not create difficulties in terms of plerocercoid supply for *in-vitro* experiments but it did however present problems with *in-vivo* experiments. Since infected fish from other locations exhibited a much higher degree of infection with other cestode species e.g. *Diphyllobothrium* and *Triaenophorus*. This could have produced artifacts in the assessment of *Schistocephalus* nutrient uptake *in-vivo*, therefore all the fish used for *in-vivo* experiments were taken from the same source; Fairmead.

Stress during handling in experiments and in some cases lack of oxygen during transportation, especially during the warmer months caused a number of fish deaths. The effects of anaesthetic upon glucose absorption was not regarded as serious, since Di Bendetto and Farmanfarmaian, (1975) found no interference by MS222 on intestinal glucose absorption in trout (*Salmo gairdneri*) using approximately similar external concentrations.

The incidence of infected fish found was rather low compared to the reports of other workers. Arme and Owen (1967) found infection rates as high as 100% and Pennycuick (1971a) recorded figures of just under 100%. In this investigation rates never rose above a level of 23% and in terms of multiple infections , never greater than sixteen plerocercoids in a single host.

Collection of samples by means of the beam trawl was most effective during the winter months but proved disadvantageous when large numbers of fish were netted and had to be separated. Hand nets were more effective for obtaining infected fish from the surface of ponds and much more efficient during the summer months when heavily infected fish were often found in the shallower regions around the perimeter.

Results

The standard errors for many in-vivo experimental results were quite large indicating the great variability between individual fish. Consequently analysis of these showed few instances where differences in distribution pattern in tissues between D-glucose and 3-O-M glucose were significant. No conclusions can be clearly drawn from this distribution for fish tissues except the gradual disappearance of glucose from the stomach region and in some cases an apparent accumulation of labelled material, for example in liver tissue.

Essentially the results show that a proportion of ^{14}C from glucose molecules, taken up by the fish host, eventually find their way into plerocercoid tissue. Over a 24 hour period the proportion of ^{14}C from labelled D-glucose found in parasite glycogen gradually increases, which means that carbohydrate of host origin can be used for glycogenesis in the plerocercoid.

As low levels of D-glucose derived ^{14}C are found in parasite tissue from 10 minute up to 2.5 hour incubation periods, this suggests that an initial rapid absorption of small quantities of glucose through the intestine may be occurring. The highest

concentration of ^{14}C in parasite tissue however is shown at 4.5 hours (Fig.5) which means that the bulk of material has taken at least 2.5 hours to be assimilated and distributed throughout the fish body, further measurements being necessary to determine this accurately. Radiochromatograms of plerocercoid homogenates failed to identify the nature of the labelled material due to very low activity levels.

Experiments using the non-metabolisable sugar 3-O-M glucose show no corresponding increase for parasite tissue as with the D-glucose experiments. A number of points can be inferred from this; these are, that 3-O-M glucose is not subject to the same absorption and distribution patterns in fish gut as D-glucose, or labelled by-products of D-glucose metabolism have been absorbed by the parasite or simply that D-glucose is absorbed more readily than 3-O-M glucose. As fish are capable of oxidising D-glucose to other compounds, it is likely that the higher levels of ^{14}C from D-glucose over the levels of ^{14}C from 3-O-M glucose are in part due to this.

The occurrence of two peaks of activity on the D-glucose graph (Fig.5) could be explained by a number of factors.

(1) The production of at least two by-products containing ^{14}C molecules with different absorption rates by the parasite or different formation rates by the host tissue. Lactic acid is produced by plerocercoids <u>in-vitro</u> (Table.6) and although experimental evidence is not conclusive, succinic acid is probably also produced (Fig. 2). It is also likely that a number of other compounds are formed considering the variety produced by other tapeworm species (Agosin, 1957; von Brand and Bowman, 1961).

(2) A greater excretion rate of labelled material by plerocercoids between 4.5 and 24 hours.

(3) Delay in absorption from the intestine of glucose or absorption from depots in host tissue, for example liver or gonads. In-vitro experiments indicate that glucose absorption by the intestine is impaired in infected fish (Table. 8).Arme and Owen (1967) have noted a reduction in liver weight for infected fish and gonad development is also known to be retarded (Kerr, 1948; Arme and Owen, 1967; Pennycuick, 1971d).

The answer is most probably a combination of these factors though in what proportion is uncertain.

The concentration of 3-O-M glucose absorbed by parasites in-vivo is very low at all periods, with the highest value being approximately 2% at 24 hours.

Overturf (1966) using rats infected with H. diminuta calculated that the in-vivo uptake of glucose by the tapeworm removed only a very small amount of the glucose available in the host gut lumen. Hymenolepis in that case was competing with the intestinal absorption mechanism of the host, whereas in this investigation Schistocephalus is in competition with the serosal surface of the gut and other viscera of its host.

The in-vitro experiments with glucose uptake over a range of concentrations (Fig.8) demonstrate the existence of a saturation level for uptake. Lineweaver-Burk reciprocal plots (Fig.9) show the affinity of the glucose binding site to have a Kt of 0.66 mM. This is close to values obtained for glucose uptake by C. verticillatum, that is 0.61 mM, (Pappas and Read, 1972). In general these figures suggest a greater affinity for this substrate exists in S. solidus plerocercoids and C. verticillatum than in those tapeworms found in warm-blooded hosts e.g. H.diminuta (Read, 1961; Read et al 1974), H. microstoma (Pappas and Freeman, 1975), in which Kt values are

higher. T.crassiceps however have an even lower Kt value than any of those mentioned (Pappas et al,1973). It is interesting to note that both T. crassiceps larvae and S. solidus plerocercoids are found in the body cavity in their respective hosts. Whether the relatively low Kt values are indirectly related to this fact is not certain although the glucose levels in these sites are unlikely to reach those found in the gut lumen.

Another point related to this comes from the findings of extended incubation experiments in solutions containing D-glucose, (Fig. 7). These clearly show that an accumulation of labelled material takes place. There is no indication of a saturation point from these results, probably due to the low glucose concentration used (0.8 mM). Hopkins (1952) concluded that a threshold value existed for glucose absorption by Schistocephalus plerocercoids, this being approximately 0.1%, below which no absorption occurred. The results from this present study appear to contradict his findings. These experiments however were performed using temperatures likely to be found in the fish host (20°C) whereas those carried out by Hopkins involved incubating plerocercoids at temperatures of 40°C. It is possible that the difference is due to the action of two separate enzyme systems as postulated by Sinha and Hopkins (1967) in which the threshold value for glucose absorption is much lower during growth within the fish body cavity than are the glucose levels required for maturation in the gut of the final host. This would generally be in agreement with the points raised concerning Kt values and the glucose levels found within the body cavity. Confirmation however rests on determining these figures and the Kt values for glucose uptake during plerocercoid maturation.

Previous *in-vitro* studies with *S. solidus* have involved mainly investigations of plerocercoid growth rates (Braten, 1966; Orr and Hopkins, 1969; Meakins and Walkey, 1973) and glycogen metabolism of plerocercoids (Hopkins, 1950).

Meakins and Walkey (1973) have suggested that a 'growth capacity' exists in the body cavity of the stickleback, based on the finding that a reduction in parasite growth rate is shown with increasing size and increasing numbers. While the decrease with size can be accounted for partly by the lowering of growth rates, the lowering of growth rates for plerocercoids in multiple infections points either to a growth inhibition mechanism produced by the parasites themselves or to a limitation caused by a finite supply of nutrients in the host body cavity. If plerocercoids are capable intrinsic of 'contact digestion', and most of the evidence points against this for tapeworms (Read, 1973) then the supply of food is not likely to be a limiting factor. If however parasites depend to a greater degreeon nutrients dissolved and suspended in the body fluid of the coelom, this is more likely to be a limiting factor, similar to the carbohydrate dependent growth pattern of *Hymenolepis* species (Read, 1959; Read and Phifer, 1959) manifested in the 'crowding effect'(Read, 1951). Although carbohydrate is known to be the limiting factor for growth in *Hymenolepis* species it would require more detailed investigation to determine whether this is the case in *Schistocephalus* or whether other reasons such as the accumulation and disposal problems of waste products from parasite metabolism were the cause.

The highest percentage of labelled material found in plerocercoids from *in-vivo* experiments, 23%, is close to the figure of 25% calculated by Markov (1961) for absorption of blood glucose

by Ligula intestinalis in fish weighing 250g. With smaller fish he concluded that this figure would be larger causing greater host mortality. These results were based on in-vitro experiments and probably represent a maximum estimate since McCaig and Hopkins (1965) found that glycogen levels in small Schistocephalus plerocercoids was increased under in-vitro conditions over those developed in-vivo. This was explained by the fact that either far less glucose is available to the plerocercoid in-vivo or that the regulatory mechanism is being overwhelmed in-vitro.

Pennycuick (1971d) calculated that sticklebacks infected with Schistocephalus were on average 25% lower in weight than uninfected fish. Correlation of this weight loss to depletion of host carbohydrate reserves would be an oversimplification of the situation without consideration for the role of other nutrients i.e. lipids and proteins or the influence of other parasites in this question. Studies by Pritchard and Porteous (1977) however, using rat intestine, have demonstrated that 25% of glucose absorbed by the intestine is transported through the intact tissue into the external serosal solution. Another 25% is accumulated temporarily in the mucosal tissue and the remaining 50% metabolised. Although these findings cannot be compared directly to the conditions of absorption and metabolism in fish it does provide some evidence that a significant proportion of glucose can be transported through the intestine into the body cavity.

Although the figure of 23% glucose removed by plerocercoids in this study seems to be a high proportion of the host food, Nagai and Ikeda (1972) have shown that the oxidation of amino-acids provides the primary source of energy in carp. Also most of the energy needs of salmonids is derived from lipids or from glucose

recently derived from lipids, (Fontaine and Hatey quoted by Love, 1974). If this were also the case for G. aculeatus metabolism then such a high level of consumption of carbohydrate-derived material by plerocercoids would not be as serious an energy loss to the host as it appears to be.

D-glucose uptake by plerocercoids at temperatures ranging from 10 - 40°C does not show a direct relationship, instead two peaks of uptake rate are evident; one at 20°C and another (over twice that of the first) at 40°C.(Fig.6).

The existence of two enzyme systems postulated by Sinha and Hopkins (1967) is based on plerocercoid growth patterns at different temperatures. One system is thought to control growth rates (this being near the maximum temperature which plerocercoids would encounter in the fish, where most growth occurs). The other enzyme system would probably control maturation and possess a peak activity around 40°C, (this being approximately the temperature that plerocercoids would meet in the final host).

Hopkins (1952) has already demonstrated that plerocercoids maintained in culture media at 40°C and allowed to mature, absorb glucose glucose from the media. The evidence being that glucose gradually disappeared from incubation media and glycogen reserves in the plerocercoid were conserved. He concluded that as the conservation of these reserves in glucose media closely resembled those from worms cultured in-vivo then worms were capable of absorbing nutrient from the final host during maturation.

The absorption of glucose in relation to temperature (Fig.6) would seem to add support to the'two enzyme hypothesis' as it is likely that glucose metabolism is closely related to the enzymes controlling both growth and maturation.

The demonstration of increased oxygen uptake rates with increased temperature (Davies and Walkey, 1966) contrasts with the results for glucose uptake and temperature in the present work. However Q_{10} values for plerocercoids (Davies and Walkey, 1966) do show some correlation with glucose uptake and appear to support the 'two enzyme' theory of Sinha and Hopkins,(1967); increases in these values being recorded from the 20-30 to 30-40°C range. Davies and Walkey also point out that the level of oxidative metabolism for plerocercoids is significantly lower than that of free-living poikilotherms, so the role of oxygen is not entirely clear and is difficult to relate to glucose metabolism at present.

Experiments to determine whether plerocercoids are capable of oxidising glucose completely to CO_2 proved negative; insignificant amounts of $^{14}CO_2$ being produced when plerocercoids were incubated aerobically in the presence of labelled D-glucose. This would seem to indicate that a fully functional TCA cycle or pentose phosphate pathway is not operative in Schistocephalus despite reports by Korting and Barrett (1976) that a complete sequence of TCA cycle enzymes is present in plerocercoids.

Significant quantities of lactate were produced by plerocercoids (Table. 6) but no pyruvate could be detected. It is not clear whether lactate is the predominant by-product of glucose metabolism as the production and proportion of other possible metabolites was not measured.

Chromatograms of extracts from plerocercoids incubated in ^{14}C-D-glucose solutions presented evidence that most of the labelled material is not in fact found as lactate within a 30 minute incubation period. Most of the radioactivity was located at the Rf

points for succinate and glucose. As no quantitative measurements were made of succinate levels it cannot be stated for certain whether this actually forms the major end product of glucose metabolism. These findings infer however that although the TCA cycle does not appear to be fully functional the glycolytic sequence of enzymes is. This is generally the situation found in most tapeworms and although all produce CO_2 as a metabolic by-product, its origin is not clear.

Nearly all tapeworms, in common with Schistocephalus are capable of producing organic acids, the nature and quantity of these varying according to substrate metabolised, environmental conditions and stage of development (Read, 1956; Agosin, 1957; Laurie, 1957; von Brand and Bowman, 1961).

In relation to the apparent production of succinate by Schistocephalus it is known that formation of this product is increased when H. diminuta is incubated in the presence of CO_2 (Fairbairn et al, 1961), this being a result of CO_2 fixation. A similar mechanism is also known to operate in the scoleces of E. granulosis (Agosin and Repetto, 1963, 1965) with $^{14}CO_2$ being incorporated into carbohydrates, proteins and lipids.

Although the significance of this has not been fully explained it is thought that fumarate, an intermediate product in the formation of succinate is responsible for the re-oxidation of NADH, an important component in glucose metabolism and formed during glycolysis.

It would be interesting to determine the relationship between CO_2 and glucose metabolism in Schistocephalus although McCaig and Hopkins (1965) found that growth rates of plerocercoids

were relatively unaffected by low CO_2 tensions (0.04%). This is considerably lower than the level of of CO_2 necessary for active glycogenesis in H. diminuta (Fairbairn et al, 1961). It would appear therefore that growth and presumably glycogenesis in Schistocephalus is not dependent upon the presence of CO_2 at such concentrations as those for H. diminuta. This suggests that CO_2 fixation is either unimportant in glucose metabolism for this species or that the CO_2 levels necessary are not critical.

Evidence that small plerocercoids possess a higher metabolic rate than the larger forms is presented by the results for D-glucose uptake and worm dry weight (fig.10).This evidence supports the findings of McCaig and Hopkins (1965) who demonstrated a negative exponential relationship between growth rate and dry weight in Schistocephalus. Further evidence is provided by Sinha and Hopkins (1967) who calculated the in-vitro growth rates for plerocercoids, and found 400% increase for 2mg worms at 23°C over 8 days, whereas 32mg worms only increased by 25-30% over the same period. Meakins and Walkey (1973) demonstrated the existence of an inverse relationship between growth and dry weight in-vivo for plerocercoids. A similar relationship was also shown for oxygen uptake and dry weight under in-vitro conditions, (Davies and Walkey, 1966).

The results for plerocercoids incubated in non-labelled glucose media (Table.3) shows a clear drop in glucose content of media over the one hour incubation period. With a final glucose value in the plerocercoid greater than that in the incubating media and almost treble that found in control worms, there seems little doubt that plerocercoids can accumulate glucose against a

concentration difference. A point which must be considered however, is whether in fact the influx is maintained in part by the continuous removal of glucose molecules in the parasite tissue by enzymatic breakdown. Chromatograms of plerocercoid extracts from in-vitro experiments (Fig.2)show an apparently low recovery of unaltered D-glucose after a 30 minute incubation period. This could account for the movement of some glucose into the plerocercoid.

Facilitated mechanisms of solute transfer tend to be selective and sometimes energy-dependent. A selective mechanism can control and restrict solute transport across the cell membrane; due in theory to the limited number of transport sites. The absorption rate then reaches a level above which it cannot rise; i.e. saturation point.

Over the range of concentrations used in these experiments no diffusion component seemed to be in operation, therefore no correction was made for this.

Both the existence of saturation levels for glucose uptake and the apparent influx against a concentration difference are strong evidence that the mechanism involved in Schistocephalus plerocercoids is an 'active mechanism'.

Other features of active uptake mechanisms are their relative stereospecificity for the compounds transported and their subsequent sensitivity to inhibition by isomers and metabolic poisons. The stereospecificity of the glucose transport site in tapeworms has already been shown to possess similarities with the mechanism for glucose transport invertebrate intestinal cells. According to studies by Wilson and Crane (1958) and Crane (1960), a hydroxyl group in the glucose configuration at C-2 together with

a pyranose structure are essential for active transport via the glucose 'site'. This structure also appears to be necessary for the monosaccharide 'active transport' mechanism of H. diminuta and a number of other tapeworm species (Read, 1961; Pappas et al, 1973; Pappas and Freeman, 1975). According to Pappas et al, (1973) glucose and galactose enter the tissue of T. crassiceps larvae by means of a single mediated system, indicated by the fact that percentage inhibition of both sugars was essentially similar when using the same inhibitor. Starling (1975) states that glucose and galactose mutually inhibit the uptake of their ^{14}C-labelled counterparts in H. diminuta; this again suggesting that both sugars are absorbed through a common transport locus. Further evidence to support this is provided by Arme et al (1973) who found that glucose uptake was inhibited by galactose in H. diminuta. However there is also evidence to suggest that separate absorption sites exist for glucose and galactose. Phifer (1960c) found no inhibition of glucose uptake by galactose with H. diminuta even when galactose concentrations were twice those of glucose. Rothman (1959b)working on the same species found galactose fermentation to be unaffected by bile salts, in contrast to glucose fermentation. von Brand et al (1964) demonstrated glucose absorption in T. taeniaeformis to be independent of external concentration whereas galactose was not.

The assessment of galactose and 3-O-M glucose as potential inhibitors of D-glucose uptake by Schistocephalus gave inconsistent and inconclusive results, especially in the case of galactose. The findings show that when galactose is present at the same concentration as glucose an apparent stimulation of glucose uptake occurs; increasing with higher concentrations of sugar (Fig 13). An explanation for this is difficult to provide though it may possibly

be due to some form of allosterism in which the binding of galactose influences the transport of glucose in a synergistic manner, or the involvement of a polyvalent carrier complex, (Wong, 1965) reacting with both glucose and galactose. Another possible explanation for this phenomenon is that the glucose absorption mechanism has been supplied with an additional energy source in the form of galactose. Phifer (1960c) found that starved H. diminuta absorbed glucose at higher rates than unstarved worms when pre-incubated in a utilisable energy source i.e. media containing glucose or galactose. This reaction could presumably be involved with the mechanism for active uptake of glucose in Schistocephalus assuming that, galactose is being absorbed by plerocercoids and is also metabolised. Although galactose is fermented by H. diminuta (Phifer, 1957) it is not glycogenic (Read, 1967) and its role in carbohydrate metabolism is thought to be limited (Dunkley and Mettrick, 1969). With galactose concentration twenty times that of glucose no evidence appeared to suggest inhibition of glucose uptake (Table.5) There was however a significant reduction in glucose uptake when galactose was present in media at ten times the concentration of glucose (Table.5). Clearly more extensive investigations concerning the effect of galactose upon glucose metabolism must be performed to resolve these contradictory results and determine whether or not there is any consistently significant interaction between the two sugars. Attempts to repeat Read's work with H. diminuta have also led to variable and contradictory results.

The action of 3-O-M glucose upon D-glucose absorption by Schistocephalus is also inconsistent. Significant inhibition of uptake was shown with only two out of the four concentrations used (Fig.12). Therefore although there is some indication that inhibition

of D-glucose absorption is caused, the evidence is far from conclusive.

The effects of 3-O-M glucose on glucose uptake in other tapeworms varies according to the species. Read (1961) reported that glucose absorption was inhibited by this sugar for H. diminuta although this could not be demonstrated by Phifer (1960c). Its effect upon D-glucose uptake by other tapeworms is mainly non-inhibitory, this being the case for C. verticillatum (Fisher and Read, 1971). These authors concluded that 3-O-M glucose uptake was not mediated. Similarly Pappas and Freeman (1975) could not demonstrate any inhibition caused by this sugar in H. microstoma for D-glucose uptake. T. crassiceps larvae show essentially the same reaction (Pappas et al, 1973). An interesting point concerning D-glucose absorption by C. verticillatum is that the lack of inhibition by 3-O-M glucose and the fact that cellobiose and maltose cause inhibition, is considered as evidence that the C-3 of the glucose molecule is necessary for reacting with the glucose transport complex, in contrast to that of H. diminuta in which an unaltered C-2 configuration is regarded as important (Fisher and Read, 1971).

Studies with the metabolic inhibitors DNP, ouabain and phlorizin demonstrate that they are all capable of inhibiting glucose uptake by Schistocephalus plerocercoids. An overall decrease in glucose uptake was noted over the concentration range 1 - 10 mM ouabain or DNP, (Figs.14,15) there is little difference between 5 mM and 10 mM for both these compounds suggesting that of the concentrations used , 5 mM was probably the level causing maximum inhibition. The effects of these compounds are indicative

of energy-dependent reactions involved in the glucose transport process.

Ouabain is known to block the sodium ion extrusion mechanism in certain vertebrate epithelial cells (Koefoed - Johnsen, 1957) and can also inhibit the glucose transport system in certain tapeworms. Pappas and Read, (1973) found that larvae of T. crassiceps were fully permeable to the compound but that this had no apparent effect on glucose uptake or accumulation. Dike and Read, (1971b)found no inhibition of glucose uptake for H.diminuta in 2 minute incubations using this glycoside. However, Fisher and Read (1971) demonstrated a significant inhibition of glucose transport and accumulation in C. verticillatum caused by ouabain in both 2 minute and 30 minute incubations. In this case the inhibition was considered to be competitive in nature. They also suggested that the inhibition was a result of ouabain reacting with the transport mechanism, this indirectly interfering with the sodium extrusion mechanism causing sodium to accumulate. Sodium extrusion is thought to occur through two mechanisms in this species; a 'slow' and a 'fast', with ouabain affecting the latter.

Glucose uptake by H. microstoma is not affected by ouabain (Pappas and Freeman, 1975) although its effect on glucose metabolism in schistosomes (Uglem and Read, 1975) and Isseroff et al (1972) suggests that its effect is dependent on the amount of ouabain actually absorbed by the helminth; inhibition being apparent only after a period of pre-incubation.

In the present work plerocercoids were only exposed to inhibitor during the 30 minute incubation period, therefore it is not possible to assess the full effect of this compound on glucose uptake without pre-incubation and short-term experiments.

Crane (1965) suggested that sodium-coupled glucose influx into animal cells is controlled by differences between internal and external sodium concentrations rather than a glucose concentration difference.

Glucose uptake by plerocercoids in the present study was significantly inhibited (95.5%) when worms were incubated in media containing potassium as a replacement cation for sodium (Fig.19A) Glucose uptake is also reduced when lithium is substituted for sodium (Fig.19B) inhibition being 43.7%. This implies the existence of a transport mechanism dependent on sodium and which can be partially replaced by lithium. In the interpretation of these results, consideration must be given to inhibition caused by increased potassium concentration rather than lack of sodium. This is unlikely however as potassium has been reported to stimulate active uptake of glucose in vertebrate intestinal cells (Czaky and Ho, 1966). Further support for the hypothesis of Crane (1965) is provided by Pappas and Read (1972). These authors investigated the possibility of reversing glucose flow by altering the sodium gradient. When C. verticillatum, pre-incubated in sodium and glucose containing media were then incubated in sodium-free media with a higher glucose concentration, there was an apparent net efflux of glucose, even against a concentration difference.

Read et al (1974) have shown that in H. diminuta a decrease in the external sodium concentration caused a lowering of the maximal velocity for glucose uptake similar to lowering the glucose concentration. The Kt value was relatively unaffected. A similar relationship between the maximal velocity for glucose absorption and sodium concentration was also noted for C. verticillatum (Pappas and Read, 1972). Therefore it would seem at least in these cases that sodium levels have very little effect on the binding of

glucose to the transport system but can affect the rate at which glucose is transported across the membrane.

Glucose uptake by T. crassiceps larvae is completely inhibited in sodium-free media and is not replaced by tris, choline or potassium (Pappas et al, 1973). This dependence on sodium is essentially the same for adult and larval T. taeniaeformis (von Brand et al, 1964; von Brand and Gibbs, 1966), H. diminuta (Dike and Read,1971b; Read et al, 1974), H. microstoma (Pappas and Freeman, 1975) and C. verticillatum (Fisher and Read, 1971; Pappas and Read, 1972).

Evidence for coupled influx of sodium and glucose as shown for H. diminuta (Read et al, 1974) and C. verticillatum (Fisher and Read, 1971) is not available in the case of Schistocephalus although from the results demonstrating sodium-dependence it would seem likely that a similar relationship exists as in other species.

DNP is apparently a slightly more effective inhibitor of glucose uptake in Schistocephalus than ouabain, with an inhibition level of 80.7% compared to 69.3% of ouabain. The action of DNP is thought also to depend on permeation of the parasite tissue (Fisher and Read, 1971) as inhibition in C. verticillatum was noted only after a ten minute pre-incubation period in media containing the inhibitor. Evidence to support this theory is provided by Phifer (1960a) who showed that inhibition of glucose uptake by H. diminuta occurred only after extended incubation periods of 30 minutes. Similarly Arme et al (1973) demonstrated DNP inhibition of glucose uptake in the cysticercoid larvae of H. diminuta only after a two hour pre-incubation period with inhibitor.

The action of DNP is generally thought to be a result of

interference in the production of phosphate compounds in the oxidative metabolic cycle, although whether such an action is the cause of inhibition of glucose metabolism in Schistocephalus and other tapeworms is not clear.

Phlorizin is a much more effective inhibitor of glucose uptake by Schistocephalus than the other compounds tested, with a 92.2% inhibition at 10^{-2}mM compared to 69.3% and 80.7% for ouabain and DNP respectively when used at concentrations of 10^{-1}mM. This reaction is not unusual as phlorizin is also known to be a strong inhibitor of the glucose uptake mechanism in nearly all of the tapeworms investigated (Pappas et al, 1973; Fisher and Read, 1971; Laurie, 1961; McCracken and Lumsden, 1974; Phifer, 1960a,c; Read et al, 1974).

It appears that phlorizin activity occurs at the helminth surface as inhibition can be reversed by washing in ringer (Laurie, 1957). Inhibition of glucose fermentation by phlorizin was considered to be non-competitive in nature for H. diminuta (Laurie, 1957) although Phifer (1960a) could not determine whether the uptake of glucose was inhibited competitively or non-competitively. In contrast to this, Read et al (1974) and McCracken and Lumsden (1974) came to the conclusion that phlorizin is a competitive inhibitor of glucose uptake in H. diminuta. Fisher and Read (1971) regard phlorizin as a competitive inhibitor of glucose absorption in C. verticillatum. Pappas and Freeman (1975) however demonstrated that the effect of this glycoside was only partially competitive for glucose uptake by H. microstoma.

The action of phlorizin on the glucose metabolism of Schistocephalus appears to be non-competitive in nature as the

maximal velocity for glucose uptake is different in the presence of inhibitor from that of controls. This difference in Vmax values implies that phlorizin is acting indirectly to inhibit glucose uptake by removing catalytic factors essential to the process. It is not possible without short-term incubation experiments to determine whether the action of phlorizin is similar to proposed surface active reactions as in other species.

The mechanism of glucose uptake by Schistocephalus appears to be generally similar to that described for other tapeworms, in relation to sodium dependence and phlorizin sensitivity,with to a lesser extent the effects of DNP and ouabain. The relationship between glucose uptake and galactose interaction in Schistocephalus however is not consistent either in its action or in comparison to the effect of galactose uptake in other tapeworms. Therefore it is not possible from present evidence or without investigating the effects of other sugar analogues to speculate on the stereospecificity of the glucose transport mechanism in plerocercoids.

The in-vitro experiments to investigate regional glucose uptake rates in plerocercoids showed a significant difference between the four main larval sections (Table.4). There is a gradual increase in the uptake rate from the anterior to the posterior region.

Studies by Daugherty and Taylor (1956) demonstrated a difference in regional distribution of glycogen along the strobila of H. diminuta. One of the reasons for this was attributed to a possible differential rate of metabolism along the length of the worm. Rapid decreases in glycogen content during periods of starvation were correlated with sections of the worm where growth

is most rapid i.e. behind the scolex and in the gravid proglottids. Read and Rothman (1958) found that a linear gradient in endogenous metabolic rate along the strobila was present in *T. taeniaeformis* and *Cittotaenia* species. Phifer (1960b) has suggested that the highest rate of synthesis occurs in the 'neck' region of *H. diminuta* where new proglottids are formed, resulting in greater glucose absorption rates than in posterior sections.

Unlike cyclophyllidean tapeworms such as *H. diminuta* in which the gradual proliferation of proglottids occurs behind the scolex, proglottid formation takes place in *Schistocephalus* at the plerocercoid stage by demarcation, within a short period, along the length of the worm.

Orr and Hopkins (1969) have reported that there is a significant increase in the proglottid number in *Schistocephalus* plerocercoids after initial formation from the range 60 - 80 to 75 - 95. Dubinina (1959) states that "a slight increase occurs due to formation of single segments at the rear end". Although as Orr and Hopkins (1969) point out, this is an ambiguous description in that the rear end could mean either the terminal segment or more generally, the rear portion.

The mean number of proglottids for the four plerocercoids used in this study was 72 ± 2.1, i.e. within the lower range for initial formation . It is possible therefore that the higher rate of metabolic activity which exists in the last quarter of the plerocercoid body could be connected with the formation of new proglottids. This requires verification as the increased activity could simply be an expression of growth and glycogenesis in already existing proglottids.

Evidence that plerocercoids are capable of affecting the nutrient reserves of the fish host is presented from measurements of glycogen levels in fish livers. A significant decrease in liver glycogen content is apparent for infected fish (Table.7). The reduction in liver weight of infected fish described by Arme and Owen (1967) can probably be partly accounted for by this loss of glycogen. Walkey and Meakins , (1970) have also stated that infection with Schistocephalus resulted in a greater depletion of the fish food reserves. This situation may be due to a direct removal of glycogen from the liver or indirectly by a constant drain on nutrients preventing glycogen build up from occurring. Some of the weight loss described by Pennycuick (1971d) can probably also be attributed to the inhibition of gonad development in infected fish. Delay in oocyte development of infected fish has been reported by Arme and Owen (1967). It would appear from these findings therefore that plerocercoid development, to some extent, is at the expense of fish oogenesis, spermatogenesis and food reserves.

The reason for reduction in the rate of glucose uptake by fish intestinal tissues (Table.8) is not known. One explanation may be that as the worm is often large enough to displace the heart and liver (Arme and Owen, 1967) in addition to distention of the host body, it is possible that some physical damage is caused by pressure on the gut mucosa, this in turn affecting absorption. Alternatively this decrease may be an indirect result of lowered metabolic efficiency due to the effects of waste product produced by the parasite. Walkey and Meakins (1970) calculated that infected fish had a lower energy transformation efficiency than uninfected fish. This however was overcompensated by the higher efficiency

of energy transformation by the plerocercoids. The low glucose uptake rate by infected fish intestine could therefore be simply a general reflection of impaired physiological mechanisms, e.g. metabolic acidosis. As pointed out by Meakins and Walkey (1975) the production of either CO_2 or fermentation acids by plerocercoids is likely to lower the pH of perivisceral fluid and in turn that of the blood, leading to a reduction in affinity of haemoglobin in fish blood for oxygen. The increased oxygen consumption of fish infected with plerocercoids (Davies and Walkey, 1966; Lester, 1971) possibly being related to such a reaction.

In general the absorption of glucose by plerocercoids shows features essentially similar to those found in other tapeworm species. Uptake appears to be mediated, energy-dependent and requires the presence of sodium. The significance of this has been discussed by Smyth (1962) who considers that active transport mechanisms would possess the advantage over other processes of absorbing glucose when present at low levels and when competing with the host cells. Schistocephalus is obviously not in direct competition with the host intestinal mucosal cells, nor is it exposed to the high concentration of glucose and other nutrients that can occur in the gut. What is unlikely to be disputed is that an adequate supply of nutients is provided for plerocercoids in the body cavity, as indicated by the enormous size which these larvae achieve. The nature of these nutrients absorbed by plerocercoids in-vivo is uncertain, although a significant proportion are of carbohydrate origin as shown by the in-vivo experimental results.

Glucose alone does not appear to be responsible for growth of plerocercoids in-vitro according to Mason (1965) who found that growth did not occur in saline media containing only glucose or

galactose. The addition of serum or amino acids was necessary for growth to take place. In saline media containing serum, growth apparently was erratic but became more stable on the addition of glucose. An interesting point described by Mason (1965) is that glycogenesis was stimulated on the addition of ribose, sucrose or glucose to the media. As sucrose and ribose are unlikely to be absorbed through the glucose transport mechanism, it is probable that a number of alternative transport systems operate in plerocercoids for nutrient uptake. A variety of these have been identified for H. diminuta; summarised by Arme (1975) and involve systems for amino acids, fatty acids, riboflavin, thiamine, pyrimidene and purine. In addition (L. T. Threadgold, 1977, personal communication) found that plerocercoids of Schistocephalus are capable of absorbing particles by pinocytosis; one of the materials absorbed being a low molecular weight protein. It appears possible therefore that some carbohydrates may also be taken up in this manner.

Glucose uptake and metabolism in Schistocephalus is clearly not a simple process and many questions still have to be answered concerning the detailed reactions involved. These include stereospecificity of the transport mechanism, enzyme systems employed, the nature and importance of other carbohydrates within the perivisceral cavity and problems of excretion.

The fact however that an active uptake mechanism for glucose has evolved in Schistocephalus, does seem to suggest that this is an important food source for plerocercoids and is likely to operate in conjunction with other transport systems, similar to those described in H. diminuta for successful growth and development.

Statistics used

Standard errors

All readings where a mean value ± standard error is given were calculated by the following equations for standard deviation and standard error.

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{N}}$$

$$S.\,E. = \frac{s}{N}$$

s = standard deviation.

N = number of samples.

S. E. = standard error.

t - test analysis

t - test analysis was applied to examine the significance of differences between two sets of values, according to the formula:

$$t = \frac{\bar{x}^1 - \bar{x}^2}{\sqrt{\frac{v^1}{n^1} + \frac{v^2}{n^2}}}$$

$\bar{x}$ = mean.

v = variance.

n = number of samples.

The level of significance for 't' is determined according to the degrees of freedom for each sample and by the t-table of distribution.

Correlation coefficient and Regression

Coefficient of correlation between two variables was determined by the equation:

$$r = \frac{\Sigma\left((x - \bar{x})\ (y - \bar{y})\right)}{\sqrt{\left(\Sigma (x - \bar{x})^2\ \Sigma (y - \bar{y})^2\right)}}$$

where x = variable 1
y = " " 2

The regression lines were calculated according to the method of least squares and values determined using the equation:

$y = a + bx$ where b = slope
a = intercept of line on y axis.

For rate of D-glucose uptake/dry weight

$y = \log y$ and $x = \log x$

Analysis of variance

The following method of analysis was used

Source of variance	Sum of squares	Degrees of freedom	Mean squares
Between columns	B - D	u - 1	a
Residual	A - B	u (v - 1)	b
Total	A - D	uv - 1	

$A = \sum$ squares of observations

$$B = \frac{\sum \text{squares of column totals}}{N}$$

$$D = \frac{\sum(\text{column totals})^2}{N}$$

Variance ratio test $= F = \frac{a}{b}$

The significance of calculated values was determined by comparison with values in the table of variance ratio.

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Percentage distribution of ^{14}C from D-glucose in major chemical fractions of plerocercoid

Four plerocercoids incubated in-vitro in ^{14}C-D-glucose media were separated into four main chemical fractions;'free-pool', lipid, protein and polysaccharide to determine the distribution of labelled material in each; based on the method of Graff (1964).

Procedure

'Free-pool' compounds were collected by extraction of plerocercoids in 70% ethanol for 24 hours.

The lipid fraction was obtained after each plerocercoid had been washed in fresh 70% ethanol and homogenised separately in another 10 ml of 70% etanol. The homogenate was centrifuged and the supernatant added to the first extract for 'free-pool' compounds. Five volumes of (ether - 70% ethanol 3:1 v/v) was then added to the precipitate and after vigorous shaking this was centrifuged. Samples of the supernatant collected were analysed for radioactivity (lipid).

To obtain protein,10ml of 10% trichloroacetic acid was added to the precipitate and this heated to 80°C for 15 minutes. After cooling and centrifuging the supernatant was removed leaving a protein precipitate. This precipitate was digested by the Nitric Acid Method (p.26) and samples analysed for radioactive content. The supernatant was boiled with four volumes of KOH. After cooling 1.2 volumes of 95% ethanol was added and again heated to boiling. The precipitate formed from this was centrifuged, washed in two volumes of 95% ethanol, isolated and resuspended in distilled water for analysis of radioactivity.

Results

	C.P.M.	% Total Counts ± S.E.
Free-pool compounds	6735	89 ± 27.3
Polysaccharide	600.5	7.9 ± 3.5
Protein	32	0.4 ± 0.09
Lipid	189.5	2.5 ± 0.7

C.P.M. figures are means for four plerocercoids

Radioactive content of fish blood and coelomic fluid

Attempts were made to obtain data concerning levels of ^{14}C-derived from D-glucose in blood and perivisceral fluid from infected sticklebacks. It was hoped to correlate these findings with in-vivo results and therefore obtain more information about distribution patterns in fish and parasite tissues.

Although a variety of techniques were tried (using heparinised micropipettes) including cardiac puncture, decapitation to expose the dorsal aorta and injection into the cuvieran vein, none were able to provide adequate samples of blood for analysis. A similar problem in terms of quantity arose in attempts to obtain samples of coelomic fluid. The largest amount of fluid collected from one fish was 5ul which on subsequent analysis contained no measureable radioactivity. After these preliminary investigations attempts to

analyse blood and perivisceral fluid were abandoned due to the relatively high wastage of infected fish involved.

Abberant behaviour of plerocercoids

During the main investigation one plerocercoid was discovered with its anterior (approximately $\frac{1}{4}$) firmly embedded in the host fish intestine just behind the stomach region. On closer investigation and after histological examination it was found that the portion inside the gut was missing and had presumably been digested by the host. Despite this fact the worm was still alive and moving when the fish was dissected. Observations by Vik (1954), who found a small plerocercoid embedded in a larger one and Threlfall (1968) who found a plerocercoid that had forced its way through the myotomes of its fish host,are incidents which reflect the potential penetrating behaviour of these larvae. Whether force alone is being used by the worm or whether enzymatic action is involved requires further study. It is unlikely that such behaviour can be of any benefit to the parasite or to the host,especially in this case where the parasite appears to have suffered more damage than the fish. Such behaviour can only be described as abberant considering the relatively low incidence and apparent detrimental effects upon both host and parasite.

In-vivo

Percentage distribution of ^{14}C from D-glucose (CPM/mg).

Incubation period	H	T	Tr	S	I	L	P
	Infected fish Tissue (means for 3 - 7 fish)						
0.13 hours	3.5 ± 2.6	11.0 ± 4.0	2.5 ± 1.5	67.0 ± 9.0	10.1 ± 2.9	5.0 ± 1.0	1.9 ± 1.0
0.5 "	3.4 ± 1.2	2.4 ± 0.7	1.6 ± 0.3	57.0 ± 12.0	10.8 ± 4.0	23.0 ± 12.0	1.8 ± 0.9
1.0 "	3.9 ± 1.5	3.9 ± 1.6	2.3 ± 1.0	46.0 ± 13.0	31.3 ± 10.0	9.9 ± 3.9	2.7 ± 1.6
1.5 "	4.2 ± 7.1	4.1 ± 0.9	7.1 ± 1.2	56.8 ± 0.9	13.0 ± 3.6	13.6 ± 4.1	1.6 ± 0.8
2.5 "	9.0 ± 2.7	3.2 ± 0.7	4.3 ± 1.3	44.0 ± 2.0	26.3 ± 6.6	11.3 ± 2.9	1.9 ± 0.7
4.5 "	9.0 ± 1.2	7.6 ± 4.3	10.0 ± 5.1	30.1 ±10.5	9.0 ± 3.2	10.6 ± 4.0	23.6 ± 9.8
7.0 "	11.9 ± 0.8	13.2 ± 2.7	5.9 ± 0.9	28.9 ± 2.1	10.0 ± 5.9	11.7 ± 3.4	18.4 ± 2.5
10.0 "	23.7 ± 8.0	16.2 ± 2.7	12.8 ± 2.5	12.3 ± 1.3	13.9 ± 4.7	16.9 ± 3.1	4.2 ± 3.8
18.0 "	4.3 ± 3.7	9.2 ± 0.8	11.0 ± 2.0	13.2 ± 1.1	9.0 ± 2.1	4.5 ± 1.6	9.8 ± 1.3
24.0 "	10.1 ± 1.9	7.0 ± 1.8	6.0 ± 2.3	13.0 ± 0.9	33.5 ± 6.5	17.4 ± 3.9	13.0 ± 1.1
	Uninfected fish tissues (means for 5 - 10 fish)						
1.0 "	3.1 ± 0.4	23.7 ± 9.0	34.3 ± 9.7	23.7 ± 4.0	8.5 ±2.3	6.7 ± 1.9	
2.5 "	3.1 ± 0.7	7.2 ± 3.0	3.8 ± 0.5	38.8 ± 17.0	34.4 ± 8.6	12.7 ± 2.7	
10.0 "	7.2 ± 1.8	4.4 ± 1.5	3.5 ± 1.3	49.9 ± 12.0	26.6 ± 8.4	8.4 ± 1.4	

cont.

24 hours	7.4 ± 2.9	7.5 ± 2.5	3.7 ± 3.0	41.7 ± 9.2	15.9 ± 3.9	23.8 ± 6.6	

Percentage distribution of ^{14}C from 3-0-M glucose (CPM/mg)

Incubation period	Infected fish tissues (means for 3 - 6 fish)						
	H	T	Tr	S	I	L	P
0.13 hours	0.7 ± 0.1	0.3 ± 0.06	0.7 ± 0.09	47.5 ± 11.0	47.3 ± 10.7	3.4 ± 1.7	0.08 ± 0.4
0.5 "	3.1 ± 1.1	3.7 ± 0.4	2.0 ± 0.8	47.0 ± 15.0	32.0 ± 8.0	11.9 ± 4.4	0.3 ± 0.1
1.0 "	1.9 ± 0.5	3.7 ± 0.7	1.8 ± 0.1	52.0 ± 8.9	28.6 ± 8.6	11.9 ± 1.8	0.16 ± 0.02
1.5 "	4.5 ± 1.3	5.0 ± 1.7	3.8 ± 1.6	45.6 ± 9.3	31.0 ± 10.0	9.9 ± 1.5	0.15 ± 0.03
2.5 "	5.9 ± 1.4	5.1 ± 1.7	3.8 ± 1.0	52.7 ± 10.5	21.6 ± 7.2	9.9 ±3.4	1.0 ± 0.6
18.0 "	12.1 ± 2.8	10.0 ± 2.6	9.0 ± 1.3	28.0 ± 10.2	24.0 ± 6.2	16.0 ± 5.0	0.9 ± 0.07
24.0 "	17.0 ± 9.9	8.6 ∓ 0.7	5.7 ± 0.4	31.5 ± 2.0	16.0 ± 4.0	18.3 ± 2.5	2.88 ± 0.7

	Uninfected fish tissues (means for 4 fish)					
1.0 "	2.2 ± 0.6	0.4 ± 0.1	1.2 ± 0.2	46.6 ± 17.0	47.1 ± 17.0	2.3 ± 0.1
2.5 "	6.5 ± 0.5	5.0 ± 0.07	4.5 ± 0.7	49.0 ± 6.4	27.0 ± 4.0	7.2 ± 0.3
10.0 "	11.0 ± 4.4	8.7 ± 0.9	6.0 ± 2.3	38.0 ± 6.2	21.5 ± 9.7	14.8 ±
24.0 "	10.4 ± 1.7	10.7 ± 1.4	5.4 ± 0.3	45.6 ± 3.0	4.4 ± 0.3	23.5 ± 0.3

Key: H - head, T - tail, Tr - trunk, S - stomach
I - intestine, L - liver, P - parasite.

In-vivo infected fish tissues.

t - values for D-glucose and 3-O-M glucose

Incub. period	H	T	Tr	S	I	L	P
0.13hr.	1.19	2.1	1.3	1.6	1.4	0.8	1.0
0.5 "	0.18	1.2	0.1	0.4	2.2	0.5	1.8
1.0 "	3.6 *	0.1	0.4	0.3	0.2	0.3	1.5
1.5 "	22.5*	0.4	1.6	0.9	1.6	0.7	1.8
2.5 "	1.0	1.0	0.3	0.8	0.3	0.3	0.7
18.0 "	7.1 *	0.1	3.2*	1.9	1.3	0.9	6.7*
24.0 "	0.7	0.8	0.1	2.4*	2.1	0.1	7.7*

* denotes significant at 0.02 level (p).

Key: H - head, T - tail, Tr - trunk, S - stomach,

I - intestine, L - liver, P - parasite.

^{14}C glycogen from plerocercoids in-vivo

Total CPM/mg from individual plerocercoids (means for three determinations).	Mean.	CPM in glycogen fraction.	Mean and % total
1138, 976, 1040, 2742	1474	47, 29, 133, 183	98 ± 31 (6.65%)
196, 256, 9992, 242, 442, 42	1861	16, 81, 189, 9. 109. 12	69 ± 32 (3.73%)
600, 486, 1982	1022	46, 99, 45	63.3 ± 14 (6.2%)
42, 256, 124, 1600, 162	436.8	11, 74, 15, 231, 66	79 ± 29 (18.2%)
62, 2012, 178, 350, 50	530.4	20, 448, 17, 194, 7	137 ± 76 (25.9%)

D-glucose uptake and temperature (CPM/mg, means for three readings x 6).

Worm number	Temperature °C 10	20	30	40
1	54	512	90	100
2	5.4	674	198	1565
3	27.8	46	436	879
means	29± 11	410± 153	241± 83	848± 344

S O S of observations = 4191520 $\frac{\text{Squares of column totals}}{N}$ = 2840501

		D.F.	M.S.
total S O S	= 2438140		
between treatment SOS	= 1087121	2	543560
residual S O S	= 1351019	8	168877

variance ratio = 3.22 Table F for(p = 0.2)is 2.0

Glucose uptake with time

CPM/mg/hr.

Time (hours)

	0.5	2.0	5.0	7.0
	923	1716	1286	3104
	688	881	8808	12760
	900	6585	4736	44224
	660	6868	8203	5480
	2912	1569	8554	23560
	-	4828	-	-
means ±	1216	3741	6200	17825
s.e.	388	315	1292	6800

Glucose uptake with varying concentration

CPM/mg/0.5hr.

D-glucose (mM)

	0.8	1.6	5.0	10.0
	30	76	540	84
	13	16	16	178
	42	4	80	29
	52	410	65	29
	108	440	71	409
means ±	49	159	199.8	173
s.e.	14	24	26	57

Regression data $\Sigma x \cdot 10^3 = 2170$ $\Sigma y \cdot 10^3 = 37.3$

$\Sigma x^2 = 19.96 \cdot 10^5$ $\Sigma y^2 = 503$ $\Sigma xy = 30963$

$\Sigma dx^2 = 819675$ $\Sigma dy^2 = 153.7$ $\Sigma dxdy = 10684$

$r = 3.06$ $b = 0.013$

from regression line $y = 2.29 + 0.013x$

$\frac{1}{Kt} = 1.5$ $Kt = 0.66$

D-glucose uptake /concentration difference

Incubation period	Absorbence values		
	worm 1	worm 2	worm 3
0 min.	0.288	0.288	0.288
2 "	0.175	0.213	0.236
12 "	0.164	0.159	0.185
22 "	0.157	0.154	0.206
40 "	0.158	0.172	0.206
60 "	0.155	0.102	0.124
worm extracts	0.6	0.39	0.42
control "	0.184	0.153	0.14

standard 0.207

calculation $100 \times \frac{\text{optical density (sample)}}{\text{o.d. (standard)}} = \text{mg/100ml}$

				mean	± se
2 min.	84	102	114	100	7.1
12 "	79	76	89	81	3.2
22 "	75	74	99	82	6.6
40 "	76	83	99	86	5.4
60 "	74	49	59	60	5.9
exp. worm extracts	289	188	202	226	25.8
control " "	89	74	68	77	5.1

Means for experimental and control worm extracts are not significantly different. Worm glucose extracts are significantly different from final glucose concentration of media (60 min) at the 0.05 level.

Uptake of D-glucose/dry weight/0.5 hr.

plerocercoid weight (x)	CPM/mg (y)	log x^2	log y^2
17.3	38.0	1.532	2.493
32.2	7.4	2.273	0.755
16.1	13.5	1.456	1.277
12.2	15.0	1.18	1.383
23.1	4.2	1.859	0.388
10.4	29.6	1.034	2.164
34.7	10.1	2.372	1.008
20.2	16.9	1.704	1.507
20.8	5.9	1.731	0.594
24.2	7.2	1.914	0.734
22.3	7.3	1.817	0.745
233.5	155.1	18.877	13.056

Regression data

$\Sigma \log xy = 14.768$ $\Sigma dx^2 = 0.252$ $\Sigma dy^2 = 0.878$

$\Sigma dxdy = -0.291$

$b = -1.154$ $r = -0.619$, significant at 0.05

for regression of y on x $y = 2.553 - 1.154x$

Uptake of 3-O-M glucose/dry weight/0.5 hr.

x	y
23.9	5.1
37.7	10.2
24.5	13.7
28.7	10.1
21.2	8.6
42.9	7.3
31.2	9.3
32.9	12.6
27.8	7.5
31.1	5.7
27.6	7.0
37.2	9.7

$r = 0.046$ not significant.

D-glucose uptake/ along strobila CPM/mg/hour

worm section	worm number			
	1	2	3	4
A (ant.)	204	156	104	142
B	200	146	104	242
C	322	204	126	196
D (post.)	356	194	136	266

S O S of observations = 10382 Squares of column totals / N = 10255

			D.F.	M.S.
total SOS	=	382		
between columns SOS	=	255	3	85
residual SOS	=	127	12	10.5

variance ratio = 8.1 Table F (for p = 0.01) is 6.0

D-glucose uptake/3-0-M glucose CPM/mg/0.5 hours

Experimental mM D-glucose + 3-0-M glucose				Control mM D-glucose			
0.1	0.5	2.5	5.0	0.1	0.5	2.5	5.0
6.5	5.0	49	410	36	197	1080	89
18.0	61.0	94	35	53	80	174	478
45.0	47.0	230	433	47	112	403	60
-	-	19	-				

means ± s.e.

	0.1	0.5	2.5	5.0
Exp.	23 ± 9	38 ± 14	98 ± 46	292 ± 105
Control.	45 ± 4	129 ± 28.2	552 ± 221	209 ± 110
t-values	2.17	3.72*	2.0	0.001

* significant at 0.05

D-glucose uptake with galactose   CPM/mg/0.5 hr.

Experimental mM glucose + galactose				Control mM glucose			
1.0	5.0	8.0	10.0	1.0	5.0	8.0	10.0
5.8	36.0	10.0	18.0	3.55	0.41	1.05	9.47
15.6	2.4	22.5	59.0	2.05	2.88	4.45	1.91
3.7	22.4	84.0	17.0	0.04	15.4	4.05	4.79
10.2	27.0	57.0	42.0	0.22	-	8.36	-

means ± s.e.

	1.0	5.0	8.0	10.0
Exp.	8.8 ± 2.2	22 ± 6	43 ± 14.5	34 ± 8.7
Control	1.5 ± 0.7	6.2 ± 1.4	4.4 ± 1.3	5.3 ± 1.8
t-values	3.12*	2.49*	2.65*	3.01*

* significant at 0.05

Regression data experimental

$\sum x = 24.1 \quad \sum y = 18.11 \quad \sum x^2 = 190 \quad \sum y^2 = 90.4$

$\sum xy = 121.9$

$\sum dx^2 = 73.8 \quad \sum dy^2 = 24.8 \quad \sum dxdy = 34.6$

$r = 0.8 \quad b = 0.46 \quad y = 1.41 + 0.46x$

Regression data control

$\sum x = 24.1 \quad \sum y = 109.4 \quad \sum x^2 = 190 \quad \sum y^2 = 3569$

$\sum xy = 809.2$

$\sum dx^2 = 73.8 \quad \sum dy^2 = 1175 \quad \sum dxdy = 274.6$

$r = 0.93 \quad b = 3.72 \quad y = 3.95 + 3.72x$

	CPM/mg	mean	corrected mean ± s.e. CPM/mg/hour
glucose 1mM	756.9, 643, 79.9	493.2	1972 ± 680
glucose 1mM galactose 10mM	573.9, 37.6, 429	346.8	1387 ± 520 *
glucose 1mM galactose 20mM	185.9, 563.4, 640	463	1852 ± 456

* significantly different from control at 0.05 level

Effect of ouabain on D-glucose uptake

ouabain		mean	corrected mean ± s.e. CPM/mg/hour
0.1mM	6168, 2014, 968	3050	6100 ± 2594
0.5mM	88, 85, 201	128	256 ± 74
1.0mM	78, 1126, 141	448	896 ± 552
% inhibition glucose control			8802 CPM/mg/hr

Effect of DNP on D-glucose uptake

DNP		mean	corrected mean ± s.e. CPM/mg/hour
0.1mM	307, 373, 735,	471	942 ± 216
0.5mM	194, 12, 120	108	216 ± 84
1.0mM	101, 160, 282, 141	171	342 ± 67
% inhibition glucose control			1167 CPM/mg/hr

Effect of phlorizin on D-glucose uptake. (CPM/mg/0.5hr x 10^{-1})

Experimental mM glucose				Control mM glucose			
0.1	0.5	1.0	2.0	0.1	0.5	1.0	2.0
9.0	31.0	7.0	75.0	82.4	205.0	156.4	325.0
14.0	32.0	13.4	69.0	108.0	789.0	385.0	1145.0
11.0	28.9	6.4	79.0	64.0	209.0	414.0	69.0
			56.0			527.0	1445.0

means ± s.e.

	0.1	0.5	1.0	2.0
Exp.	11.5 ± 1	30.8 ± 0.8	8.9 ± 1.8	69 ± 3.7
Control	84.8 ± 10	401 ± 158	495 ± 131	746 ± 288
t-values	2.32	2.34	3.71*	2.87*

* significant at 0.05

Regression data experimental (reciprocals)

$\sum x = 13.5$ $\sum y = 0.244$ $\sum x^2 = 105.25$ $\sum y^2 = 0.021$

$\sum xy = 1.043$ $\sum dx^2 = 59.6$ $\sum dy^2 = .0062$

$\sum dxdy = 0.219$ $r = 0.350$ $b = 0.0036$

$$y = 0.0489 + 0.0036x$$

regression data control (reciprocals): $\sum x = 13.5$ $\sum y = 0.016$ $\sum x^2 = 105.25$ $\sum y^2 = 7.5 \times 10^{-4}$
$\sum xy = 0.117$ $\sum dx^2 = 59.6$ $\sum dy^2 = 6.8 \times 10^{-4}$
$\sum dxdy = 0.06$ $r = 0.3$ $b = 0.001$

$y = 0.0009 + 0.001$

phlorizin	CPM/mg/hour	means ± s.e.
0.1×10^{-3}	3040, 3880, 9020	5310 ± 1520
1.0 "	371, 2353, 3228	1984 ± 690
10.0 "	20, 352, 185, 660	330 ± 118
100.0 "	112, 20, 61	64 ± 21

Effect of sodium on glucose uptake

	CPM/mg/hour	means ± s.e.	t-values
Lithium			
Exp.	994, 1916, 1168	1358 ± 198	3.82*
Control	2820, 2720, 2410, 1010	2240 ± 362	
Potassium			
Exp.	2, 2, 60	21.2 ± 15.6	2.8*
Control	314, 134, 646	364 ± 124	

* significant at 0.05

Lactate production

Incubation period	worm 1	worm 2	worm 3
	Absorbence values		
2 minutes	0.193	0.25	0.265
12 "	0.115	0.267	0.286
22 "	0.231	0.281	0.229
40 "	0.267	0.293	0.289
60 "	0.253	0.291	0.266

standard 0.184

calculation   49.9 x O.D. = mg/100ml   at 340 nm

mg/100 ml lactate

Incub. period	worm 1	worm 2	worm 3	mean ± s.e.
2 minutes	9.6	12.4	13.2	11.7 ± 0.89
12 "	5.7	13.3	14.2	11.0 ± 2.2
22 "	11.5	14.0	11.4	12.3 ± 0.69
40 "	13.3	14.6	14.4	14.1 ± 0.32
60 "	12.6	14.5	13.2	13.4 ± 0.45

Glycogen content of fish livers

Infected.

Absorbence	mg glycogen/100 g tissue	Absorbence standard
0.869	7160	0.222
0.119	1179	"
0.107	3237	"
0.271	1650	"
0.099	950	"
0.164	1796	"
0.547	5461	"
0.172	6002	0.084
0.009	1967 mean = 3290	"

Uninfected

Absorbence	mg glycogen/100 g tissue	Absorbence standard
0.192	5808	0.222
0.208	2810	"
0.214	11884	"
0.173	41255	"
0.178	8908	"
0.073	24065	0.091
0.14	4510	"
0.003	33214	0.084
0.225	8928 mean = 15600	"

Glucose uptake by fish intestine

		mean
Infected CPM/mg/hour	754, 58, 299, 262, 372, 245, 142, 325, 112	285 ± 64
Uninfected CPM/mg/hour	204, 322, 676, 484, 314, 606, 869, 752, 561	532 ± 69

Lateral plate number of fish

source: Epping Forest

Fish No.	Lateral plate No.(mean for both sides).
1	4
2	2
3	3
4	4
5	13
6	3
7	3
8	3
9	3
10	3
11	4
12	3
13	4
14	4
15	3
16	4
17	3
18	3
19	3
20	3
21	2
22	5

mean = 3.7 ± 0.5

source: Queen Mary Reservoir

Fish No.	Lateral plate No.(mean for both sides).
1	10
2	11
3	14
4	13
5	11
6	13
7	10
8	11
9	12
10	10

mean = 11.5 ± 0.6

Calibration data

Scintillation counter efficiency. 93.5%

Scintillation 'cocktail' efficiency. 68.25%

Quenching correction by internal standard (toluene- C^{14}, 0.502 uCi/g toluene).

QUEEN MARY
COLLEGE
LIBRARY