OBSERVATIONS UPON THE AMERICAN REPRESENTATIVE OF MACROSTOMUM TUBA

WILLIAM A. KEPNER AND M. W. STIFF
Miller School of Biology, University of Virginia

ONE TEXT FIGURE AND ONE PLATE (FIVE FIGURES)

AUTHORS' ABSTRACT

What appears to be a variety of Macrostomum tuba has been described in this paper. There is but a single retinula in each eye. This visual cell displays three regions: rhabdome, ellipsoid, and myoid, as does a vertebrate's retinula. Moreover, the accessory pigment associated with the retinula of M. tuba is applied to the rhabdome and not to the ellipsoid and myoid; this likewise is similar to the distribution of the pigment about the vertebrate's retinula. An analogy, therefore, may be drawn between the visual cell of the rhabdocoele and that of the vertebrate. This is so strong as to suggest homology. Several features peculiar to this variety are: first, the chitinous, distal ring of the penis; second, the fact that shell and yolk droplets seem to be elaborated by each cell destined to become an oocyte.

CONTENTS

INTRODUCTION

Macrostomum tuba is a flatworm of the family Microstomidae of the order Rhabdocoelida, which has not been described before as from the United States.

Our specimens differ essentially from the species as described in but one particular—the penis. For this reason we may be justified in considering this animal a variety of the species Macrostomum tuba.

The purpose of this paper is to describe the retinula and the morphology of the reproductive system of Macrostomum tuba with the formation of its egg. Other anatomical features will be touched upon but briefly.

METHODS

The animals dealt with in this paper were found exclusively in Chamberlain's Pond, near the university.

A portion of the animals were put aside in Stender dishes and fed about twice weekly on fragments of annelids. These cultures are still flourishing, but the animals in the aquarium disappeared after about six weeks.

Only two fixing fluids were used in preparing animals for slides: Goldsmith's and Bouin's. Of the two, Goldsmith's proved to be more effective and was used in at least 95 per cent of the preparations.

Much difficulty was experienced in killing the animals in a distended condition. It was eventually found that 15 per cent alcohol anaesthetized the animals satisfactorily and also caused them to clear the enteron of food. At this point the fixing fluid was applied for five minutes, and the animals were caught in a life-like attitude. Each grade of alcohol and xylene was applied for about two minutes, after which the animals were placed in a paraffin bath for two minutes.

The specimens were sectioned, for the most part, frontally and sagitally at 5 and 7 μ. Delafield's haematoxylin gave good results and was used almost exclusively.

GENERAL DESCRIPTION

Macrostomum tuba is an elongated, spindle-shaped animal, greatly flattened dorsoventrally, with a length of about 3 mm. in the mature, distended condition. The anterior end is rounded, while the posterior end is spatulate and thinner than the general body. The opening of the mouth (fig. 2, *m*) is situated on the ventral surface of the animal near the middle of the upper third of the body. The oval pharynx is conspicuous and highly muscular (fig. 2, *ph*).

The epidermal epithelium is covered with numerous, concentrated cilia. The whole surface of the animal is studded with large rhabdites, more numerous at the extremities. The enteron, extending from the pharynx to the anteroposterior end of the spatulate region of the body (fig. 2, *en*), is a simple, blind sac, characteristic of the rhabdocoeles of this group. Its lateral borders are more or less diverticulated. It is slightly opaque in the living animal and can be readily distinguished. The mesenchyme is very similar to the usual type found in most of the rhabdocoeles. Since the reproductive organs occupy most of the pseudocoele between the enteron and the body wall, the mesenchyme is limited, for the most part, to the anterior and posterior ends of the animal. There are several types of glands to be found in this species, which will not be described here. In fact, details of any of the above-mentioned features will not be given, as they have been adequately described elsewhere.

THE RETINULA

Hesse ('97) described a rhabdome-like region of the retinula that lay within the pigment cup of the triclad eye. Kepner and Taliaferro ('16) discovered that at the base of the rhabdome of Prorhynchus (Geocentrophora?) applanatus there is a refractive, lens-shaped, middle region, thus recognizing three regions of the retinula of the turbellarian eye. Jänichen ('96), as Taliaferro ('20) indicated, "was probably the first to describe the middle region in the retinula of the turbellarian eye as a definite structure" (p. 61). It was left, however, for Kepner and Foshee ('17) to name these three regions as rhabdome, ellipsoid, and myoid, and to indicate a striking analogy, in this manner, existing between the retinula of the vertebrate and that of the rhabdocoele. Kepner and Lawrence ('18) extended this analogy when they discovered that the retinula of the rhabdocoele Polycystis goettei Bresslau presented rhabdome, ellipsoid, and myoid. The American investigators, therefore, were inclined to consider that the turbellarian retinula presented a striking analogy with that of the vertebrate retinula. The appearance, however, of Steinböck's excellent "Monographie der Prorhynchidae (Turbellaria)" ('27) somewhat weakened the convictions of some of us concerning the presence of the three regions of the turbellarian retinula. Steinböck was unable, for example, to find an ellipsoid in the retinula of the European Geocentrophora applanata.[1] Barrett ('29), therefore, made a second study of the eye of the American Geocentrophora applanata and was able to demonstrate the ellipsoid. The study of Macrostomum tuba has revealed an interesting feature in this connection, for when examined under a 4-mm. or water-immersion objective a lens-shaped, highly refractive, colorless body may be seen projecting beyond the mouth of the simple pigment cup of the eye. This refractive object (fig. 3, *el*) resembled the refractive ellipsoid that Kepner and Taliaferro ('16) found in a living, greatly compressed Prorhynchus (Geocentrophora) applanatus. A straw-colored (by transmitted light) region (fig. 3, *r*) practically filled the lumen of the pigment cup of the living specimen. The living animal, therefore, showed a pigment cup (fig. 3, *pc*), a straw-colored structure lying within the lumen of the cup and a refractive lens-like body projecting from the mouth of the pigment cup. The fixed material showed that there was but a single retinula associated with each pigment cup, and that this retinula was very clearly differentiated into rhabdome, ellipsoid, and myoid (fig. 4, *r, el, my*).

We were thus able to extend the close analogy that is held to exist between the rhabdocoele's retinula and that of the vertebrate. In figure 1, B, it may be seen that the retinula of Geocentrophora (?) applanata has its three regions lying within the path of light so that the axis of the retinula is parallel to the rays of light that enter it from the myoid's extremity. The myoid of Polycystis goetti (C) is somewhat deflected from the path of the light rays. In Macrostomum tuba (D) the myoid is more greatly deflected, but not as yet attenuated as Taliaferro found it to be in Planaria (E). This tendency on the part of the myoid to become deflected does not destroy the analogy to which attention has been called by

[1] Steinböck ('27) changes 'Prorhynchus applanatus' to 'Geocentrophora applanta.' The senior author is not convinced that what he and his former students had taken to be P. applanatus is, after all, the old European P. applanatus.

previous workers. Kepner and Foshee ('17) and Kepner and Lawrence ('18), however, overlooked the possibility of carrying the analogy that exists between the turbellarian and vertebrate retinulae a step further. It is of interest to observe that in all of the turbellarian eyes the pigment is not extended along the retinulae beyond the ellipsoid (fig. 1, B, C, D, E). This is likewise the case in the vertebrate retinula

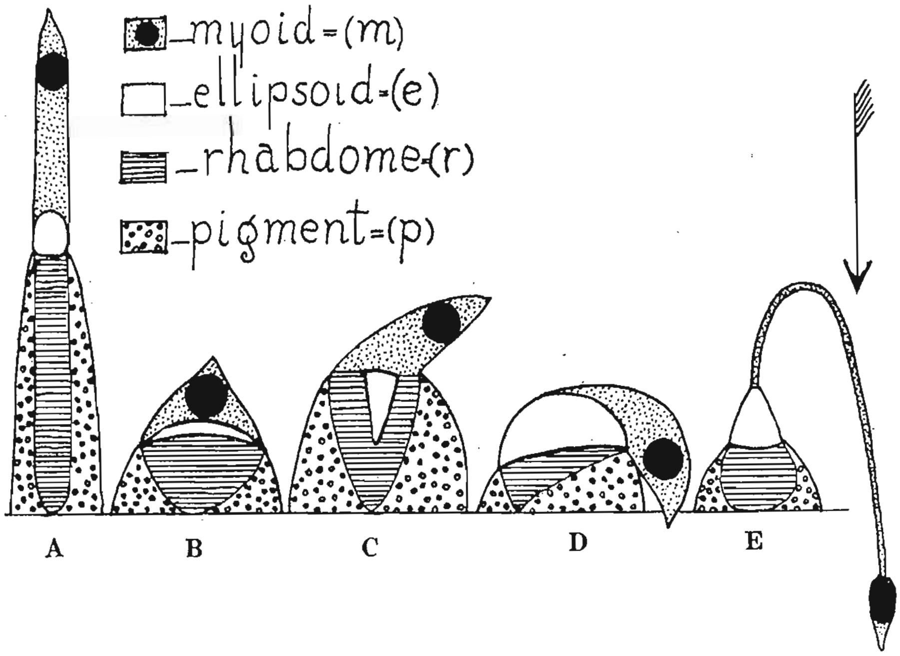


Fig. 1 Diagram to show the anatomy of the retinulae of, A) a vertebrate; B) Geocentrophora applanata; C) Polycistis goettei; D) Macrostomum tuba; E) a planarian. E is after Taliaferro. The arrow indicates the path of light that falls upon all of the retinulae in life.

(fig. 1, A). It thus appears that the more we learn of the retinula of our American Turbellaria, the more striking becomes the analogy between it and the vertebrate's retinula. Indeed, the analogy is so strong as to suggest homology.

MALE REPRODUCTIVE SYSTEM

The male reproductive system consists of paired testes (figs. 2 and 6, *t*) that lie between the enteron and the body wall, stretching diagonally from the anterior end of the enteron to the anterior margin of the ovaries. It is difficult to observe the testes in the living animal except at a time when they contain many well-formed spermatozoa. Posteriorly, the testes form small channels, which connect with the vasa deferentia (figs. 2 and 6, *vd*). The vasa deferentia run posteriorly along the outer margin of the ovaries and unite beyond the posterior extremity of the enteron to enter the seminal vesicle (figs. 2 and 6, *sv*). The latter is an elongated, flexed tube which, in the living animal, can be readily seen lying just posterior to the enteron. A large vesiculum granulorum (figs. 2 and 6, *vg*) is always present, connecting the penis (figs. 2 and 6, *p*) with the seminal vesicle. The penis, a long, chitinous structure, extends diagonally across the pseudocoele with its posterior end in communication with the male genital pore along the midventral line. The posterior end of the chitinous wall of the penis is peculiar in that it is reinforced by a heavy ring of chitin. As far as can be determined, such a penis has never been described in connection with Macrostomum tuba. The closest approach to this peculiarity of the penis is given by Ruhl ('27) in his figure 13. But even in this illustration the sharply defined terminal zone or ring of chitin that we see is not shown for the penis of the European M. tuba. This, therefore, is an anatomical feature that departs from descriptions previously given and makes it possible to consider this a variety of the species.

There is no doubt whatever that a vesiculum granulorum exists in Macrostomum tuba. In all of the living forms studied it has been conspicuously present as a separate and well-defined organ. It may also be definitely seen on the prepared slides.

FEMALE REPRODUCTIVE SYSTEM

Paired ovaries (figs. 2 and 6, *o*) lie on either side of the enteron, directly behind the testes. They are diverticulated, compact structures, and appear in the living form to be quite translucent. An oviduct (figs. 2 and 6, *od*) extends from the mesial posterior end of each ovary. These oviducts join in the midventral line and form an enlargement or common oviduct (fig. 5, *cod*) that communicates with the female genital atrium.

As stated above, the two oviducts unite to form an oval receptacle. In this receptacle or common oviduct the egg lodges before slipping into the female genital atrium. The female genital atrium (figs. 2, 5, and 6, *ga*) is a spheroidal structure lined by large, columnar cells. The lumen of the atrium usually contains sperm.

The female genital pore lies under the female genital atrium. The egg passes through this opening to the exterior. The opening is surrounded by numerous glands which have an affinity for eosin. Meixner ('23) states that these glands secrete a coarsely granular substance on the eggshell as the egg is deposited. This secretion hardens into an irregular jelly-like sheath which incompletely surrounds the eggs and serves to attach it to the substratum.

EGG FORMATION

The oogonia are very small with large nuclei. Each nucleus contains a nucleolus, staining darkly with haematoxylin. The initial development of the oogonium takes place within the ovary. As the oogonium grows, it slowly descends into the oviduct. Prior to this, however, the oogonium has taken on shell droplets and yolk granules. There are various theories as to the origin of these bodies. Meixner ('23) describes the yolk and shell formation and states that the pale yellow shell droplets are formed in rich masses by the young, indifferent germ cells, and that these masses are discharged between the oogonia and are to be found in the ovary and in the oviduct, where they are picked up by the pseudopodial processes of the egg. He concludes that, in macrostomum, the production of the shell substance and at least a part of the yolk material is carried on by the indifferent germ cells. These cells function for life as shell or yolk cells, but they keep their ability undamaged to develop into egg cells when necessary.

If this method of shell production holds good in Macrostomum tuba, it is certainly not sufficient for the great amount of shell material needed for the developing eggs. We have seen but few free shell droplets in the ovaries and none in the oviducts. It is our opinion that each oogonium elaborates its own shell and yolk material and that there are no indifferent germ cells that perform this function for the benefit of the oogonia. Germ cells disintegrate at times, as do body cells, and thereby incidentally expel shell material. This would explain the presence of the very few shell droplets seen in the ovaries.

As the oogonium starts its development, shell droplets are elaborated, and by the time the enlarged oogonium reaches the oviduct, the cytoplasmic body is profusely sprinkled with shell droplets. But, at this period, they move to the periphery of the egg, thus forming its shell.

The shell-covered oocyte drops into the common oviduct over the female genital atrium, whence it descends into the atrium. We could not determine what took place at this point in the atrium in the living animal. But, in our slides, the columnar epithelial cells seemed to have disappeared in all regions except that near the oviduct opening. Even here the cells seemed distorted. At first it was thought that the epithelial cells had disintegrated; but upon closer examination we found a very small ring of granular material around the inner edge of the atrium, and in this ring there were scattered, at fairly regular intervals, nuclei. This evidence forced us to conclude that the epithelial cells were still present, but greatly contracted along their axis, so as to be almost flat. Comparative measurements of the preatrial oocyte and the atrium showed conclusively that the atrium must distend to receive the oocyte. This distention, then, of the muscular female genital atrium is accompanied by a great flattening of the epithelial cells, thus providing a lumen capable of accommodating the egg. As many as four oocytes may develop simultaneously in an oviduct, but in the common oviduct never more than one oocyte rests at a time.

SUMMARY

What appears to be a variety of Macrostomum tuba has been described in this paper. There is but a single retinula in each eye. This visual cell displays three regions: rhabdome, ellipsoid, and myoid, as does a vertebrate's retinula. Moreover, the accessory pigment associated with the retinula of M. tuba is applied to the rhabdome and not to the ellipsoid and myoid; this likewise is similar to the distribution of the pigment about the vertebrate's retinula. An analogy, therefore, that may be drawn between the visual cell of the rhabdocoele and that of the vertebrate that is so strong as to suggest homology. Several features peculiar to this variety are: first, the chitinous, distal ring of the penis; secondly, the fact that shell and yolk droplets seem to be elaborated by each cell destined to become an oocyte.

LITERATURE CITED

BARRETT, W. C., JR. 1929 The retinula cell of the turbellarian Prorhynchus applanatus Kennel. Trans. Am. Mic. Soc., vol. 48, pp. 66–69.

HESSE, RICHARD 1897 Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. II. Die Augen der Platyhelminthes insbesondere der tricladen Turbellarien. Zeit. f. wiss. Zool., Bd. 62.

JÄNICHEN, E. 1896 Beiträge zur Kenntnis des Turbellarianauges. Zeit. f. wiss. Zool., Bd. 63, S. 250–288.

KEPNER, WM. A., AND A. M. FOSHEE 1917 Effects of light and darkness on the eye of Prorhynchus applanatus Kennel. J. Exp. Zoöl., vol. 23, pp. 519–531.

KEPNER, WM. A., AND JOHN S. LAWRENCE 1918 The eye of Polycystis goettei Bresslau. J. Morh., vol. 30, pp. 465–473.

KEPNER, WM. A., AND W. H. TALIAFERRO 1916 Organs of special sense of Prorhynchus applanatus Kennel. J. Morph., vol. 27, pp. 163–177.

MEIXNER, JOSEF 1923 Über das Ovarium von Microstomum lineare und die Abscheidungsfolge des Schalen- und Dottermaterials bei rhabdocoelen Turbellarien. Zool. Anz., Bd. 58, S. 195–213.

RUHL, L. 1927 Regenerationserscheinungen an Rhabdocoelen. Zool. Anz., Bd. 72, S. 160–175.

STEINBÖCK, OTTO 1927 Monographie der Prorhynchidae (Turbellaria). Zeit. f. Morph. und Ökol. der Tiere, Bd. 8, S. 538–662.

TALIAFERRO, WM. HAY 1920 Reactions to light in Planaria maculata, with special reference to the function and structure of the eyes. J. Exp. Zoöl., vol. 31, pp. 59–116.

VON GRAFF, L. 1882 Monographie der Turbellarien. I. Rhabdocoelida. Leipzig.

WARD AND WHIPPLE 1918 Fresh-water biology. New York.

PLATE 1

EXPLANATION OF FIGURES

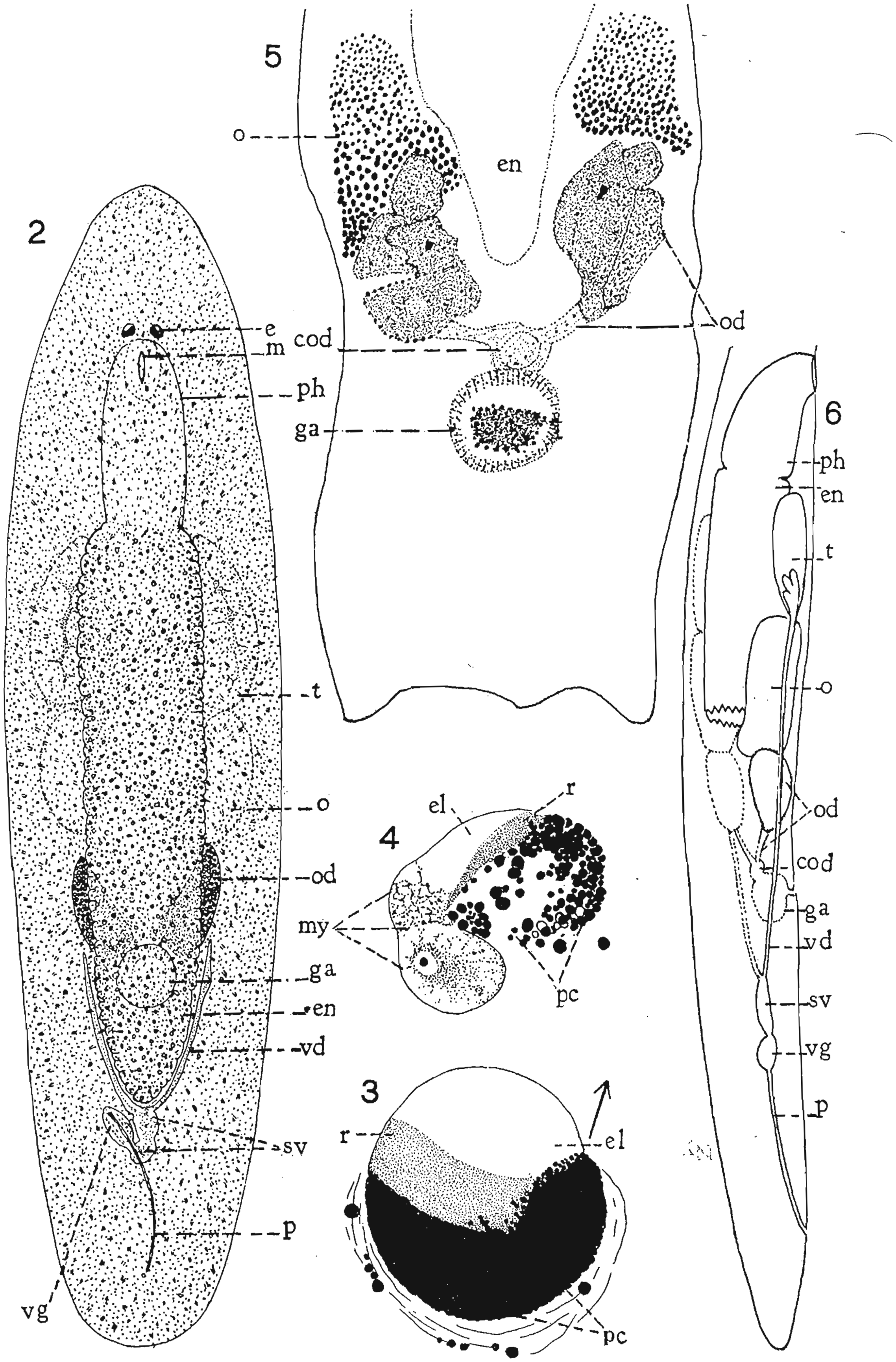
2 Dorsal aspect of Macrostomum tuba. *e,* eye; *en,* enteron; *ga,* female genital atrium; *m,* mouth; *o,* ovary; *od,* oviduct containing an oocyte; *p,* penis; *ph,* pharynx; *sv,* seminal vesicle; *t,* testis; *vd,* vas deferens; *vg,* vesiculum granulorum. × 50.

3 Dorsal aspect of eye of living specimen. *el,* ellipsoid; *pc,* pigment cell; *r,* rhabdome. × 950.

4 Section of eye involving margins of ellipsoid (*el*) and rhabdome (*r*) and the myoid (*my*). × 900.

5 Frontal section. *cod,* common oviduct; *en,* enteron; *ga,* female genital atrium; *od,* oviduct. × 100.

6 Diagram of anatomy of genital systems, right aspect. *cod,* common oviduct; *en,* enteron with its posterior end represented as having been removed; *ga,* female genital atrium; *o,* ovary; *od,* oviduct (right) with an oocyte in its anterior region; *p,* penis; *ph,* pharynx; *sv,* seminal vesicle; *t,* testis; *vd,* vas deferens; *vg,* vesiculum granulorum. × 50.


5
o
en
od
cod
ga
2
e
m
ph
t
o
od
ga
en
vd
sv
p
vg
6
ph
en
t
o
od
cod
ga
vd
sv
vg
p
4
el
r
my
pc
3
r
el
pc