PURIFIED PROTEIN DERIVATIVE OF TUBERCULIN INDUCES IMMUNOGLOBULIN PRODUCTION IN NORMAL MOUSE SPLEEN CELLS*

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Two major events occur in the response of the immune system to antigenic stimulation, proliferation of cells and secretion of soluble products. The relative importance of these two events is not well known. Mitogens have been found to provide valuable tools for analysis of these problems (for review, see reference 1). The substances most well studied are phytohemagglutinin (PHA)† and concanavalin A, which have been found to selectively activate thymus-derived (T) cells (2-6), and lipopolysaccharides of bacterial origin (LPS), which have been found to selectively activate bursal equivalent or bone marrow-derived (B) cells (2, 7). Pokeweed mitogen seems to activate both cell types (4).

Recently we reported (8) that purified protein derivative (PPD) tuberculin, which has previously been associated only with a number of supposed T cell activities in immune situations, induces DNA synthesis in nonimmune mouse B cells, i.e., acts as a B cell mitogen. The present work reports our analysis of a second major activity of PPD, namely induction of immunoglobulin synthesis.

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1 Abbreviations used in this paper: B cells, bursal equivalent or bone marrow-derived lymphocytes; BSS, balanced salt solution; HGG, human gamma globulin; HRBC, horse erythrocytes; LPS, lipopolysaccharides of bacterial origin; NNP, 4-hydroxy-3,5-dinitrophenacetyl; PBS, phosphate-buffered saline; PFC, plaque-forming cells; PHA, phytohemagglutinin; PPD, purified protein derivative; SRBC, sheep erythrocytes; T cells, thymus-derived lymphocytes; TNP, 2,4,6-trinitrophenyl.
Materials and Methods

Animals.—Unless otherwise stated, we used F1 hybrid mice (C3H/HeJ × CBA) of both sexes, usually between 8–16 wk of age (same sex and age within each experiment).

Antigens and Mitogens.—PPD tuberculin RT32 was supplied by the Statens Seruminstitut, Copenhagen, as sterile solution 1 mg/ml. When higher concentrations were needed the same tuberculin in powder form was dissolved in phosphate-buffered saline (PBS). In some experiments we also used two tuberculin lots (42/23 and 62/23, in the Results section termed lot a and lot b) with well-defined potencies in immune systems. Sheep erythrocytes (SRBC) and horse erythrocytes (HRBC) were stored in Alsever’s solution and washed three times in balanced salt solution (BSS) before use.

4-Hydroxy-3,5-dinitrophenacetyl (NNP) human gamma globulin (HGG) was prepared as described previously (9). NNP-SRBC were prepared as described by Pasanen and Mäkelä (10). The cells used were no 2 cells, i.e. having a moderately high epitope density (for coupling: 0.1 mg/ml of NNP-azide in a suspension of 10% SRBC in BSS).

PHA was obtained from Wellcome Foundation Ltd., Beckenham, Kent, England, and used in final dilution 1:100. LPS from Escherichia coli was obtained by the phenol-water extraction method of Westphal et al. (11, 12).

Cell Cultures.—The procedure was essentially that of Mishell and Dutton (13). Spleens were removed aseptically, minced with forceps in BSS, and further dispersed by gentle pipetting. After sedimentation of larger pieces, cells were washed twice in BSS and counted in a hemacytometer with addition of trypan blue for viability testing. They were then suspended at 10^7 viable, nucleated cells per milliliter in medium with 10% fetal calf serum (Flow Laboratories, Rockville, Md.; lot 42005). 1 ml cultures in plastic Petri dishes (Falcon Plastics, Div. B-D Laboratories, Los Angeles, Calif.; 3002) were prepared in triplicate and incubated in an atmosphere of 5% CO2 in air at 37°C with continuous rocking. The cultures were given nutritional “cocktail” daily. Cells were harvested using a rubber policeman, washed twice, and counted as described above.

In experiments with mitomycin treatment of cells before culture, this was performed with 25 µg mitomycin C/ml in a suspension of 10^6 cells/ml, 30 min at 37°C, followed by two washings in PBS.

Hemolytic Plaque Assay.—This assay (14, 15) was employed with some modifications: 0.6 ml of 0.5% agar in BSS with 0.05% diethylaminoethyl-dextran was kept in glass tubes at 47°C. 0.2 ml of washed spleen cells (usually pooled cells from three cultures), 0.03 ml of RBC 1:8 in BSS, and 0.05 ml of guinea pig serum 1:4 in BSS were added. The suspension was mixed with a preheated pipette and three 0.2 ml spots were spread in a plastic Petri dish. The Petri dishes were incubated at 37°C for 2–3 h and read in indirect light. Unless otherwise stated, values given are plaque-forming cells (PFC)/10^6 recovered, viable cells. Some variations in the cell recoveries occurred with a tendency for higher cell recovery in cultures with PPD than in control cultures. The plaques were complement-dependent, and when checked microscopically, always contained a centrally located, nucleated cell.

Experiments with inhibition of NNP-PFC by free NNP-gHGG (16) were done by adding to the assay mixture 0.05 ml of dilutions from 10^-6 to 10^-7 of a NNP-gHGG solution 3.5 mg/ml. Thus, the 10^-6 dilution represents a NNP-gHGG concentration in the gel of 190 µg/ml.

Detection of Immunoglobulin by Immunofluorescence Technique.—Cultured cells were washed twice and centrifuged gently onto microscopic slides. After drying the slides were fixed in methanol. After drying, fluorescein-labeled anti-mouse Ig antibody (sheep origin, Statens Bakteriologiska Laboratorium, Stockholm) was added and the slides were incubated for 30 min in a moist atmosphere at 37°C. After washing, the slides were mounted in phosphate-buffered

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glycerol. Reading was performed, using a Leitz Orthoplan microscope (E. Leitz, Inc., Rock-leigh, N. J.). 3-500 cells were examined and the percentage of fluorescent cells was determined.

RESULTS

Nonimmune mouse spleen cells cultured for 48 h in the presence of PPD yielded significantly increased numbers of PFC against SRBC, HRBC, and NNP-SRBC, in comparison with control cultures (Table I). Similar degrees of increase have also been obtained with 2,4,6-trinitrophenyl (TNP)-SRBC (not shown). The use of hapten-coupled erythrocytes in this assay system results in much greater numbers of PFC compared with nonhaptenated erythrocytes. This is probably due to the high epitope density of hapten on the red cell surface, which permits binding and therefore lysis by very low avidity anti-NNP immunoglobulins (10). Since this greatly increases the sensitivity of the assay system by permitting detection of a broader spectrum of Ig-secreting cells, we chose to use NNP-SRBC throughout this investigation.

TABLE I
Numbers of PFC/10⁶ Cells against Heterologous Erythrocytes and Hapten-Coupled Erythrocytes after 48 h Incubation of Normal Mouse Spleen Cells with PPD Tuberculin

| PPD concn (µg/ml) | Target cells |
|------------------|--------------|
|                  | SRBC         | HRBC         | NNP-SRBC     |
| 0                | 34 ± 12*     | 14 ± 3       | 761 ± 285    |
| 10               | 111 ± 26     | 57 ± 10      | 1326 ± 374   |
| 100              | 138 ± 17     | 99 ± 16      | 2300 ± 159   |

* Values given are mean values of results obtained in three separate experiments ± SE.

Kinetics and Dose Dependence of PPD-Induced Immunoglobulin Production.—The first detectable increase in PFC occurred 15–20 h after initiation of cultures (Fig. 1). With the highest PPD concentration used, 100 µg/ml, the response reached a maximum at 48 h, whereas lower PPD concentrations resulted in later peak responses (Fig. 2). Consequently, the dose curves had different shapes depending on the duration of culture before assay (Fig. 3). A higher PPD concentration than those shown, 1 mg/ml, resulted in essentially the same response as with 100 µg/ml.

Thus, compared with the PFC responses in cultures without PPD, PPD induces a response which starts 8 h earlier and reaches much higher levels (Fig. 1). Nevertheless, as can be seen from Figs. 1–3 and as previously reported (9), there is a substantial increase of PFC also in the absence of PPD. In more recent experiments we have found that this background increase requires the presence of fetal calf serum, whereas removal of fetal calf serum does not affect the PPD response (data to be published).
Characteristics of the PPD-Induced Immunoglobulin.—Only direct plaques were obtained with PPD-stimulated, nonimmune cells. The relative avidity of immunoglobulins was measured by inhibition of anti-NNP PFC with various concentrations of soluble NNP_51HGG in the assay mixture. As seen in Fig. 4, the concentration of hapten-protein required to inhibit 50% of the PPD-induced PFC was 100 times higher than that needed to inhibit 50% of antigen-induced PFC. These results indicate that the avidity of PPD-induced anti-NNP Ig is much lower than that of antigen-induced anti-NNP Ig. Free PPD in the gel did not cause any inhibition of PFC.

Besides determining the responses to a selected number of antigenic determinants, we also wanted to get an estimate of how many B cells were actually triggered by PPD. This was done by determining the numbers of cells containing immunoglobulin (examined with fluorescein-labeled anti-mouse Ig antibody). Using this technique we found in three experiments that 40–70% of the cells contained immunoglobulin after 48 h of culture with PPD, compared with 5–15% in cultures without PPD and below 5% in cultures with PHA.

Comparison of PPD, LPS, and PHA.—Fig. 5 compares culture responses (anti-NNP PFC) to PPD, LPS, and PHA. Essentially similar responses were obtained with PPD and LPS, whereas PHA actually depressed the response in
comparison with controls, in analogy with recent findings by others (R. W. Dutton, personal communication).

**Dependence on DNA Synthesis.**—Mitomycin-treated spleen cells were cultured with or without PPD for 24 and 48 h. Both the numbers of anti-NNP PFC and thymidine incorporation were determined. The results are given in Table II. It can be seen that the PPD-stimulated NNP-PFC remained unaltered after mitomycin treatment, whereas thymidine uptake was depressed with approximately 90%. In fact, the stimulatory effect of PPD at 48 h, expressed as factor of stimulation, was more obvious after mitomycin treatment, because the response was depressed in control cultures. It should be noted, however, that the cell loss in the cultures of mitomycin-treated cells was considerably more pronounced than in untreated cultures (viable cell recovery [expressed as percentage of cells put in culture]: non-mitomycin-treated cells approx. 30%, mitomycin-treated below 10%). Furthermore, the depression of thymidine uptake was not 100%, which must be considered in the interpretation of these results.
Effects of Tuberculins of Different Potency.—To determine if the activity on B cells correlated with the ability of the substance to elicit immune reactions, we tested two lots of PPD with different immunological potencies. From experiments in immune systems (skin tests in BCG-vaccinated guinea pigs and DNA synthesis in lymphocytes from tuberculin-positive humans) it had been clearly established that lot a was definitely more potent than lot b in an immune situa-

![Graph](image)

Fig. 3. The response of normal mouse spleen cells to various concentrations of PPD after various times in culture. Values given represent mean values of results obtained in three different experiments ± SE. (The value for 0.1 µg/ml is from one experiment only.)

The results when nonimmune spleen cells were cultured with these PPD preparations showed the same relation, i.e., lot a induced greater numbers of PFC than lot b (Table III).

In Vivo Experiments.—To determine the in vivo efficiency of PPD as a B cell stimulator, mice were injected with various doses of PPD. Their spleens were removed 24–96 h after injection and assayed for PFC against SRBC and NNP-SRBC. In three experiments the values after PPD injection (5 mg/animal intravenously) were 50–70 anti-SRBC PFC/10^6 cells (1–3 PFC/10^6 in controls) and 115–280 anti-NNP PFC/10^6 cells (30–50/10^6 in controls). The response
reached a maximum 48 h after injection (Fig. 6). In other experiments we have also obtained increased numbers of PFC after injection of BCG.

DISCUSSION

PPD induces a rapid activation of lymphocytes with the capacity to secrete immunoglobulins. We conclude that this is not due to antigenic properties of

![Graph showing the effect of NNP65HGG dilution on the percentage of control PFC.](image)

**Fig. 4.** Normal mouse spleen cells were cultured for 48 h with PPD 100 µg/ml, whereafter the numbers of anti-NNP PFC were determined in the presence of various dilutions of NNP65HGG in the gel (to the assay tubes containing the suspension of agar + spleen cells + erythrocytes + complement, total volume = 0.9 ml were added 0.05 ml of dilutions from a solution of NNP65HGG of 3.2 mg/ml). The values given are the percentage of anti-NNP PFC at various NNP65HGG dilutions compared with an assay without NNP65HGG. As comparison is shown similar inhibition data from an experiment with HGG-primed cells cultured in the presence of an optimal concentration of NNP65HGG.

PPD since (a) the response is multispecific, against every determinant tested (SRBC, HRBC, NNP, TNP), (b) there is a rapid induction of the response, which is in contrast to findings in specific immune responses in vitro (13), and (c) the avidity of the Ig produced after PPD stimulation is low, in contrast to that of antigen-induced Ig (Fig. 4). This low avidity is similar to that obtained after stimulation with another B cell mitogen, LPS (1). Also the very high numbers of PFC induced by PPD in a sensitive anti-hapten system, approaching 0.5% of the cells in a nonimmune situation, indicate that a larger B cell population is involved than could be induced by any one antigen. This taken together...
Fig. 5. Normal mouse spleen cells were cultured with the mitogens shown in the figure and the numbers of anti-NNP PFC determined after various times in culture.

**TABLE II**

**Effect of Mitomycin Treatment on the Reactivity of Normal Mouse Spleen Cells to PPD Tuberculin**

| PPD (μg/ml) | 24 h in culture | 48 h in culture |
|------------|----------------|----------------|
|            | Untreated      | Mitomycin treated | Untreated      | Mitomycin treated |
| 0          | 109 ± 37*      | 162 ± 45         | 1035 ± 281     | 353 ± 156         |
| 100        | 313 ± 153      | 510 ± 171        | 2483 ± 336     | 2465 ± 271        |

* Values given are mean values of results obtained in four separate experiments. In two of these experiments [3H]thymidine uptake was determined and mitomycin treatment was found to cause reduction with 85–97%. with the immunofluorescence data strongly argues that PPD activates a majority of B cells.

PPD-stimulated B cells undergo both DNA synthesis, as reported in detail elsewhere (8), and a rapid induction of immunoglobulin secretion which begins
TABLE III
Effect of Two Tuberculins with Different Potencies on the Development of Anti-NNP PFC in 48 h Cultures of Normal Mouse Spleen Cells

| Exp. no. | PPD* | Anti-NNP PFC/10^6 cells |
|----------|------|-------------------------|
|          |      | a    | b    |
| 1        | N.D. | 2000 | 740  |
| 2        | 907  | 3173 | 1893 |
| 3        | 1040 | 6067 | 2973 |

* The two tuberculin lots had previously been tested in immune situations (skin tests in BCG-vaccinated guinea pigs and DNA synthesis in lymphocytes from tuberculin-positive humans). In these situations lot a was found to give rise to much stronger responses than lot b (for reference, see Materials and Methods). The concentration used was 100 μg/ml.

Fig. 6. Mice were injected with 5 mg of PPD i.v. and the numbers of PFC against SRBC and NNP-SRBC determined 24-96 h after injection (pooled spleens from two to three animals for each point).

within 20 h of culture and peaks on day 2 or 3 depending on the PPD concentration used (Fig. 3). The variation in the time for peak responses with different concentrations may indicate either that lower numbers of trigger molecules may cause slower induction of protein synthesis or that lower concentrations may require a longer time to reach effective concentrations at the cellular receptors. The bifunctional effects on DNA and Ig synthesis parallel those reported for LPS in mouse B cells (2, 7) and may indicate either two stimulatory components, acting via two separately activated cell receptors, one inducing division
and one inducing protein synthesis, or an intimate link between these processes, requiring only one receptor/signal component. The results of mitomycin treatment may indicate that little DNA synthesis is required for optimal induction of immunoglobulin synthesis, arguing against an absolute link between these processes. This also agrees with the report that only a single mitotic cycle is required for immune induction of Ig synthesis (17). However, the present data should be interpreted with some care, first because of the pronounced cell losses, second because the reduction of thymidine uptake was not 100%. If the findings of unchanged numbers of PFC/10⁶ recovered cells are put together with these two facts, two interpretations are possible. Either the PFC had not gone through DNA synthesis, i.e. the thymidine uptake had occurred in other cells, or the PFC represented a fraction of cells which in some way had been able to escape the blocking effect of mitomycin treatment on DNA synthesis, and thus had gone through one or more cycles of DNA synthesis during differentiation to Ig-producing cells. The present results do not allow a decision between these alternatives, but experiments are in progress to analyze this problem.

PPD is a complex mixture of substances, involving a number of proteins, polysaccharides, and nucleic acids (see e.g. reference 18). Which component of this mixture that is associated with the effect on B cells remains to be clarified. It seems probable that this component may be highly active since 1 µg/ml of the whole mixture gave close to maximal degrees of stimulation (Fig. 3). In this context it is of interest to note that the PPD-induced PFC responses occur over a wide range of concentrations (1–1000 µg/ml) and that no decrease of the response was encountered with the highest concentration tested. A broad range of effective concentrations is also obtained with the other B cell mitogen, LPS (2), whereas T cell mitogens exert their effect over a much more narrow concentration range, higher and lower concentrations than the optimal resulting in sharply decreased responses (2).

The finding that B cell activation occurred after injection of PPD in vivo indicates that the mitogenic effect could have relevance for events in tuberculous infection. Further support for this hypothesis emerges from findings of enhanced antibody production to a variety of antigens in natural and experimental tuberculous infection (19, 20). The mechanism of action of adjuvants of various types, including mycobacteria, have been claimed to involve primarily macrophages (21, 22) or T cells (23). The present results indicate that direct stimulation of B cells is another possibility to take into consideration.

PPD has been widely used as a model substance for studies of various lymphocyte activities associated with delayed hypersensitivity, which is commonly regarded to be a T cell-dependent phenomenon (for review, see reference 24). Various soluble substances, such as migration-inhibiting factor (25, 26) and mitogenic factors of various kinds (27, 28), have been found in supernatants from PPD-stimulated lymphocyte cultures. These effects are commonly regarded as being due to PPD acting as antigen on specifically PPD-reactive T
cells. It appears from the results presented here that a clear distinction between these “immune” effects and the B cell mitogenic effect is critical in the interpretation of the action of the various factors.

In conclusion we thank that PPD, which is a readily available, standardized material, will provide a new tool for activating B cells, possibly without division. This will permit analyses of the induction processes in B cells and probably also analysis of the Ig specificities that are present in “virgin” immunocompetent B cells.

SUMMARY

Purified protein derivative (PPD) tuberculin induced immunoglobulin production in cultures of nonimmune mouse spleen cells, as measured by plaque-forming cells (PFC) against sheep erythrocytes (SRBC), horse erythrocytes, and 4-hydroxy-3,5-dinitrophenacetyl-SRBC. The increase started between 15 and 20 h of culture and reached a peak at 48–72 h. Higher PPD concentrations resulted in earlier peak responses than low concentrations. The Ig produced was mainly 19S and of very low avidity. The response elicited by PPD was of the same type as that caused by lipopolysaccharide of bacterial origin. Mitomycin treatment of cells before culture did not change the numbers of PFC/10^6 recovered cells but the cell recovery was considerably lower.

Also injection of PPD in vivo resulted in increased numbers of PFC.

On the basis of these results it is suggested that PPD nonspecifically activates a majority of the B cell population to proliferation and immunoglobulin synthesis.

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REFERENCES

1. Andersson, J., O. Sjöberg, and G. Möller. 1972. Mitogens as probes for immunocyte activation and cellular cooperation. Transplant. Rev. 11:131.

2. Andersson, J., G. Möller, and O. Sjöberg. 1972. Selective induction of DNA synthesis in T and B lymphocytes. Cell. Immunol. 4:381.

3. Janossy, G., and M. F. Greaves. 1971. Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. Clin. Exp. Immunol. 9:483.

4. Janossy, G., and M. F. Greaves. 1972. Lymphocyte activation II. Discriminating stimulation of lymphocyte subpopulations by phytomitogens and heterologous antisera. Clin. Exp. Immunol. 10:525.

5. Blomgren, H., and E. Svedmyr. 1971. Evidence for thymic dependence of PHA-reactive cells in spleen and lymph nodes and independence in bone marrow. J. Immunol. 106:835.

6. Stobo, J. D., A. S. Rosenthal, and W. E. Paul. 1972. Functional heterogeneity of murine lymphoid cells. I. Responsiveness to and surface binding of concanavalin A and phytohaemagglutinin. J. Immunol. 108:1.
7. Andersson, J., O. Sjöberg, and G. Møller. 1972. Induction of immunoglobulin and antibody synthesis in vitro by lipopolysaccharides Eur. J. Immunol. 2:349.
8. Sultzer, B. M., and B. S. Nilsson. 1972. PPD tuberculin—a B cell mitogen. Nature (Lond.). In press.
9. Bullock, W. W., and E. Møller. 1972. Spontaneous B-cell activation due to loss of normal mouse serum suppressor. Eur. J. Immunol. In press.
10. Pasanen, V. J., and O. Mäkelä. 1969. Effect of the number of haptens coupled to each erythrocyte on haemolytic plaque formation. Immunology. 16:399.
11. Westphal, O., O. Lüderitz, and F. Bister. 1952. Über die Extraktion von Bakterien mit Phenol-Wasser. Z. Naturforsch. Teil. B. 7:148.
12. Westphal, O., and K. Jaun. 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. 5:33.
13. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126:423.
14. Jerne, N. K., A. A. Nordin, and C. Henry. 1963. The agar plaque technique for recognizing antibody producing cells. In Cell Bound Antibodies. B. Amos and H. Koprowski, editors. Wistar Institute Press, Philadelphia, Pa.
15. Ingraham, J. S., and A. Bussard. 1964. Application of a localized hemolysin reaction for specific detection of individual antibody-forming cells. J. Exp. Med. 119:667.
16. Andersson, B. 1970. Studies on the regulation of avidity at the level of the single antibody-forming cell. J. Exp. Med. 132:77.
17. Segal, S., A. Globerson, and M. Feldman. 1969. The minimal cycles of cell replication which determine antibody production. Isr. J. Med. Sci. 5:444.
18. Seibert, F. B. 1949. The isolation of three different proteins and two polysaccharides from tuberculin by alcohol fractionation. Their chemical and biological properties. Am. Rev. Tuberc. 59:86.
19. Raffel, S. 1956. Immunopathology of tuberculosis. Am. Rev. Tuberc. Pulm. Dis. 74(Suppl.):60.
20. Bergqvist, S., and T. Packalén. 1958. The antibody response of the tuberculous guinea-pig to non-tuberculous antigens. Acta Pathol. Microbiol. Scand. 43:73.
21. Spitznagel, J. K., and A. C. Allison. 1970. Mode of action of adjuvants: effects on antibody responses to macrophage-associated bovine serum albumin. J. Immunol. 104:128.
22. Unnue, E. R., B. A. Askonas, and A. C. Allison. 1969. A role of macrophages in the stimulation of immune responses by adjuvants. J. Immunol. 103:71.
23. Allison, A. C., and A. J. S. Davies. 1971. Requirement of thymus-dependent lymphocytes for potentiation by adjuvants of antibody formation. Nature (Lond.). 233:330.
24. Peterson, R. D. A., M. D. Cooper, and R. A. Good. 1965. The pathogenesis of immunologic deficiency diseases. Am. J. Med. 38:579.
25. David, J. R., S. Al-Askari, H. S. Lawrence, and L. Thomas. 1964. Delayed hypersensitivity in vitro. I. The specificity of the inhibition of cell migration by antigens. J. Immunol. 93:264.
26. Thor, D. E., R. E. Jureziz, S. R. Veach, E. Miller, and S. Dray. 1968. Cell migration inhibition factor released by antigen from human peripheral lymphocytes. *Nature (Lond.)*. 219:755.

27. Valentine, F. T., and H. S. Lawrence. 1969. Lymphocyte stimulation: transfer of cellular hypersensitivity to antigen in vitro. *Science (Wash. D. C.)*. 165:1014.

28. Janis, M., and F. H. Bach. 1970. Potentiation of in vitro lymphocyte reactivity. *Nature (Lond.)*. 225:238.