gen treated recipients was not a non-specific effect is indicated by the fact that injection of the anitgenic material back into the spleen donors did not induce accelerated rejection of kidney homotransplants from indifferent animals.

To our knowledge the isolation of a subcellular, transplantation antigenic preparation from the dog has not previously been reported. The fact that cultures of both rabbit (1,2) and dog spleen have yielded media with similar antigenic activities suggests that the release of transplantation antigenic material into the surrounding fluid may be a general result of the cultivation of mammalian spleen cells *in vitro*.

It now appears likely that the intravenous route is the most effective for induction of tolerance(3,7). Since the Millipore filtered dog antigenic medium is apparently entirely non-toxic upon intravenous infusion it may be potentially useful in future experimental attempts to induce adult immunological tolerance to organ and tissue grafts when used in combination with short courses of immunosuppressive drug therapy, or sublethal total body radiation.

*Summary.* 1. Millipore filtered tissue culture medium in which dog spleen cells have been cultivated *in vitro* was found to contain subcellular material with transplantation antigenic activity which could be concentrated by ultracentrifugation. Antigenic activity was demonstrated by the accelerated rejection by antigen recipients of a kidney homotransplant from the original spleen donor. 2. The antigenic material did not induce accelerated rejection of kidney homotransplants when injected back into the original spleen donor. 3. The Millipore filtered culture medium containing antigenic activity was apparently nontoxic when infused intravenously into homologous recipients.

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## Influence of Mercaptoethanol Treatment on Skin Sensitizing and Complement Binding Ability of 7 S Anti-Dinitrophenol-Bovine Gamma Globulin Antibody.* (29275)

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Rabbit 7 S immune globulin can be split by papain digestion(19) into two fragments containing the specific antigen combining activity, and a third fragment, which has no antigen combining sites, but which is essential for complement fixation(22) and skin sensitization(17). This third fragment is the one involved in precipitation of aggregated 7 S gamma globulin by rheumatoid factor(4). It was previously shown that mercaptoethanol (ME) treatment of 7 S immune globulin affects its complement binding capacity without diminishing its reactivity with rheumatoid factor(23).

In the present study the influence of ME on the skin sensitizing and the complement fixing ability of 7 S antibody and the role of complement in passive cutaneous anaphylactic

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reactions in guinea pigs and mice are examined.

*Materials and methods.* Antigens: dinitrophenol-bovine serum albumin (DNP-BSA) was used for quantitative complement fixation, immuno electrophoresis and passive cutaneous anaphylaxis. DNP was bound to BSA in a molar ratio of 30 to 1(15). For reverse PCA dinitrophenol-rabbit gamma globulin (DNP-RGG) was used as antigen (9 DNP groups per molecule of antigen, (14)).

Immune serum: Rabbit anti dinitrophenol-bovine gamma globulin antibody (anti-DNP-BGG) was used throughout this study. It contained 2 mg antibody protein/ml as measured by the quantitative microprecipitation technique of Heidelberger and Kendall(6). The antibody was shown to be a 7 S globulin by immunoelectrophoretic analysis: DNP-BSA in one trough produced a single arc corresponding to the gamma 2 arc obtained in the other trough with a mouse immune serum against rabbit whole serum. A 7 S fraction of this antiserum was obtained by DEAE-cellulose chromatography(3).

2-Mercaptoethanol (ME) diluted in buffered saline (pH 7) was added to the serum, which was diluted 1:5 in buffered saline (pH 7), to a final concentration of 0.1 M. After 2 hours incubation at room temperature the samples were dialyzed against iodacetamide (0.02 M in buffered saline) and subsequently against buffered saline.

Quantitative complement fixation was performed according to the method of Mayer *et al*(8,9,10) using 100-130 C′ 50 units/10 ml in the equivalence zone.

Decomplementation of mice was done according to Frick *et al*(5). Human gamma globulin (Cohn's fraction II) in a solution of 25 mg per ml was heat aggregated at 62°C for 20 min 0.1 ml per 20 g body weight was injected intraperitoneally and, in some animals, intravenously. The animals were used 4 hours after injection of human gamma-globulin.

Passive cutaneous anaphylaxis (PCA) was performed in untreated and decomplemented Albino Swiss Webster mice weighing 25-35 g (13). 0.05 ml of untreated and of ME treated antiserum or 0.05 ml of untreated and of ME treated 7 S fractions were injected intradermally. Various antibody dilutions were used so that one intradermal dose contained 5-44 $\mu$g of antibody protein. After a latent period of one hour 0.25 ml of a mixture containing equal amounts of Evans blue dye and DNP-BSA (420 $\mu$g/mouse) were injected intravenously. After 35 min the animals were killed, skinned and the diameter of the spots on the inside of the skin was measured in mm and listed. PCA in the guinea pigs was done in the usual way(12): 3 hours after intradermal injection of 0.1 ml of untreated and ME treated antiserum (used in dilutions of 1:100 to 1:8000), 250 $\mu$g DNP-BSA were given i.v. together with 0.5 ml Evans blue.

Reverse PCA was performed in guinea pigs using DNP-RGG as antigen for intradermal injection(14). The amount of antigen administered varied from 0.05, 0.1, 0.5, 1.0 to 10.0 $\mu$g/0.1 ml. After a latent period of 3-7 hours 0.5 ml of native or ME treated antibody was injected intravenously.

*Results.* a) *Effect of ME treatment of anti DNP-BGG serum on PCA in untreated and decomplemented mice and untreated guinea pigs.* Table I summarizes the results. In a preliminary study the smallest amount of antibody producing regularly PCA reactions in mice was found to be 20 $\mu$g/0.05 ml. This amount was used throughout the experiment. Results obtained in normal and decomplemented mice were similar, no matter whether decomplementation was performed by i.v. or i.p. administration of aggregated human gamma globulin. With ME treated immune serum no PCA reaction was observed in untreated mice. In decomplemented mice 2 animals reacted very weakly. As seen in Table I dialysis of the serum against iodoacetamide did not affect the reaction significantly.

In a second series of experiments higher concentrations of antibody (44 $\mu$g/0.05 ml) were used. With this concentration weak reactions were obtained with the ME treated fraction (Table II).

The influence of the amount of antibody on PCA reaction in mice using ME treated and native antibody in normal and decomplemented mice is shown in Fig. 1. In guinea pigs 4 times more antibody was necessary to elicit a PCA when ME treated serum was

TABLE I. Influence of ME-treatment of Whole Serum (Anti DNP-BGG) on PCA in Intact and Decomplemented Mice.

| | | PCA reactions (mm diameter) elicited with 20 μg of antibody treated with: | | |
|---|---|---|---|---|
| | | ME | Iodo | Saline |
| Intact mice No. | 1 | 0 | 8 | 10 |
| | 2 | 0 | 9 | 12 |
| | 3 | 0 | tr. | 3 |
| | 4 | 0 | 8 | 10 |
| | 5 | 0 | 8 | 12 |
| | 6 | 0 | 8 | 10 |
| Decomplemented mice No. | 7 | 0 | 6 (pale) | 9 |
| | 8 | 0 | 10 | 12 |
| | 9 | 0 | 9 | 10 |
| | 10 | 0 | 6 (pale) | 8 |
| | 11 | 8 (pale) | 10 | 10 |
| | 12 | 0 | 12 | 12 |
| | 13 | 0 | 10 | 10 |
| | 14 | 0 | 8 | 10 |
| | 15 | 3 (pale) | 15 | 15 |
| | 16 | 0 | 10 | 10 |

ME: sample treated with ME, dialyzed against iodoacetamide in buffered saline and consequently against buffered saline.
Iodo: sample dialyzed against iodoacetamide in buffered saline and consequently against buffered saline.
Saline: sample dialyzed against buffered saline.

used (Table III). b) *Effect of ME treatment of anti DNP-BGG antibody on quantitative complement-fixation.* Under the experimental conditions used, complement fixation was practically abolished by ME treatment of whole serum and also of the gamma globulin fraction obtained by chromatography (Table IV). c) *Effect of ME treatment of anti DNP-BGG serum on reverse PCA in guinea pigs.* This experiment was done to determine whether diminished PCA reaction with ME

TABLE II. Influence of ME-treatment of a 7 S Fraction (Anti DNP-BGG) on PCA in Mice.

| | | PCA reactions (mm diameter) elicited with 44 μg of antibody treated with: | |
|---|---|---|---|
| | | Iodo | ME |
| Intact mice No. | 1 | 17 | 10 (pale) |
| | 2 | 15 | 10 " |
| | 3 | 18 | 10 " |
| Decomplemented mice No. | 4 | 15 | 13 |
| | 5 | 12 | 10 " |
| | 6 | 15 | 13 " |
| | 7 | 15 | n.d. |

Iodo: sample treated with iodoacetamide as indicated in Table I.
ME: sample treated with ME as indicated in Table I.
n.d.: not done.

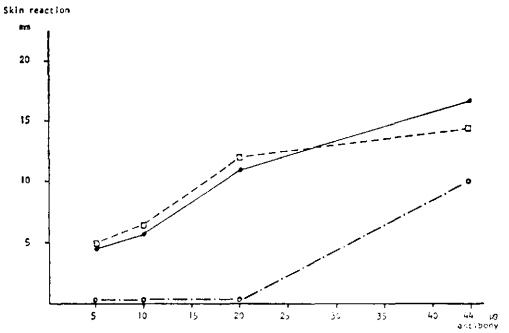


FIG. 1. PCA in untreated and decomplemented mice with native serum and in untreated mice with ME treated serum using various amounts of antibody. ●———● normal mice, serum inactivated □ - - - □ decomplemented mice, serum inactivated; ○—•—○ normal mice, serum inactivated and treated with ME; abscissa: μg antibody; ordinate: diameter of halo in mm; all points represent average values obtained from at least 5 animals.

treated serum was due to an altered immune reaction or to a diminution of skin sensitizing capacity of antibody. ME treatment did not influence reverse PCA (Table V).

*Discussion.* ME treatment resulted in complete suppression of PCA in mice, when threshold amounts of antibody were used. With higher doses it became apparent that the suppression was not complete: Three to four times more antibody was necessary to obtain a reaction comparable to that produced by native antibody (Fig. 1). Similar results were obtained in guinea pigs. In this species also 4 times more of ME treated than of na-

TABLE III. Influence of ME-treatment of Whole Serum (Anti DNP-BGG) on PCA in Guinea Pigs.

| | Iodo | | | | ME | | | |
|---|---|---|---|---|---|---|---|---|
| Animals No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Serum dilutions | | | | | | | | |
| 1:100 | 30 | 25 | 17 | 25 | 15 | 17 | 15 | 20 |
| 1:500 | 15 | 10 | 15 | 15 | 10 | 0 | 0 | 17 |
| 1:1000 | 15 | 10 | 10 | 10 | 0 | 0 | 0 | 0 |
| 1:2000 | 10 | 0 | 10 | 10 | 0 | 0 | 0 | 0 |
| 1:4000 | 8 | 0 | tr. | 8 | 0 | 0 | 0 | 0 |
| 1:8000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Iodo: treated with idoacetamide as indicated.
ME: treated with ME as indicated.

TABLE IV. Influence of ME-treatment of Whole Serum and 7 S Fraction (Anti DNP-BGG) on PCA in Mice and on Complement Fixation.

| | Amt of antibody tested | C'50 fixed | PCA |
|---|---|---|---|
| Whole serum untreated | 20 μg | 52 | +++ |
| Whole treated with ME | 20 " | 0 | (±) |
| 7 S fraction untreated | 44 " | 113 | ++++ |
| 7 S treated with ME | 44 " | (4) | ++ |

tive antibody was necessary to trigger PCA reaction (Table III).

Two different mechanisms of ME action may be responsible for this diminution of PCA: 1. The biological consequences of the antigen-antibody reaction may be altered. 2. The skin sensitizing properties of the antibody may be impaired. To answer this question reverse PCA was used with DNP-RGG as antigen. Rabbit gamma globulin (RGG) is known to sensitize well the skin of guinea pigs, so that subsequent injection of antibody can challenge the reaction. If the biological consequences of antigen antibody reaction were impaired by ME treatment of the antibody, a diminished reverse PCA should result. Since no difference was found in the severity of reverse PCA between ME treated and native antibody using different amounts of antigen for intradermal injection (Table V), it may be concluded that the diminution is due to an impaired tissue fixation of ME treated antibody.

TABLE V. Influence of ME-treatment of Whole Serum (Anti DNP-BGG) on Reverse PCA in Guinea Pigs.

| | Iodoacetamide treated sample | | | ME treated sample | | |
|---|---|---|---|---|---|---|
| Animal No. | 1 | 2 | 3 | 4 | 5 | 6 |
| Intradermal dose of DNP-RGG | | | | | | |
| 10* | n.d. | 15 | 22 | 13 | 15 | 25 |
| 1 | 20 | 20 | 25 | 15 | 15 | 30 |
| .5 | 22 (pale) | 15 | 20 | 6 | 13 | 20 |
| .1 | 0 | 0 | 10 | 0 | 0 | 20 |
| .05 | 0 | 0 | 0 | 0 | 0 | 10 |
| .02 | 0 | 0 | 0 | 0 | 0 | 0 |

* Latent period = 3 hr; latent period for other doses of antigen = 7 hr.
n.d. = not done.

Similar results were obtained by Ovary and Taranta(18) with pepsin treated rabbit antibody against human gamma globulin. With pepsin treated antibody PCA reaction was not possible, while reverse PCA was readily obtained with the treated antibody. ME treatment of the anti DNP-BGG antibody resulted in practically complete loss of complement fixing capacity. It should be mentioned that ME does not abolish the complement fixing capacity of all antibodies to the same extent. Thus the loss was only 75% in the case of a rabbit anti rat erythrocyte serum (24). Schur and Becker(20) observed a reduction in complement fixation of less than 20% after treating a rabbit anti human albumin serum with cystein. Ishizaka *et al*(7) found no loss at all with a rabbit anti human gamma globulin serum.

The fact that ME treatment of the antibody used in this study resulted in practically complete loss of its complement fixing capacity is important in evaluation of the role of complement in PCA reaction. Since reverse PCA was not affected in guinea pigs, complement does not seem to play a decisive role in this species. Also in mice it does not seem to be of importance, since PCA reaction occurred in decomplemented animals with the same intensity as in untreated ones. It should be noticed that decomplementation of mice has been shown to impair considerably complement dependent phagocytosis of the RES.

Our findings are in line with results obtained with guinea pig antibodies(1,2,16). Guinea pigs may produce 2 types of 7 S globulin antibodies but only the electrophoretically fast moving gamma globulin (gamma 1 antibody) sensitizes the skin for PCA. However the latter antibody does not fix complement. The electrophoretically slower moving antibody (gamma 2) does not sensitize the guinea pig, but fixes complement. Species differences must be important, because complement seems to enhance PCA in rats, as shown by Osler *et al*(11).

*Summary.* Treatment of a 7 S anti DNP-BGG antibody with 2-ME resulted in a

marked diminution of its ability to trigger positive PCA reactions in mice and guinea pigs; 3 to 4 times more ME treated antibody was necessary to obtain a reaction comparable to that produced by native antibody. In the reverse PCA technique, ME treatment was without influence on the skin reaction. Therefore, the diminution of the direct PCA reaction is not the result of an impaired antigen antibody reaction, but rather the consequence of a diminished tissue fixation of ME treated antibody. Complement fixing capacity of the antibody was practically abolished by ME treatment. From this fact and from results of PCA reactions in decomplemented animals, it follows that PCA reactions in mice and guinea pigs require little, if any complement.

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## Effect of Ethionine-Induced Pancreatic Damage on Intestinal Enzyme Activity of the Rat. (29276)

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Dl-ethionine, the ethyl analog of methionine, produces pancreatic exocrine damage when added to the diet of experimental animals. In the dog, this effect has been demonstrated histologically and by assay of pancreatic enzymes obtained by cannulation of the pancreatic duct(1-3). In the rat however, the effect of dl-ethionine has been demonstrated only histologically(4-7). The purpose of the present study was to evaluate alterations of intestinal pancreatic enzyme activity as an indicator of pancreatic exocrine damage.

*Material and methods.* Twelve male Wistar strain rats, weighing 200-225 g each, were separated into 2 equal groups. Control animals were fed a basic diet as described by Kinney *et al*(7). Test animals received dl-ethionine in a concentration of 0.5 g/100 g of basic diet. Diets and water were administered *ad lib* for a period of 25 days. At the end of this period and without prior fasting,