THE IMMUNOLOGICAL RESPONSE OF SOUTH AFRICAN *BULINUS NATALENSIS* ANTIGENS TO DIPLOID AND POLYPLOID *BULINUS* ANTISERA

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ABSTRACT

Foot muscle antigens of the diploid *Bulinus natalensis* from Lake Sibayi showed complete immunological correspondence with antiserum prepared from foot muscle tissues of the diploid *B. tropicus*. On the other hand, strong "non-identity" reactions occurred when *B. natalensis* antigens were tested with the antiserum prepared from foot muscle of the tetraploid *B. coulboisi*. It is therefore concluded that *B. natalensis* is indeed a member of the "*tropicus* species group", as its chromosome number would imply, and not a diploid member of the more northern polyploid "*truncatus* species group", as several morphological characters might suggest.

INTRODUCTION

Various members of the African freshwater gastropod genus *Bulinus* (Planorbidae) are intermediate hosts of human urinary bilharziasis in Africa and the Near East. One particular section of this genus (traditionally referred to as the subgenus *Bulinus* s.s., although if subgenera are to be maintained, it should nomenclaturally more correctly be called *Isidora*) is remarkable cytologically because it contains a polyploid series. Only tetraploids (n=36, the *truncatus* species group) are known north of the Sahara, only diploids (n=18, the *tropicus* species group) are known from Africa south of Tanzania, and in the latitudes between are found both diploids and tetraploids (Burch 1964, 1967b). Higher polyploids occur in the Ethiopian zoogeographical region, i.e., hexaploids (n=54) in Ethiopia and octoploids (n=72) in Ethiopia and West Aden (Burch 1967a; Brown and Burch 1967). *Bulinus truncatus* (n=36) serves as the intermediate host for *Schistosoma haematobium* in its area of distribution, and *Bulinus* sp. (n=72) has been experimentally infected in the laboratory (Lo 1968), but the diploid species of this "subgenus" (i.e., the *B. tropicus* group) generally are not considered to be susceptible to infection with *S. haematobium* (e.g., see Dawood and Gismann 1956; Cridland 1955, 1957; Lo 1968). In southern Africa there occur forms of *Bulinus* which, although diploid, have certain morphological characters (mainly radular mesocone shape and shell shape) of the more northern *truncatus* species group (Schutte 1965, 1966). Additionally, one population (from Nelspruit) has been infected with several species of *Schistosoma* of the *haematobium* group (having terminal spined eggs) (Pitchford 1965). These taxonomically troublesome forms have been referred to the *truncatus* species group (Schutte 1965), or more recently to the *natalensis* species group (Brown, et al. 1967).

* Supported by a research grant (AI 07279) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

Taxonomic assessment of the relationships of African species of the *Bulinus tropicus*, *B. natalensis* and *B. truncatus* species groups is difficult, and systematics of the whole *Bulinus* complex in Africa is tenuous at best. The radular mesocones may have some value as taxonomic characters (Mandahl-Barth 1965; Schutte 1965, 1966; Brown et al. 1967), but they are not infallible, and in fact, in some cases, they would appear to have very limited usefulness (Burch and Lindsay 1970). In the latter study it was found that diploid (2n=36, n=18) populations of presumed *B. natalensis*, with radular characters of the tetraploid (n=36) *truncatus* species group, showed immunological correspondence with diploid antisera of *B. tropicus*, but not with tetraploid or octoploid antisera. However, because of the difficulty in distinguishing between *B. tropicus* and *B. natalensis*, especially in specimens lab-raised for many generations (see discussion of this problem in the appendix to Burch and Lindsay 1970), we were especially interested in making an antiserum to a population of *B. tropicus* of more certain identity, and to test its immunological response to tissue extracts (antigen) from specimens of *B. natalensis*, also of more certain identity. It is on the results of that study that this report is based.

MATERIALS AND METHODS

Specimens of *Bulinus natalensis* collected by D. S. Brown and G. Oberholzer from Lake Sibayi, Zululand, Republic of South Africa, were the snails of special concern in this study.* Crude antigen extracts were prepared from pooled foot muscle using a Biosonic homogeniser.

These antigens were tested for reactions with antisera prepared from (1) the diploid (n=18) *B. tropicus* originally from De Villiers cement reservoir, Malelane, Nelspruit, Transvaal, South Africa; (2) a diploid (n=18) species, originally from Crocodile Creek, Lake McIlwaine, Rhodesia, referable most probably to *B. tropicus*, but possibly to *B. natalensis* (see appendix to Burch and Lindsay 1970), and (3) the tetraploid (n=36) *B. coulboisi*, originally from Mwanza, Tanzania. The antisera (2) and (3) above were the same as those used by Burch and Lindsay (1970), and the procedures for production of antisera, preparation of test antigens and the agar gel, and setting up the plates for the micro-Ouchterlony double diffusion test were also the same as described there.

Briefly, the procedures were as follows: antibodies against snail antigens were produced in virgin female rabbits by two consecutive series of five one-ml injections of snail foot muscle in increasing concentrations. The antigen injections consisted of the supernatant liquid obtained after centrifugation of the homogenised snail foot tissue. The resulting serum containing the antibodies was sterilised by filtration after which an anti-bacterial agent (merthiolate, i.e., sodium-ethylmercurithiosalicylate; 0·001 %) was added. Plates for the double diffusion test were prepared by fastening a glass ring 21 mm in inside diameter and

* Brown, Oberholzer and Van Eeden (1971) and Brown (pers. comm.) consider the Lake Sibayi specimens to conform to the nominal species *B. zuluensis* Melvin and Ponsonby, which they regard as a synonym of *B. natalensis*.

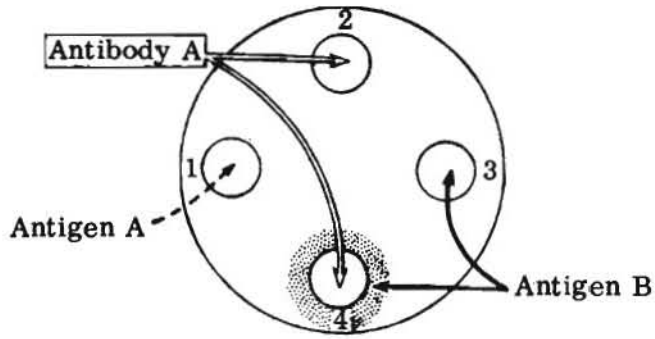

FIGURE 1 (left)
Photograph of a typical shell of *Bulinus natalensis* from Lake Sibayi, showing the characteristically reduced spire and straight columella. Although this shell was from a laboratory-raised specimen, it has the typical shell-shape characteristics of the wild population. This particular shell, of average size, was 6 mm in height.

FIGURE 2 (right)
Procedure for filling the wells with antigen and antisera in the micro-Ouchterlony double diffusion test. Preabsorbed antigen is around well 4. Excess antigen for the preabsorption was removed before the antibody was placed in well 4.

10 mm in height, to a microscope slide. The semi-solid medium used to fill the rings to about 5 mm was a 1% agar gel with 0·4% NaCl and 0·0001% merthiolate.

Four wells were cut in the gel and filled with antigens or antiserum according to the scheme illustrated in Figure 2. Full-strength antisera were used. The fluid containing the antigen was the supernatant obtained from centrifuging 0·1 g snail foot tissue homogenised in 1·0 ml snail physiological saline. Proteins occurring in one species but not in the other could be observed by the precipitin reaction of diffusing protein fractions between wells 1 and 4, because the anti-serum in well 4 had to pass through a surrounding barrier of pre-absorbed heterologous antigen, allowing only those antibodies through to react with antigens from well 1 that were not common to the two populations. Thus, we would determine whether or not the two species in question had developed immunologically detectable differences in foot muscle proteins during their evolutionary divergence. Therefore, the presence ("non-identity") or absence ("identity") of precipitin bands between wells 1 and 4 were of prime interest to us.

Chromosome numbers of the snails were determined from acetic-orcein squash preparations of ovotestis tissues.

RESULTS

The antigen-antibody reactions between the antigens of *Bulinus natalensis* from Lake Sibayi and the three antisera are shown in Figure 3. At least five antigen-antibody systems could be identified in all three instances, indicating strong antisera. When testing *B. natalensis*

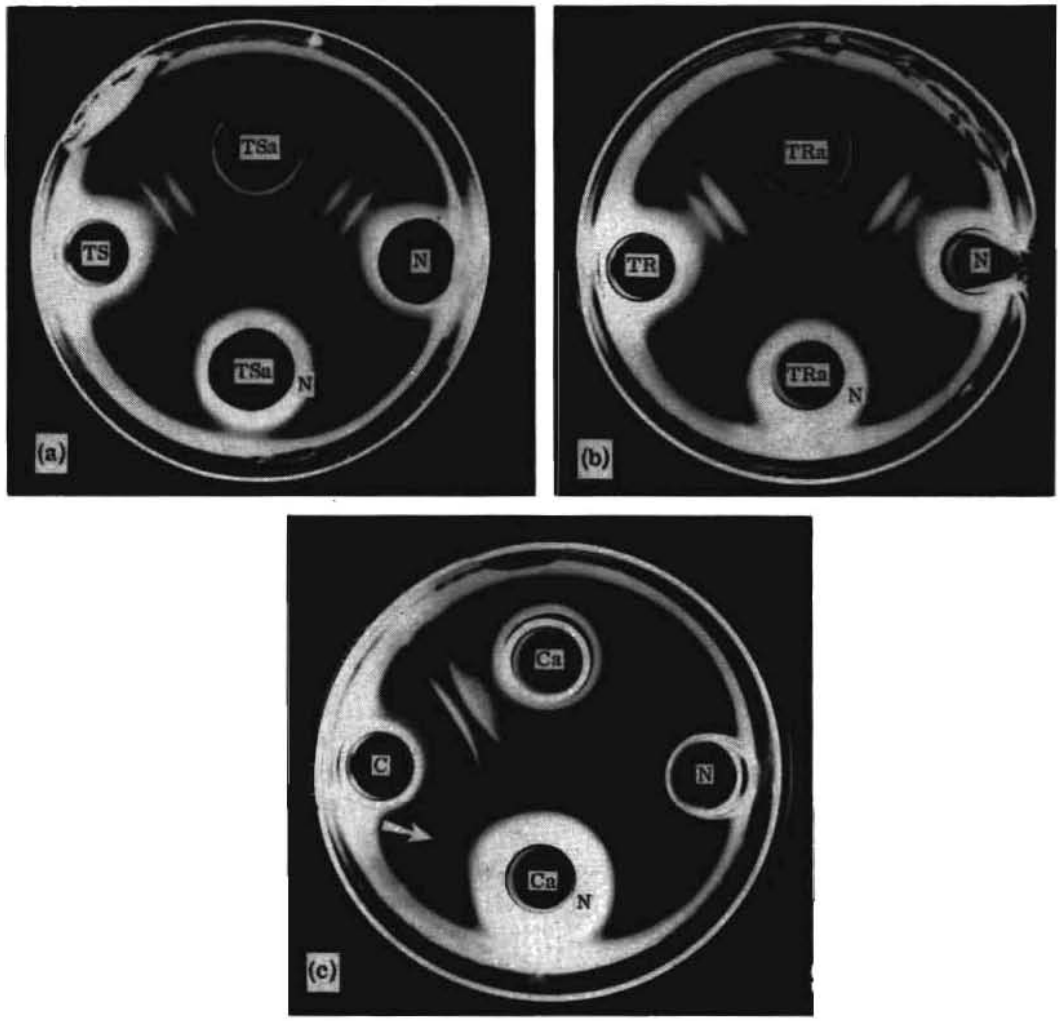

FIGURE 3
Precipitation reactions of *Bulinus natalensis* [n=18] antigens with antisera of (*a*) *B. tropicus* (S. Africa) [n=18], (*b*) *B. tropicus* (Rhodesia) [n=18], and (*c*) *B. coulboisi* (Tanzania) [n=36]. Note the "non-identity" reactions in c (arrow), and their absence in a and b.
N = *B. natalensis* antigen; TS = *B. tropicus* (S. Africa) antigen; TSa = *B. tropicus* (S. Africa) antiserum; TR = *B. tropicus* (Rhodesia) antigen; TRa = *B. tropicus* (Rhodesia) antiserum; C = *B. coulboisi* antigen; Ca = *B. coulboisi* antiserum.

antigen with *B. coulboisi* (n=36) antisera a strong "non-identity" reaction (arrow) occurred between wells 1 and 4, i.e., between wells labelled C and Ca (Fig. 3, c), representing the presence of non-homologous foot muscle proteins between the diploid (n=18) *B. natalensis* and the tetraploid (n=36) *B. coulboisi*. Significantly, no "non-identity" reactions occurred

when *B. natalensis* antigens reacted with antisera from the two diploid (n=18) *B. tropicus* populations (Fig. 3, a and b).

DISCUSSION AND CONCLUSION

Burch and Lindsay (1970) reported the immunological response to diploid and polyploid antisera of antigens from specimens of 37 populations of African, Near Eastern and Mediterranean *Bulinus* s.s. In these tests they never observed reactions of the "identity" type between populations having two different chromosome numbers, and, with only two possible exceptions, "non-identity" reactions occurred only between populations having different chromosome numbers; populations of the same chromosome number showed only "identity". Snails supplying diploid antigens came not only from South Africa, but from as far north as Senegal and Lake Tana, Ethiopia. Snails supplying tetraploid antigen were from the Mediterranean area and the Near East, but also from as far south as Ghana and northern Tanzania.

Of special concern in the previous study (Burch and Lindsay 1970) was whether or not the populations having the diploid chromosome number (2n=36; n=18) of the more southern *tropicus* species group, but also certain morphological similarities (radular mesocone; shell shape) with the more northern tetraploid (2n=72, n=36) *truncatus* group were related more closely to the northern than to the southern group. One of the prominent nominal species having such characteristics is *Bulinus natalensis*. However, because of the difficulty of identifying laboratory-reared specimens, there might be some doubt as to whether any of our populations could be definitely associated with the nominal concept of *B. natalensis*. For this reason the current series of experiments were carried out with specimens that belonged with greater certainty to *Bulinis natalensis* in order to establish the immunological response, as determined by these particular immunological techniques, between their antigens and antisera produced against both *B. tropicus* (n=18) and *B. coulboisi* (n=36).

Inasmuch as "non-identity" reactions did not occur between *Bulinus natalensis* and *B. tropicus*, though they did occur between *B. natalensis* and *B. coulboisi*, we may infer that *B. natalensis* is more closely related to the diploid *B. tropicus* than it is to the tetraploid species *B. coulboisi*, regardless of certain similarities of morphological detail to that species. The close immunological relationship between *B. coulboisi* and *B. truncatus truncatus*, the main form of the "*B. truncatus* species group", and the occurrence of "non-identity" reactions between *B. t. truncatus* and n=18 antisera was demonstrated by Burch and Lindsay (1970).

ACKNOWLEDGMENTS

Grateful acknowledgment is made to C. A. Wright, D. S. Brown and G. Oberholzer for making the Lake Sibayi *Bulinus natalensis* available for our study. The latter two collected the specimens, and D. S. Brown kindly offered information regarding their identification. The specimens of *B. tropicus* and *B. coulboisi* were from Museum of Zoology laboratory stocks originally provided by C. H. J. Schutte and John McClelland respectively. Special appreciation is due to Anne Gismann for critically reading the manuscript and offering helpful suggestions.

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