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Biochemistry

Hapten mimic elicits antibodies recognizing prostaglandin E_2

(isosteric hapten/immunochemical mimic/radioimmunoassay)

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ABSTRACT **Antibodies that recognized the prostaglandin (PG) E structure were elicited from rabbits. 9-Deoxy-9-methylene-$PGF_{2\alpha}$, a stable isosteric mimic of PGE_2, was conjugated to two different protein immunogens and the immune response system was duped into producing antibodies with poor recognition for prostaglandins other than the hapten mimic (9-deoxy-9-methylene-$PGF_{2\alpha}$) and its isosteric counterparts (PGE_1 and PGE_2). With this procedure, crossreaction that would ordinarily arise from the chemical or metabolic instability of an authentic PGE_2 immunogen was avoided. Antibodies raised against a keyhole limpet hemocyanin conjugate of 9-deoxy-9-methylene-$PGF_{2\alpha}$ had an average intrinsic association constant, $K_0 = 2.6 \times 10^9$ liters·mole^{-1}, for PGE_2. Crossreaction was low for a number of related prostaglandins, and a sensitive radioimmunoassay procedure with a detection limit of 6 pg was developed.**

The production of antibodies of sufficient specificity to allow direct radioimmunoassay of E series prostaglandins (PG) in biological samples is difficult. The chemical and metabolic instability of the hapten has been implicated as the source of this difficulty (1–10). Dehydration at the 11-hydroxyl position with resultant formation of A and B series prostaglandins can occur under chemical conditions prevailing during the coupling of hapten to immunogen. This often results in antibody populations recognizing all three haptens to an extent that negates specific PGE determinations. Plasma isomerase enzymes capable of altering the PGE hapten after its attachment to the immunogen have been suggested as an additional source of PGA and PGB crossreacting antibodies (6). These features, which have discredited the value of the analytical results obtained by radioimmunoassay of PGE_2, were avoided by at least three investigators (11–13), but the reasons for their success are vague. A logical, chemical way of minimizing the problems inherent in the production of antibodies for the radioimmunoassay of E series prostaglandins seemed desirable. We have tested an approach in which a stable, isosteric hapten mimic is substituted for the purpose of eliciting antibodies that bind the compound to be assayed, PGE_2. Specific, high-affinity antibodies are produced which are then useful for the radioimmunoassay of PGE_2, the isosteric counterpart of the hapten, 9-deoxy-9-methylene-$PGF_{2\alpha}$.

EXPERIMENTAL

Materials. Keyhole limpet hemocyanin and bovine serum albumin were purchased from Calbiochem (La Jolla, CA). Water-soluble carbodiimide, 1-ethyl-3(-3-dimethyl)aminopropylcarbodiimide·HCl, was purchased from Bio-Rad Laboratories (Richmond, CA). [^{3}H]PGE_2, labeled at carbons 5, 6, 8, 11, 12, 14, and 15, specific activity 117 Ci/mmol, was obtained from New England Nuclear (Boston, MA). All unlabeled prostaglandins were supplied by the chemical research laboratories of The Upjohn Company. Norit A charcoal was supplied by Sigma (St. Louis, MO) and Dextran T70 by Pharmacia (Upsalla, Sweden). Other chemicals and materials were the best grade available. Aqueous counting scintillant was purchased from Amersham/Searle (Chicago, IL).

Buffer. Phosphate buffer (0.1 M), pH 7.4, with 0.9% saline and 0.1% gelatin, was used for all incubations.

Preparation of Conjugates. Prostaglandin hapten was attached to two different protein immunogens with water-soluble carbodiimide. 9-Deoxy-9-methylene-$PGF_{2\alpha}$ (10 mg), dissolved in 1.0 ml of dimethyl formamide, bovine serum albumin (20 mg), and 1-ethyl-3(-3-dimethyl)aminopropylcarbodiimide·HCl (10 mg) were mixed in 6.0 ml of distilled water. The pH was maintained between 5.4 and 5.6 for 1 hr at 25°; then the mixture was allowed to stand overnight at 25°. The reaction between 9-deoxy-9-methylene-$PGF_{2\alpha}$ and keyhole limpet hemocyanin was at pH 7.0, and an additional 10 mg of water-soluble carbodiimide was added after the first hour of reaction. Both conjugates were dialyzed against 4 liters of distilled water for 24 hr; then they were lyophilized and stored dessicated at −20°. Previous studies had shown that 9-deoxy-9-methylene-$PGF_{2\alpha}$, the hapten mimic of PGE_2, was completely resistant to decomposition or dehydration under the conditions used to prepare the conjugates.

The epitope density was estimated by incorporating a trace (<100 pg) of [^{3}H]$PGF_{2\alpha}$ into the reaction mixture to monitor the extent of coupling between prostaglandin and protein. After reaction, a portion of the lyophilized conjugate was oxidized in a combustion chamber and the radioactivity was determined by scintillation counting. Nineteen molecules of 9-deoxy-9-methylene-$PFG_{2\alpha}$ were attached to each molecule of bovine serum albumin, assuming a molecular weight of 68,500 for the protein (14). Sixty-eight molecules of 9-deoxy-9-methylene-$PGF_{2\alpha}$ were attached to each molecule of keyhole limpet hemocyanin, assuming a molecular weight of 600,000 for the soluble protein subunit of dissociated hemocyanin at pH 7.0 (15).

Immunization. Albino New Zealand rabbits were inoculated intradermally at 30–40 sites on their flanks with 1 mg of conjugate emulsified in 1.0 ml of 0.9% sterile saline and 1.0 ml of Freund's complete adjuvant. On the day of their first inoculation, rabbits were also injected intramuscularly in their hindquarters with 1.0 ml of attenuated *Bordetella pertussis* suspension, according to Dray *et al.* (11). About 50 days after the primary inoculation, rabbits were boosted subcutaneously with 500 μg of conjugate emulsified in 1.0 ml of 0.9% saline and 1.0 ml of Freund's incomplete adjuvant. Animals were bled from the central ear artery periodically, after the booster injections, and the antiserum titer was determined by measuring the binding of [^{3}H]PGE_2. Animals were boosted when the titer showed a decline.

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Abbreviation: PG, prostaglandin.

Antibody Binding Parameters. The immunological response was determined by measuring the binding of [^{3}H]PGE_2 at a constant antiserum dilution (1:750) on all bleedings. [^{3}H]PGE_2 in phosphate/saline/gelatin (25,000 dpm/0.2 ml, 40% counting efficiency) and 0.1 ml of antiserum diluted 1:250 in phosphate/saline/gelatin were incubated for 1 hr at 25°, then for 16–24 hr at 4°. The antibody-bound and the free fractions were separated with dextran-coated charcoal (13). A binding check was made on all bleedings.

The average intrinsic association constant, K_0, and the concentration of antibody-combining sites, A_0, were determined by equilibrium dialysis according to Pinckard and Wier (16). First, the immunoglobulin component of sera from normal and inoculated rabbits was isolated by ammonium sulfate precipitation. Serum (4 ml) was incubated with saturated ammonium sulfate (2 ml) for 4 hr at 4°. After centrifugation, the supernatant containing serum albumin was discarded and the immunoglobulin pellet was dissolved in 4 ml of pH 7.4 phosphate buffer with 0.9% sodium chloride and 0.01% sodium azide. The immunoglobulin solution was dialyzed against phosphate buffer for 24 hr (4 liters, three changes of buffer). The final immunoglobulin solution from normal and inoculated rabbits was diluted 1:250.

Dialysis tubing (¼ × 5 inches) was washed continuously in distilled water for 36 hr prior to use. A buffer control solution (1.0 ml), a normal immunoglobulin control solution (1.0 ml), and an antibody solution (1.0 ml) were dialyzed against buffer solutions of [^{3}H]PGE_2. Hapten ([^{3}H]PGE_2) concentrations ranged from 1 to 2100 pg/ml. Triplicate determinations were made for each concentration. Dialysis was carried out by placing bags containing 1.0 ml of the appropriate solution (buffer control solution, normal immunoglobulin control solution, or antibody solution) in a 12 × 75 mm polystyrene tube with 3.0 ml of hapten solution. The tubes were rotated at 6 rpm for 48 hr at 4°. At equilibrium, samples were withdrawn volumetrically from each side of the dialysis bag while the temperature was maintained at 4°. The concentrations of bound and free hapten were determined by scintillation counting. The data were plotted according to Scatchard (17) using polynomial regression analysis to predict the x-intercept and the best curve through the experimental points. Calculations were performed according to Pinckard and Weir (16).

A secondary empirical binding parameter for each antiserum was determined by the method of Kahn *et al.* (18).

Radioimmunoassay Procedure. Samples (0.1 ml) or PGE_2 standards (1–500 pg/0.1 ml) and 0.1 ml of [^{3}H]PGE_2 (25,000 dpm/0.1 ml, 40% counting efficiency, specific activity 117 Ci/mmol) were added to 12 × 75 mm polypropylene tubes. Antiserum (0.1 ml) against the PGE_2 mimic, 9-deoxy-9-methylene-$PGF_{2\alpha}$, was added at a dilution (1:1000) that bound 50% of the [^{3}H]PGE_2 in the absence of competitive binding from unlabeled PGE_2. Tubes were incubated for 1 hr at 25°, then for 16–24 hr at 4°. Antibody-bound and free fractions were separated with dextran-coated charcoal (13). The amount of antibody-bound [^{3}H]PGE_2 in the supernatant was determined by scintillation counting. Computations were performed with the Rodbard (19) radioimmunoassay program with a Hewlett-Packard Model 9830A desk top computer.

RESULTS

Rabbits inoculated against either a keyhole limpet hemocyanin conjugate or a bovine serum albumin conjugate of 9-deoxy-9-methylene-$PGF_{2\alpha}$ developed antibodies that bound PGE_2, the isosteric counterpart of the hapten. The average intrinsic association constant (K_0) and the concentration of antibody binding sites (A_0) were calculated from the Scatchard plots obtained by equilibrium dialysis. Experimental points are shown in Fig. 1, with the lines predicted by second degree polynomial regression analysis. The curves were corrected for nonspecific binding (bound/free = 0.05–0.08) to the immunoglobulin component of normal rabbit serum. The immunoglobulin component of a rabbit inoculated with a keyhole limpet hemocyanin conjugate of 9-deoxy-9-methylene-$PFG_{2\alpha}$ contained an antibody-combining site concentration, A_0 = 1.5 nM for PGE_2. The average intrinsic association constant for PGE_2 determined at 50% saturation of the antibody-combining sites (bound = 0.75 nM) was $K_0 = 2.4 \times 10^9$ liters·mole^{-1}. The antibodies against the bovine serum albumin conjugate had an average intrisic association constant for PGE_2 of $K_0 = 3.8 \times 10^8$ liters·mole^{-1} with a combining site concentration, A_0 = 4.2 nM.

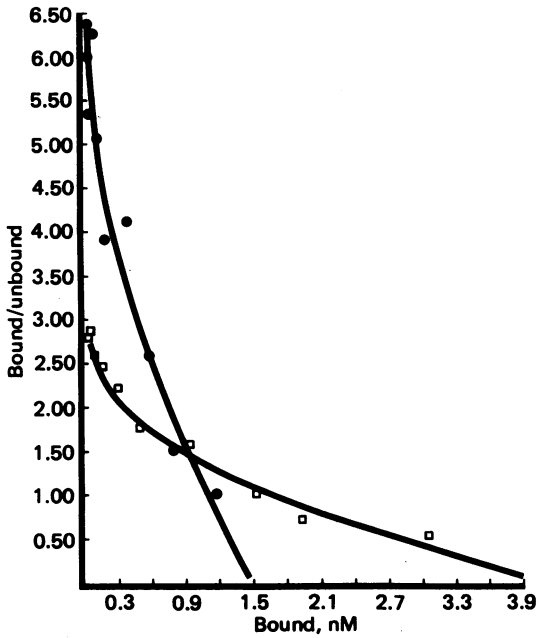

FIG. 1. Scatchard plots for the determination of K_0 and A_0 of 9-deoxy-9-methylene-$PGF_{2\alpha}$ antibodies that bound PGE_2. Data were obtained by equilibrium dialysis at 4° according to Pinckard and Weir (16). The experimental points are shown with the line predicted by second degree polynomial regression analysis. ●, Affinity of antibodies against a keyhole limpet hemocyanin conjugate toward PGE_2; □, affinity of antibodies against a bovine serum albumin conjugate toward PGE_2.

Under radioimmunoassay conditions, the maximum binding between PGE_2 hapten and antibodies is initially fixed at 50% [bound (B)/unbound (U) = 1]. The linear portion of the radioimmunoassay inhibition curve covers an approximate range, B/U = 0.66–0.10. Kahn *et al.* (18) have described a procedure to assign an empirical binding constant to the antibodies used under radioimmunoassay conditions. Strictly speaking, this association constant may not necessarily equal the average intrinsic association constant since it describes the equilibrium binding of only a limited population of antibodies. Nevertheless, it is this population which is most important during the radioimmunoassay. The binding parameter of Kahn *et al.* (18) was calculated from the Scatchard plots shown in Fig. 2. The plots were corrected for nonspecific binding to normal rabbit serum. The antibodies against the keyhole limpet hemocyanin conjugate had a qualified binding constant according to Kahn: $K = 1.0 \times 10^{10}$ liters·mole^{-1} with A_0 = 0.076 nM for PGE_2. The antibodies against the bovine serum albumin conjugate had a

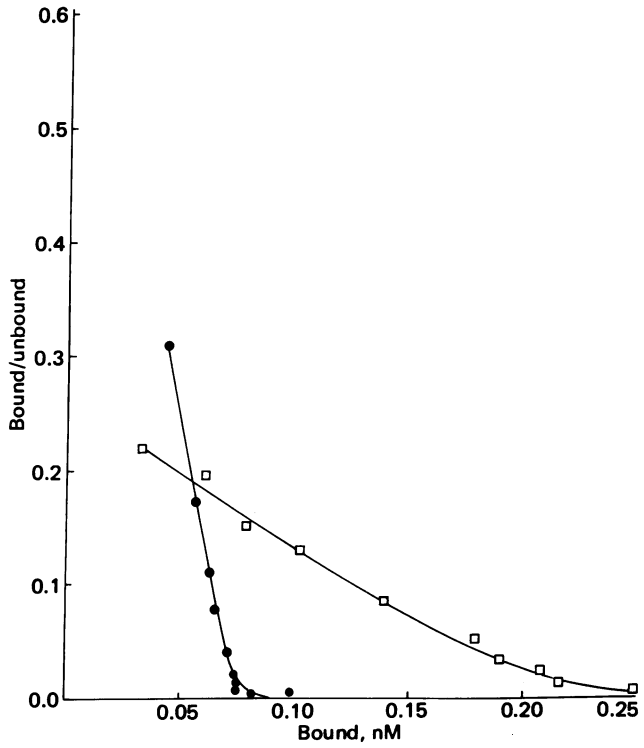

FIG. 2. Scatchard plots for determination of a binding constant according to Kahn *et al.* (18). ●, Affinity of antibodies against a keyhole limpet hemocyanin conjugate toward PGE_2; □, affinity of antibodies against a bovine serum albumin conjugate toward PGE_2.

qualified binding constant according to Kahn: $K = 1.5 \times 10^9$ liters·mole^{-1} with $A_0 = 0.2$ nM.

The association constants were sufficiently high that development of a radioimmunoassay for PGE_2 was plausible. Fig. 3 shows a radioimmunoassay inhibition curve for the high-affinity antiserum. The amount of PGE_2 required to decrease the binding (B/B_0) of [^{3}H]PGE_2 from 100% to 80% was 6 pg. The lower affinity antiserum was correspondingly less sensitive and required 30 pg of PGE_2 to decrease the binding (B/B_0) of [^{3}H]PGE_2 from 100% to 80%. The specificity of the antisera are shown in Table 1. The results confirmed the premise that crossreaction with A and B series prostaglandins, in particular, would be minimized. Crossreaction with several related prostaglandins is also low, especially for the high-affinity antiserum developed against a keyhole limpet hemocyanin conjugate. Only PGE_1, the 5,6-dihydro analog of PGE_2, crossreacts appreciably with the hapten mimic or its isosteric counterpart (PGE_2). The relatively low ratio of homo-γ-linolenic/arachidonic acid in most cell membranes (20, 21) minimizes the possible consequences of PGE_1 interference, since it is derived from homo-γ-linolenic acid.

Our radioimmunoassay has been tested primarily in its application to determine PGE_2 levels during the aggregation of blood platelets with arachidonic acid. It is well established that thromboxane B_2 is the major product derived from arachidonic acid during platelet aggregation (22–24), but for our purposes it was also necessary to measure at least one component of classical prostaglandin biosynthesis simultaneously. Platelet-rich plasma was prepared from fresh, human whole blood collected over citrate [1 part citrate (3.8%)/9 parts blood]. The platelet-rich plasma supernatant was retained after centrifugation of blood at 200 × *g* for 10 min at 25°. In a typical experiment, 2 ml of platelet-rich plasma was added to an aggregometer cuvette containing 600–1000 μg of arachidonic acid. The platelet-rich plasma was stirred at 1100 rpm at 37° and the aggregation profile was followed with a Payton aggregometer. Samples (0.1 ml) were withdrawn at intervals and immediately

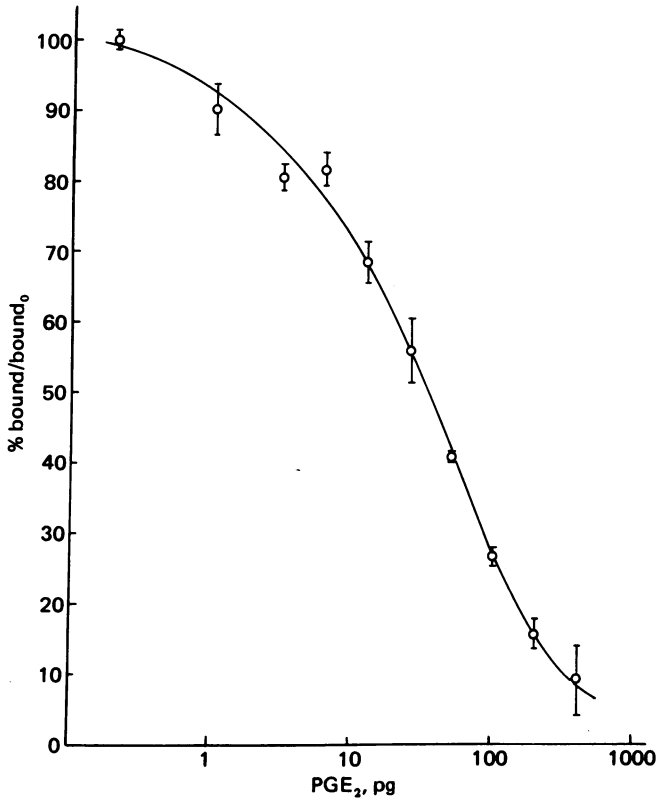

FIG. 3. PGE_2 inhibits the binding of [^{3}H]PGE_2 to antibodies formed against 9-deoxy-9-methylene-$PGF_{2\alpha}$.

Table 1. Crossreaction (%)* of antisera against 9-deoxy-9-methylene-$PGF_{2\alpha}$ relative to PGF_2

Prostaglandins	Keyhole limpet hemocyanin conjugate†	Bovine serum albumin conjugate‡
PGE_1	90	100
9-Deoxy-9-methylene-$PGF_{2\alpha}$	100	100
PGA_1	0.08	4.9
PGA_2	0.75	2.8
PGB_1	0.005	0.1
PGB_2	0.009	0.05
13,14-Dihydro-PGE_2	1.6	3.6
15-Keto-PGE_2	0.03	2.5
13,14-Dihydro-15-keto-PGE_2	0.03	0.2
PGD_1	0.02	4.9
PGD_2	0.02	10.0
$PGF_{1\alpha}$	0.09	2.9
$PGF_{1\beta}$	0.30	5.0
$PGF_{2\alpha}$	0.20	1.5
$PGF_{2\beta}$	2.0	4.4
13,14-Dihydro-$PGF_{2\alpha}$	0.34	0.2
15-Keto-$PGF_{2\alpha}$	0.01	0.2
13,14-Dihydro-15-keto-$PGF_{2\alpha}$	0.10	0.01
6-Keto-$PGF_{1\alpha}$	0.10	0.50

* % crossreaction = (ng PGE_2 required to displace 50% B/B_0 [^{3}H]PGE_2)/(ng of heterologous prostaglandin required to displace 50% B/B_0 [^{3}H]PGE_2) × 100.

† 0.035 ng displaces 50% B/B_0.

‡ 0.200 ng displaces 50% B/B_0.

Table 2. Analytical accuracy of PGE_2 radioimmunoassay

Added, pg	Found, pg (±95% confidence limits)
4.0	—*
8.0	7.4 ± 0.9
16.0	14.0 ± 1.8
32.0	26.2 ± 1.9
64.0	62.0 ± 2.9

Platelet-rich plasma was fortified with PGE_2 so that the samples processed according to the procedure in *Experimental* contained the amounts indicated in column 1. Tubes of each experiment were analyzed in sextuplicate. Five experiments were done at each concentration.

* Not statistically different from 0.

added to 0.9 ml of phosphate/saline/gelatin containing 0.1 mM flurbiprofen, a potent inhibitor of the transformations of arachidonic acid into prostaglandins or thromboxanes. These diluted samples were frozen in liquid nitrogen immediately after their withdrawal from the aggregometer cuvette. The effect of various stimulators and inhibitors of prostaglandin and thromboxane synthesis may then be determined by parallel assay of PGE_2 and thromboxane B_2.

The accuracy of the method was determined by adding known amounts of PGE_2 to platelet-rich plasma. The results are listed in Table 2. The precision was determined by analyzing several samples repeatedly. Typical results are listed in Table 3.

Table 3. Precision of replicate PGE_2 determinations by radioimmunoassay

Sample	Found, pg	± 95% confidence limits
1	14	± 11
2	14	± 2
3	36	± 3
4	52	± 7
5	7	± 4
6	23	± 3
7	9	± 1
8	44	± 5
9	49	± 10
10	32	± 10
11	15	± 3
12	74	± 9
13	6	± 2

Samples were assayed six times each according to the procedure described in *Experimental*. All samples were derived from arachidonate-induced aggregations of platelet-rich plasma. Samples (30 assays) that were not aggregated gave PGE_2 values of 1 ± 2 pg (i.e., below the limit of detection of the radioimmunoassay).

DISCUSSION

Fig. 4 depicts a proposed solution to the problems accompanying the production of high-affinity, high-specificity antibodies for PGE-like compounds. Our results support the validity of this proposal. Rabbits inoculated with conjugates of 9-deoxy-9-methylene-$PGF_{2\alpha}$ produced antibodies with association constants, K_0, from 10^8 to 10^9 liters·mole^{-1} for PGE_2, the isosteric counterpart of the hapten. K_0 for antibodies against a keyhole limpet hemocyanin conjugate was approximately 10 times greater than K_0 for a bovine serum albumin conjugate. The higher affinity antibodies were also more specific. The increased epitope density on the keyhole limpet hemocyanin conjugate (68 moles of hapten/mole of protein) relative to the bovine serum albumin conjugate (19 molecules of hapten/mole of protein) is a likely cause of these differences. Different immunogenic potencies of the proteins may also be important. Raz *et al.* (2) reported that thyroglobulin conjugates were superior to bovine serum albumin conjugates for PGE_2 antibody production. They attributed this superiority to unknown deficiencies in bovine serum albumin as an immunogenic protein in conjunction with PGE_2 hapten; however, their thyroglobulin conjugate also had an epitope density 4 times greater than the

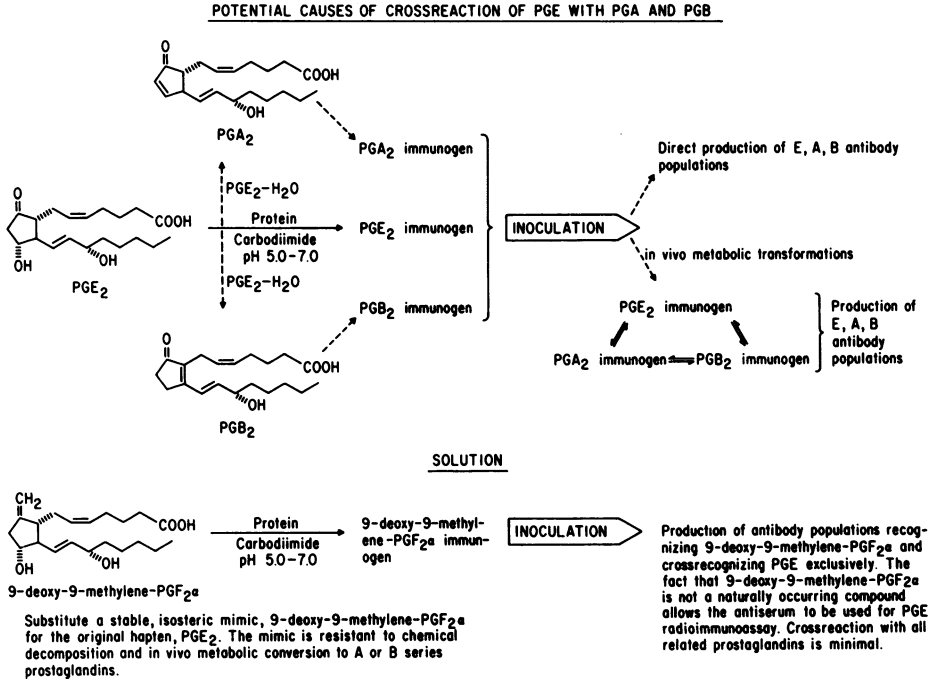

FIG. 4. Schematic presentation of a proposed method to elicit specific PGE antibodies.

corresponding bovine serum albumin conjugate. Our results are consistent on this point.

Sela (25) has reviewed the characteristics that confer immunodominance and immunopotency to antigens. Our results show that 9-deoxy-9-methylene-PGF$_{2\alpha}$ and PGE$_2$ have such nearly identical characteristics that antibodies directed against the substituted hapten mimic are useful for the radioimmunoassay of its isosteric counterpart. The sensitivity of the resultant radioimmunoassay and the inhibition range of the calibration curve are comparable to those reported by others (11–13), who have obtained affine antibodies against authentic PGE$_2$–protein conjugates. The results also support the notion that crossreaction of PGE antibodies with A and B series prostaglandins does arise from the instability of the PGE hapten.

Further studies are needed to determine the general utility of our approach. Providing that stable, isosteric hapten mimics can be found, it seems applicable, conceptually, to immunological problems where the instability of a hapten promotes crossreaction or limits the magnitude of the association constant. In the specific instance that the antibodies are intended for radioimmunoassay, the characteristics that confer immunodominance and immunopotency to the hapten mimic should be nearly identical to those of its isosteric counterpart. This condition limits the magnitude of the antibody association constant and thus the quality of the radioimmunoassay. The substituted hapten mimic should not be a known endogenous substance if the antibodies are intended for the radioimmunoassay of an endogenous substance, without including a chromatographic step. If radiolabeled hapten mimic were used instead of radiolabeled isosteric hapten, or if complement fixation were used instead of radiolabeled tracers it is possible that one might differentiate between the two.

By using a similar approach we have developed antibodies that recognize and bind the intrinsically unstable prostaglandin endoperoxide, PGH$_2$. These antibodies retard the ability of PGH$_2$ to aggregate platelets and they retard enzymatic and chemical conversion of PGH$_2$ into thromboxane B$_2$ and classical prostaglandins (26).

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1. Jaffe, B., Behrmann, H. & Parker, C. (1973) *J. Clin. Invest.* **52,** 398–405.
2. Raz, A., Schwarzman, M., Kenig-Wakshal, R. & Perl, E. (1975) *Eur. J. Biochem.* **53,** 145–150.
3. Jaffe, B., Smith, J., Newton, W. & Parker, C. (1971) *Science* **171,** 494–496.
4. Yu, S. C. & Burke, G. (1972) *Prostaglandins* **2,** 11–22.
5. Zusmann, R., Caldwell, B. & Speroff, L. (1972) *Prostaglandins* **2,** 41–53.
6. Levine, L., Guttierez-Cernosek, R. M. & Van Vunakis, H. (1971) *J. Biol. Chem.* **246,** 6782–6785.
7. Jobke, A., Peskar, B. A. & Peskar, B. M. (1973) *FEBS Lett.* **37,** 192–196.
8. Bauminger, S., Zor, U. & Lindner, H. (1973) *Prostaglandins* **4,** 313–324.
9. Ritzi, E. & Stylos, W. (1974) *Prostaglandins* **8,** 55–80.
10. Stylos, W., Howard, L., Ritzi, E. & Skarnes, R. (1974) *Prostaglandins* **6,** 1–14.
11. Dray, F., Charbonnel, B. & Maclouf, J. (1975) *Eur. J. Clin. Invest.* **5,** 311–318.
12. Christensen, P. & Leyssac, P. (1976) *Prostaglandins* **11,** 399–420.
13. Maclouf, J., Andrieu, J. & Dray, F. (1975) *FEBS Lett.* **56,** 273–278.
14. Schachman, H., Edelstein, S. (1966) *Biochemistry* **5,** 2681–2705.
15. Van Holde, K. & Cohen, J. (1965) *Biochemistry* **3,** 1803–1819.
16. Pinckard, R. & Weir, D. (1973) in *Handbook of Experimental Immunology,* Ed. Weir, D. M. (Blackwell Scientific Publications, London), Vol. 1 pp. 16.1–16.21.
17. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.* **51,** 660–672.
18. Kahn, D., Andrieu, J. B. & Dray, F. (1974) *Immunochemistry* **11,** 327–332.
19. Rodbard, D., Bridson, W. & Rayford, P. (1969) *J. Lab. Clin. Med.* **74,** 770–776.
20. Marcus, A., Ullman, H. & Safier, L. (1969) *J. Lipid Res.* **10,** 108–114.
21. Hilditch, T. P. (1956) *The Chemical Constitution of Natural Fats* (Chapman and Hall, London), 3rd Ed., p. 664.
22. Hamberg, M., Svensson, J. & Samuelsson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2994–2998.
23. Malmsten, C., Granstrom, E. & Samuelsson, B. (1976) *Biochim. Biophys. Res. Commun.* **68,** 569–576.
24. Granstrom, E., Kindhal, H. & Samuelsson, B. (1976) *Anal. Lett.* **9,** 611–627.
25. Sela, M. (1969) *Science* **166,** 1365–1374.
26. Fitzpatrick, F., Gorman, R. & Bundy, G. (1978) *Nature,* in press.