PHARMACOLOGICAL REVIEWS
Vol. 25, No. 2

Printed in U.S.A.

Antibodies to Pharmacologically Active Molecules: Specificities and Some Applications of Antiprostaglandins

LAWRENCE LEVINE*

Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts†

WITH the classical techniques of Landsteiner (19), antibodies directed toward a variety of substances of low molecular weight have been produced. Methods for synthesizing the required antigenic conjugates have been reviewed in this symposium (5). By using these principles, antibodies to several pharmacologically active substances have been prepared and are listed in table 1. For syntheses of most of the antigenic conjugates listed, an amide bond between the carrier and the hapten *via* reaction with water soluble carbodiimides was formed (10). Several macromolecular carriers were used including bovine serum albumin, human serum albumin, polylysine, and a copolymer of glutamic acid and lysine. In the case of the latter carriers, a salt complex between the positively charged antigenic conjugate and a negatively charged macromolecule is mandatory for successful production of antibodies (40).

We have used several of the antibodies listed in table 1 to study a variety of biological problems. However, this presentation will be confined to a description of the specificities of prostaglandin antiprostaglandin reactions and how we have used these antibodies to study some problems of biological and medical interest. Prostaglandins are C_{20} unsaturated fatty acids containing a cyclopentane ring and two aliphatic side chains. Substitution and unsaturation in the cyclopentane ring divide prostaglandins (PG) into various types (E, F, A, and B). The degree of unsaturation (indicated by the subscript following the letter) of the aliphatic side chains results in different classes, such as $PGF_{1\alpha}$ and $PGF_{2\alpha}$ Structures of some of the major prostaglandins are shown in figure 1. The E prostaglandins contain a keto group at C_9 and a C_{11} hydroxyl group. These compounds are sensitive to alkali and are easily dehydrated by removal of the C_{11} hydroxyl group to form PGA's. Subsequent rearrangement of the C_{10}-C_{11} double bond results in the formation of PGB. Enzymatic isomerization of PGA_1 to PGB_1 proceeds *via* the intermediate PGC_1 (15-hydroxy-9-ketoprosta-11,13-dienoic acid) (16). PGC_1 is unstable under mild alkaline conditions and is converted to PGB_1 (15-hydroxy-9-ketoprosta-8(12),13-dienoic acid).

The F prostaglandins differ from the E compounds by the presence of a hydroxyl group at C_9 instead of a keto group. Configuration of this substituent is designated as α, if the substituent is located on the same side of the cyclopentane ring as the carboxylic side chain. If the C_9 group is located on the same side of the ring as the alkyl side chain, the configuration is designated as β.

Prostaglandins are biosynthesized from essential fatty acids *via* cyclization and incorporation of molecular oxygen (1, 2, 29, 36–38) (fig. 2). The enzyme system(s) involved in the conversion of the polyenoic fatty acids to prostaglandins has been found

* American Cancer Society Professor of Biochemistry (Award No. PRP-21).

† Publication No. 886. This study was supported in part by research grant No. 1C-10L from the American Cancer Society, Inc.

TABLE 1

Antisera produced to pharmacologically active compounds in the Brandeis Biochemistry Department Laboratory

Compound	Ref.	Compound	Ref.
Angiotensin	10	Vitamin B_{12}	7
Bradykinin	10	Prostaglandin (PG)E_1*	21
3,4-Dimethoxyphenylethylamine	41	PGE_2*	†
		PGE_3*	†
Normetanephrine	26	PGA_1*	†
Serotonin	†	PGA_2*	†
2,5-Dimethoxy-4-methylamphetamine	41	$PGF_{1\alpha}$	21
		$PGF_{2\alpha}$	21
		15-keto-$PGF_{2\alpha}$	22
Mescaline	41	13,14-Dihydro-$PGF_{2\alpha}$	†
Lysergic acid	42		
Morphine	43	13,14-Dihydro-15-keto-$PGF_{2\alpha}$	†
Δ^9-THC	†		
		15-keto-PGE_2*	†

* Antibodies are directed toward the PGB structure.

† L. Levine, unpublished observations.

in numerous tissues and is associated with the microsomal fraction (29, 38).

When [^{3}H] prostaglandins, E_2 and $F_{2\alpha}$, are injected into man, they are rapidly metabolized and eliminated from the blood (11, 30, 31). PGE_2, PGE_1, and $PGF_{1\alpha}$ undergo essentially the same reactions: 1) oxidation of the C_{15} hydroxyl group (initial step) to form 15-keto-prostaglandin; 2) reduction of the C_{13}-C_{14} double bond; 3) two steps of *beta*-oxidation, and 4) *omega*-oxidation to yield 5α,7α-dihydroxy-11-keto-tetranorprosta-1,16-dioic acid, which is the major urinary metabolite of both $PGF_{2\alpha}$ and $PGF_{1\alpha}$ (fig. 3). The major urinary metabolite of PGE_2 or PGE_1 is 7-hydroxy-5,11-diketotetranorprosta-1,16-dioic acid (fig. 3). Although it is known that the first two steps precede the latter two, the order of the subsequent reactions is uncertain.

Specificities of Prostaglandin Antigen-Antibody Reactions

Increasing interest in the physiological role of prostaglandins has intensified efforts to develop techniques for quantitating these compounds and their metabolites. The applicability of a radioimmunoassay for estimating levels of steroids has proved to be invaluable. Radioimmunoassay is a simple, rapid, sensitive technique that does not require extensive purification of the sample. Although fatty acids are not good immunogens, we hoped that the somewhat more rigid structure of prostaglandins imparted by the cyclopentane ring would increase their immunogenicity. In 1970, we reported the first successful production of antibodies to certain prostaglandins (20). Shortly thereafter, several laboratories developed serological methods that could be used for measuring prostaglandins (3, 15, 17, 25, 33, 45, 46).

It is the serological specificities of the prostaglandin antigen-antibody reactions that determine the usefulness of the antibodies for assaying the prostaglandins. Antiserum to $PGF_{2\alpha}$ is both sensitive and highly specific. It detects $PGF_{2\alpha}$ in nanogram and even picogram quantities and distinguishes the different types and classes of prostaglandins and the early metabolites of $PGF_{2\alpha}$ (8, 13, 21). Cross reactions of the various prostaglandins with anti-$PGF_{2\alpha}$ demonstrate that antibodies to $PGF_{2\alpha}$ recognize modifications in: 1) the cyclopentane ring, *i.e.*, substitution of the C_9 hydroxyl group by a keto group (PGE) or change in the spatial orientation of the C_9 hydroxyl group from α to β ($PGF_{2\beta}$); 2) the C_5-C_6 double bond (absent in $PGF_{1\alpha}$); 3) the C_{15} hydroxyl group (oxidation of $PGF_{2\alpha}$ to 15-keto-$PGF_{2\alpha}$ dramatically decreases its inhibitory capacity); and 4) the C_{13}-C_{14} double bond (absent in 13,14-dihydro-$PGF_{2\alpha}$ (table 2).

Antibodies of $PGF_{1\alpha}$ exhibit approximately the same sensitivities as anti-$PGF_{2\alpha}$ but less specificity; $PGF_{2\alpha}$ cross reacts appreciably (25%) with anti-$PGF_{1\alpha}$. Therefore, recognition of the C_5-C_6 double bond by anti-$PGF_{1\alpha}$ is not as striking as that by antiserum to $PGF_{2\alpha}$. Antiserum to $PGF_{1\alpha}$ does, however, distinguish other prostaglandin types and initial $PGF_{2\alpha}$ metabolites.

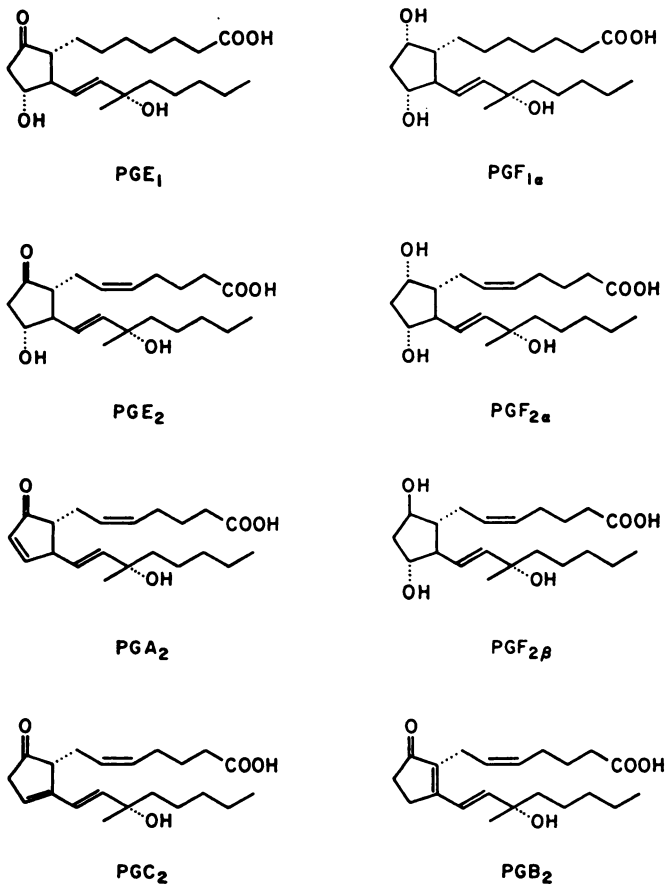

FIG. 1. Structural formulas of some prostaglandins.

Substitution of the 7-methylene group by oxygen ((+)-7-oxa-$PGF_{1\alpha}$) substantially decreases the binding energy. Its optical antipode ((−)-7-oxa-$PGF_{1\alpha}$) cross reacts even less with anti-$PGF_{1\alpha}$.

The specificities exhibited by $PGF_{1\alpha}$ and $PGF_{2\alpha}$ antisera make it possible to determine $PGF_{2\alpha}$ levels in human serum (13) without interference by the heterologous types of prostaglandins and the initial metabolites. Extracts of 78 serum samples have been assayed. Males have more circulating $PGF_{2\alpha}$ in their blood than females, but there is a wide range in the values obtained from both males and females (males: 889 ± 564 pg/ml serum; females: 272 ± 221 pg/ml serum) (24).

Many samples of blood were also assayed with anti-$PGF_{1\alpha}$ to establish that the values obtained with anti-$PGF_{2\alpha}$ reflected $PGF_{2\alpha}$ inhibition and not $PGF_{1\alpha}$ cross reactions. For example, with the anti-$PGF_{2\alpha}$ antigen-antibody reaction (fig. 4), if a blood sample inhibits 40%, 1.6 ng $PGF_{2\alpha}$ or 20 ng $PGF_{1\alpha}$ or some mixtures of these prostaglandins must be present. We can rule out the presence of 20 ng $PGF_{1\alpha}$ because the inhibition of $PGF_{1\alpha}$ anti-$PGF_{1\alpha}$ antigen-antibody reaction (fig. 4) by all serum extracts tested could be quantitatively accounted for by $PGF_{2\alpha}$ content.

As shown previously (fig. 3), the pharmacologically active prostaglandins are readily inactivated by metabolic processes (30). Therefore, radioimmunoassays for specific metabolic products would be extremely useful, especially if a metabolite were more stable in the circulatory system.

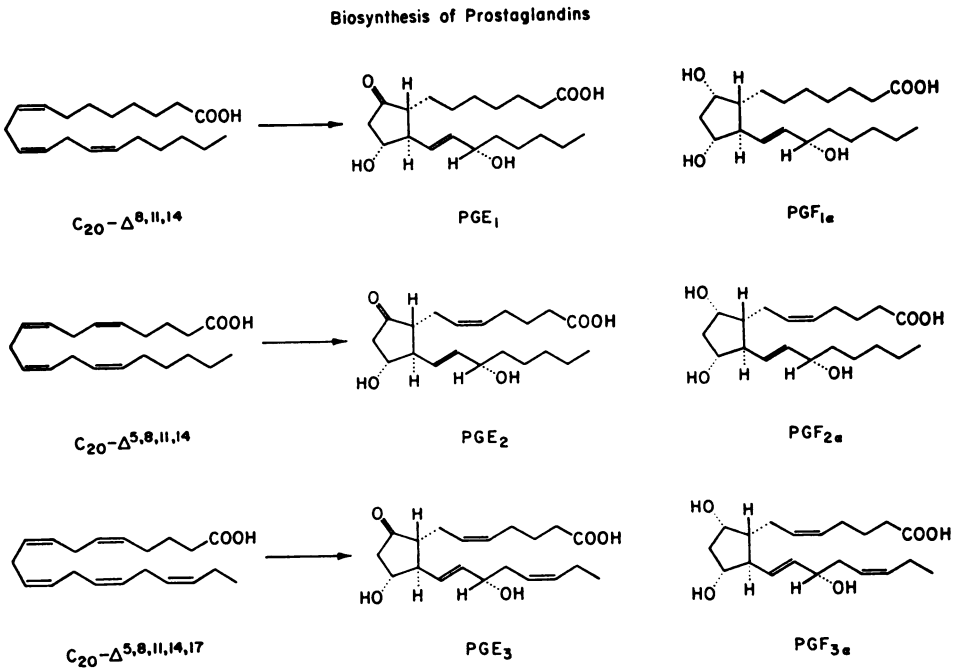

FIG. 2. The biosynthesis of primary prostaglandins from unsaturated fatty acids.

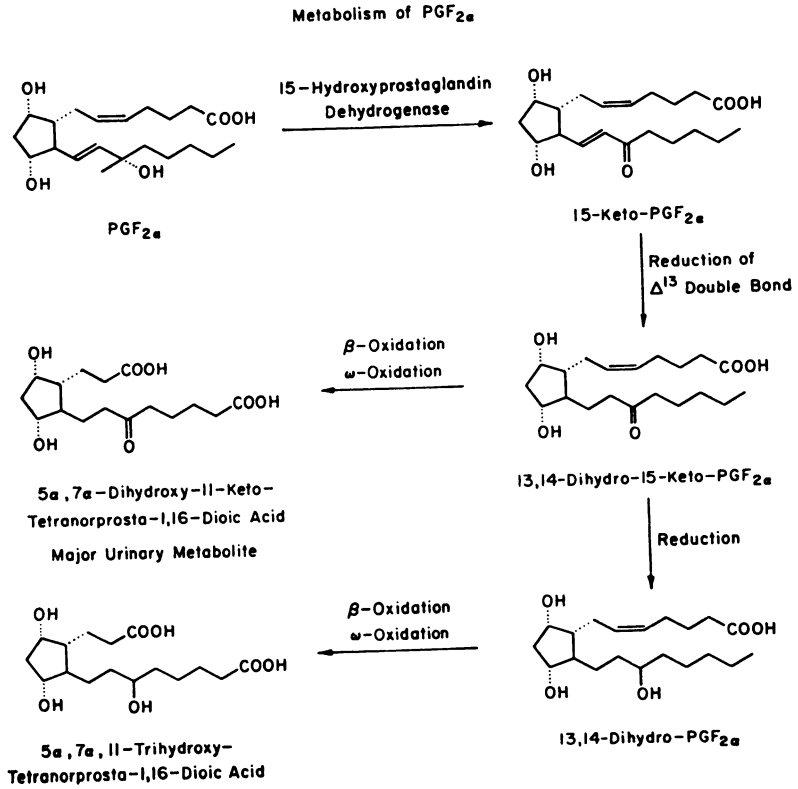

FIG. 3. The metabolism of $PGF_{2\alpha}$ in man.

Recently, we were able to prepare antibodies to 15-keto-$PGF_{2\alpha}$ in monkeys (22). At least three subsites on the prostaglandin molecule contribute to the specificity of this antiserum: 1) C_{15}, 2) C_{13}-C_{14}, and 3) C_{11}. $PGF_{2\alpha}$, which has a C_{15} hydroxyl group instead of a keto group, is a very weak inhibitor. Little interference by $PGF_{2\alpha}$ is found when biological samples are analyzed for the metabolite, 15-keto-$PGF_{2\alpha}$. The next metabolite formed from the 15-keto-$PGF_{2\alpha}$ is 13,14-dihydro-15-keto-$PGF_{2\alpha}$. This reduction step results in a striking decrease in cross reactivity with anti-15-keto-$PGF_{2\alpha}$. (To produce 50% inhibition, 40 times more 13,14-dihydro-15-keto-$PGF_{2\alpha}$ than 15-keto-$PGF_{2\alpha}$ is required.)

TABLE 2

Serological specificity of $PGF_{2\alpha}$ anti-$PGF_{2\alpha}$ (24)

$PGF_{2\alpha}$ anti-$PGF_{2\alpha}$: $\Delta F^0 = -10.44$ kcal/mole.

Prostaglandin	Picomoles Required for 50% Inhibition	K_0	$\Delta(\Delta F^0)$*	Contribution to Total Binding
		liters/mole	*kcal/mole*	%
$F_{2\alpha}$	7.1	6.5×10^7		100
$F_{1\alpha}$	150	2.9×10^6	1.8	83
15-Keto-$F_{2\alpha}$	930	5.3×10^5	2.8	73
$F_{2\beta}$	1.7×10^3	2.6×10^5	3.2	69
13,14-Dihydro-15-keto-$F_{2\alpha}$	3.7×10^3	1.3×10^5	3.6	65
E_2	8.5×10^3	5.6×10^4	4.1	61
A_2	28×10^3	1.7×10^4	4.8	54

* $\Delta(\Delta F^0) = -RT \ln \frac{50\% \text{ inhibition (heterologous)}}{50\% \text{ inhibition (homologous)}}$.

Antibodies to 15-keto-$PGF_{2\alpha}$ do not recognize the C_9 substituent of the cyclopentane ring. However, because they do recognize the C_{11} hydroxyl group, this cross reaction with 15-keto-PGE_2 presents no big problem. Alkaline treatment of 15-keto-PGE_2 results in the formation of a compound (perhaps a PGB-like compound), which cross reacts poorly. Therefore, to obtain accurate 15-keto-$PGF_{2\alpha}$ values, a sample can be analyzed before and after alkaline treatment. Alkaline treatment does not alter the inhibitory capacity of 15-keto-$PGF_{2\alpha}$.

Recently the production of antibodies in rabbits to 13,14-dihydro-15-keto-$PGF_{2\alpha}$ has been reported (12). Both the saturated 13,14-double bond and the 15-keto group are immunodominant, since 15-keto-$PGF_{2\alpha}$

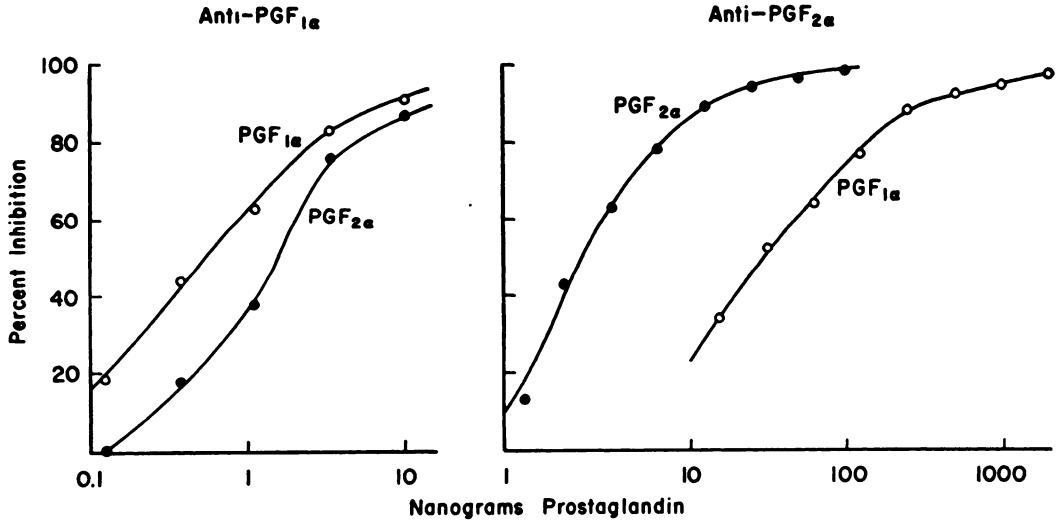

FIG. 4. (Left side) Inhibition of [^{3}H]$PGF_{1\alpha}$ anti-$PGF_{1\alpha}$ binding by $PGF_{1\alpha}$ and by $PGF_{2\alpha}$. (Right side) Inhibition of [^{3}H]$PGF_{2\alpha}$ anti-$PGF_{2\alpha}$ binding by $PGF_{2\alpha}$ and by $PGF_{1\alpha}$.

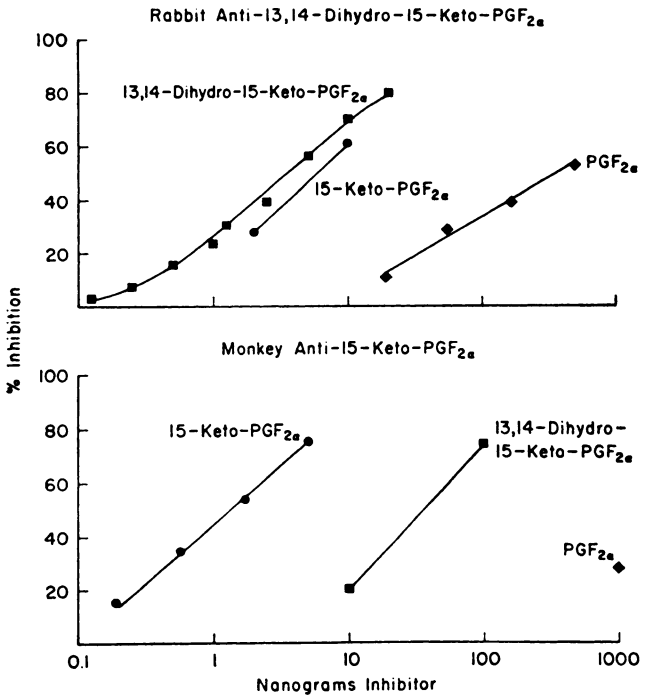

FIG. 5. (Top) Inhibition of [^{3}H]-15-keto-PGF$_{2\alpha}$ anti-13,14-dihydro-15-keto-PGF$_{2\alpha}$ binding and (bottom) inhibition of [^{3}H]-15-keto-PGF$_{2\alpha}$ anti-15-keto-PGF$_{2\alpha}$ binding by 13,14-dihydro-15-keto-PGF$_{2\alpha}$, 15-keto-PGF$_{2\alpha}$, and PGF$_{2\alpha}$.

and 13,14-dihydro-PGF$_{2\alpha}$ cross react only 3.8 and 2.5%, respectively. We have also produced antibodies in rabbits to the 13,14-dihydro-15-keto-PGF$_{2\alpha}$ metabolite. Our antibodies recognize the 15-keto group but not the reduced 13,14-double bond. The 15-keto-PGF$_{2\alpha}$ metabolite cross reacts about 80%, while PGF$_{2\alpha}$ cross reacts about 2%. Again the serological effects of the substituent on C_9 of the cyclopentane ring are not absolute; 15-keto-PGE cross reacts about 10% and the cross reaction is abolished upon treatment of the 15-keto-PGE$_2$ with alkali. With the use of both antisera, because of their specificities (fig. 5), the two metabolites can be quantitated. For example, if an unknown sample inhibited the rabbit anti-13,14-dihydro-15-keto-PGF$_{2\alpha}$ 50%, either 2 ng of 13,14-dihydro-15-keto-PGF$_{2\alpha}$ or 2.5 ng of 15-keto-PGF$_{2\alpha}$ could be present. If it were the 13,14-dihydro-15-keto-PGF$_{2\alpha}$ metabolite, the 2 ng would not inhibit the monkey anti-15-keto-PGF$_{2\alpha}$ at all. If it were 15-keto-PGF$_{2\alpha}$, 2.5 ng would produce about 70% inhibition of the monkey anti-15-keto-PGF$_{2\alpha}$. We have used both of these antisera to measure the two metabolites in biological fluids. There are no measurable amounts of 15-keto-PGF$_{2\alpha}$ but easily measurable amounts of the 13,14-dihydro-15-keto-PGF$_{2\alpha}$ metabolites in normal human sera.

Still another combination of antisera can be used to identify and quantitate a particular prostaglandin. We have recently immunized rabbits with 13,14-dihydro-PGF$_{2\alpha}$, another metabolite of PGF$_{2\alpha}$ which arises from the reduction of the 15-keto group of 13,14-dihydro-15-keto-PGF$_{2\alpha}$ (29, 31). These antibodies do not differentiate 13,14-dihydro-PGF$_{2\alpha}$ from PGF$_{2\alpha}$ (fig. 6). But anti-PGF$_{2\alpha}$ does (neither antiserum cross reacts extensively with the 15-keto-PGF$_{2\alpha}$ derivatives), so the metabolite can be quantitatively estimated in biological fluids.

We have found it difficult to prepare

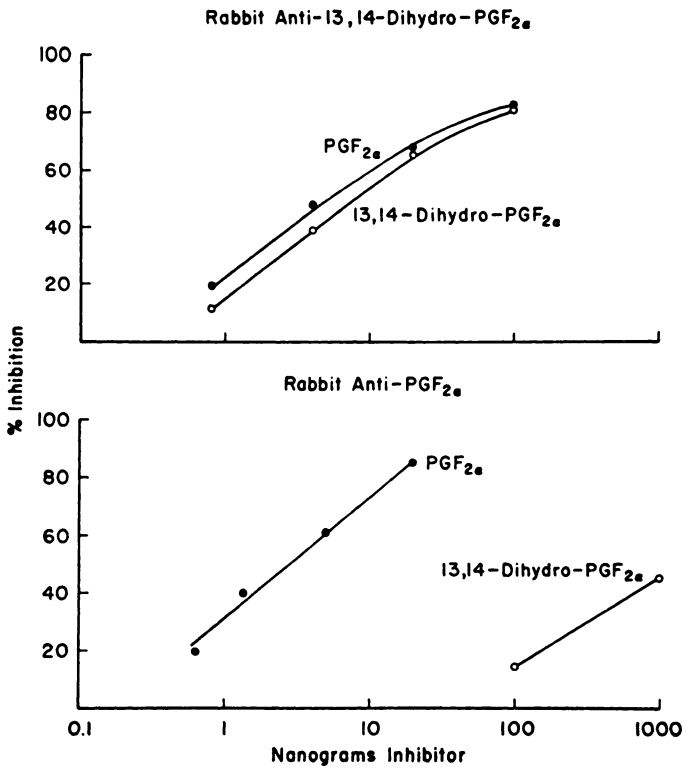

FIG. 6. (Top) Inhibition of [^{3}H]$PGF_{2\alpha}$ anti-13,14-dihydro-$PGF_{2\alpha}$ binding and (bottom) [^{3}H]-$PGF_{2\alpha}$ anti-$PGF_{2\alpha}$ binding by $PGF_{2\alpha}$ and 13,14-dihydro-$PGF_{2\alpha}$.

analytical reagents for assay of the E type of prostaglandins. Antibodies produced in rabbits, guinea pigs, and monkeys to PGE or PGA all are directed toward PGB. However, successful production of antibodies directed to PGE (15) and PGA (33, 46) have been reported. All of our antibodies directed toward PGB do differentiate the different types of prostaglandins. For example, the antisera were more sensitive to inhibition by PGB_1 than by PGA_1, which differs from PGB_1 only by the location of the unsaturated bond in the cyclopentane ring (PGA_1: 9-keto-$\Delta^{10,18}$-prostadienoic acid, PGB_1:9-keto-$\Delta^{8(12)18}$-prostadienoic acid). Approximately 15 times more PGA_1 is needed to yield equivalent inhibition. The presence of an 11-hydroxyl group on the cyclopentane ring (PGE_1) rendered this prostaglandin still less effective as an inhibitor of the antigen-antibody reaction; 22 ng were required to produce 50% inhibition compared to 70 pg of PGB_1. The F type of prostaglandin, which contains two hydroxyl groups in a saturated cyclopentane ring, was an extremely poor inhibitor of the reaction. PGB_2, which contains a second unsaturated double bond between C_5 and C_6 of the aliphatic chain, was a weaker inhibitor than PGB_1. This indicates that the antibodies in the antisera also recognize the aliphatic side chains. The other *bis*-unsaturated compounds, *i.e.*, PGA_2 and PGE_2, were also less inhibitory than the corresponding monounsaturated prostaglandins (PGA_1 and PGE_1). In addition to being quite specific, this antigen-antibody reaction, because of the high specific activity of the radioactive ligand, exhibited great sensitivity. It easily detects the presence of 10 pg of PGB_1 or 200 pg of PGA_1.

We have previously suggested (24) that production of antibodies directed toward PGE will be difficult because of the insta-

bility of PGE to chemical coupling procedures or to enzymes (dehydrases and isomerases) in blood. A combination of antisera can be used to identify PGB or PGA, and alkaline treatment or $NaBH_4$ reduction or both sometimes can be used to differentiate PGA from PGE in biological samples. The data in figure 7 show the specificities of two antisera, one immunized with a PGE_1 conjugate and the other with a PGE_3 conjugate. Both antisera produced antibodies which bind most effectively with PGB. The specificities of these two antisera become broader when analyzed by competition with a cross reacting radiolabeled prostaglandin (24, 45). When the cross reaction, [^{3}H]PGA anti-PGB_3 binding, is inhibited, PGB and PGA are equally effective; PGE is 2 logs less effective. When the competition for the binding of [^{3}H]PGB and anti-PGB_1 is analyzed, PGB is 15 times more effective than PGA and more than 1000 times more effective than PGE. Therefore, in a biological sample, PGB and PGA can be identified and quantitatively estimated by simultaneous assays with these two immune systems.

PGE and PGA are quantitatively converted to PGB by alkaline treatment. Therefore, a biological sample reacting with the anti-PGB system can be identified by assaying the sample before and after alkaline treatment with the [^{3}H]PGB anti-$PGE_1(B_1)$ reaction. A 10- to 15-fold increase in competitive effectiveness would indicate PGA was present, while a 1000-fold increase in effectiveness would suggest that PGE was being measured. In addition, PGE can be converted to PGF by treatment with sodium borohydride. PGE_2, after such reduction, is converted to a mixture of $PGF_{2\alpha}$ and $PGF_2\beta$, but $PGF_2\beta$ cross reacts $<0.5\%$ with anti-$PGF_{2\alpha}$ (21). With our antisera, after reduction of known quantities of PGE_2 with $NaBH_4$, the yield of serologically active product, when assayed with anti-$PGF_{2\alpha}$, was 50% (fig. 8). PGA or PGB treated with $NaBH_4$ did not react serologically with anti-PGF. If PGE were present in a biological sample, then $NaBH_4$ catalyzed reduc-

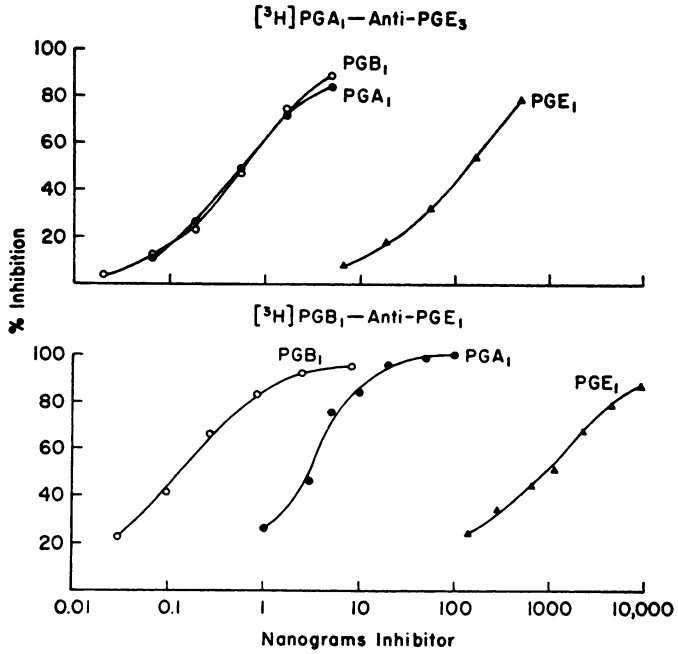

FIG. 7. (Top) Inhibition of [^{3}H]PGA anti-PGE_3 binding and (bottom) [^{3}H]PGB anti-PGA_1 binding by PGB, PGA, and PGE.

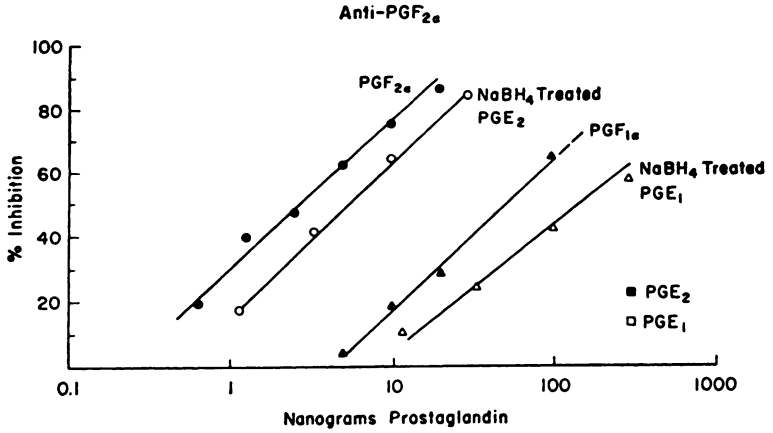

FIG. 8. Inhibition of [^{3}H]PGF$_{2\alpha}$ anti-PGF$_{2\alpha}$ binding by PGF$_{2\alpha}$, PGF$_{1\alpha}$, PGE$_1$, PGE$_2$ and PGE$_1$ and PGE$_2$ after treatment with NaBH$_4$.

tion could be used for its identification by analysis with specific anti-PGF. In addition, the relative specificities of anti-PGF$_{1\alpha}$ and anti-PGF$_{2\alpha}$ (fig. 4) make it possible to identify the PGE (converted to PGF) as either PGE$_1$ or PGE$_2$.

Some Applications of Prostaglandin Antibodies

In crude tissue homogenates or in concentrated biological fluids, it is difficult to measure activities of metabolic enzymes (fig. 3) by relatively simple methods, for example, spectrophotometric analysis. The product of the 15-hydroxy-PGF$_{2\alpha}$ dehydrogenase reaction (15-keto-PGF$_{2\alpha}$), at least in crude tissue homogenates, is identified only after chromatographic separation.

With the use of antibodies of the appropriate specificities, these activities can be measured without prior chromatographic separation of the reaction mixture, even in crude homogenates. For example, our antibodies to PGF$_{2\alpha}$ do not cross react with the dehydrogenase product, 15-keto-PGF$_{2\alpha}$ (8, 13), nor do our antibodies to the product, 15-keto-PGF$_{2\alpha}$, cross react with the substrate PGF$_{2\alpha}$ (fig. 5) (22). Therefore, incubation of PGF$_{2\alpha}$ with a 15-hydroxy-PGF$_{2\alpha}$ dehydrogenase preparation will result in the disappearance of PGF$_{2\alpha}$ serological activity, when measured with the anti-PGF$_{2\alpha}$, and the concomitant appearance of 15-keto-PGF$_{2\alpha}$ serological activity, when measured with anti-15-keto-PGF$_{2\alpha}$. If the product, 15-keto-PGF$_{2\alpha}$, is acted upon by another enzyme (the Δ13,14 reductase) and if this second product (the 13,14-dihydro-15-keto-PGF$_{2\alpha}$) does not cross react with the anti-15-keto-PGF$_{2\alpha}$ (fig. 5), its serological activity will be lost. With antibodies to the reductase product (13,14-dihydro-15-keto-PG), the serological activity will be generated as the Δ13,14 double bond is reduced. Our antibodies to 13,14-dihydro-15-keto-PG do not recognize the reduction of this Δ13,14 double bond (the 15-keto group is immunodominant), so they react just as well with 15-keto-PGF$_{2\alpha}$ and 13,14-dihydro-15-keto-PGF$_{2\alpha}$. Therefore, when the Δ13,14 double bond of 15-keto-PGF$_{2\alpha}$ is reduced, instead of a loss of serological activity, which we observe with anti-15-keto-PGF$_{2\alpha}$, generation of the new product is accompanied not by a loss in serological activity but by a continuing rise in serological activity until all of the initial substrate is converted to 13,14-dihydro-15-keto-PGF$_{2\alpha}$. The difference curve calculated from the serological activities with these two latter antibodies represents the reduction of the Δ13,14 double bond. If 13,14-dihydro-15-keto-PGF$_{2\alpha}$ is metabolized further by a 13,14-dihydro-15-keto-PGF$_{2\alpha}$ reductase to produce the 15-hydroxy

compound (13,14-dihydro-$PGF_{2\alpha}$), there will be a rise in serological activity with an antibody that can measure the product, 13,14-dihydro-$PGF_{2\alpha}$, provided that this latter product does not cross react with 15-keto-PG compounds (fig. 6). Such an enzyme should exist, since a metabolite of 13,14-dihydro-$PGF_{2\alpha}$ has been found (29, 31). Some of these changes in serological activity which accompany 15-hydroxy-$PGF_{2\alpha}$ dehydrogenase and 15-keto-$PGF_{2\alpha}$ (Δ13,14) reductase activities are shown in figure 9.

PGE is relatively unstable. In acid and mild alkali, it readily undergoes dehydration at C_{11} with rearrangement of the bonds within the cyclopentane ring to form the Δ10, 11 compound (PGA). In stronger alkali, the double bond rearranges to Δ 11,12 (PGC) (16) and then Δ8,12 (PGB). Enzymatic dehydration has been postulated, and such activities have been detected in rat kidney homogenates (4) and in human serum (24). Our antibodies to PGE_1 (fig. 7) react 15 times more effectively with PGB than PGA. Therefore, if PGA is incubated with an isomerase (14), the product, PGB, will inhibit more effectively. We have used this method to follow the conversion of PGA_1 to PGB_1 in the sera of several mammalian species (27). In addition, the enzyme, as it exists in rat serum, has been partially characterized. Similar conversions of PGA_1 to PGB_1 have been observed after incubation of PGA_1 in the sera of several species. The activities of the different species vary widely (table 3). Rat sera have the highest activity followed by rabbit and mouse sera, whereas cat plasma is less active. Monkey and horse sera, even at relatively high concentrations, convert PGA_1 only partially. Human, guinea pig, and fetal calf sera, at least at concentrations tested, were inactive.

The E and F classes of prostaglandins are formed directly from intermediates by

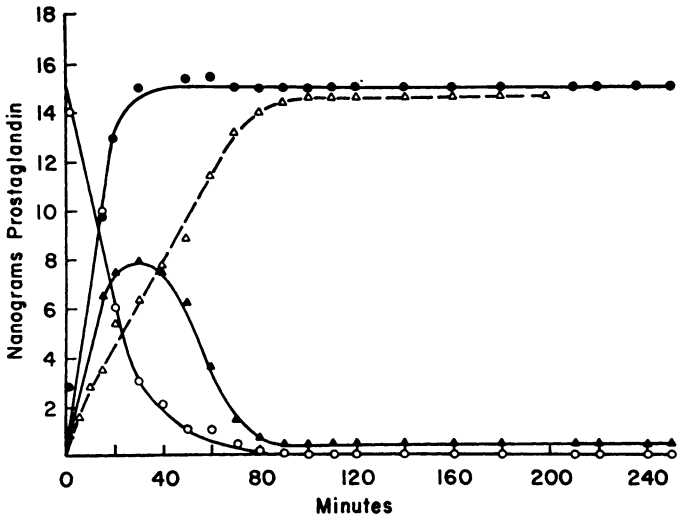

FIG. 9. Nanograms of prostaglandin or prostaglandin metabolite found in the reaction mixture of $PGF_{2\alpha}$ incubated with dog lung homogenate and NAD. (○——○): serological activity of the reaction mixture when measured with anti-$PGF_{2\alpha}$. This antiserum measures only $PGF_{2\alpha}$. (▲——▲): serological activity in the same reaction mixture when measured with anti-15-keto-$PGF_{2\alpha}$. This antiserum measures 15-keto-$PGF_{2\alpha}$ but does not cross react with 13,14-dihydro-15-keto-$PGF_{2\alpha}$; therefore, reduction of the 13,14 double bond of the 15-keto-$PGF_{2\alpha}$ results in a loss of serological activity. (●——●): serological activity of the same reaction mixture when measured with anti-13,14-dihydro-15-keto-$PGF_{2\alpha}$. This antiserum measures both 15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-$PGF_{2\alpha}$. (△- - -△): difference curve of serological activities calculated from the data obtained with the two latter antisera. Data represent the generation of 13,14-dihydro-15-keto-$PGF_{2\alpha}$.

TABLE 3

PGA_1 isomerase activity in sera of various species (27) at pH 7.5

Species	Units of Enzyme/ml Serum*
Rat	2000
Rabbit	1000
Mouse	500
Cat (plasma)	40
Horse	10
Monkey†	10
Human	<10
Guinea pig	<10
Fetal calf	<10

* Enzyme activity is expressed in units. One unit is arbitrarily defined as the enzyme activity that isomerizes 5 ng PGA_1 in 1 hr at 37°C. This is equivalent to the reciprocal of the dilution of serum that converts 50% of the added 10 ng PGA_1 to B_1.

† Serum of one animal.

way of the prostaglandin synthetic system (fig. 2), although conversion of PGE to PGF has been detected in guinea pig liver (31). With the use of antibodies to PGF, which do not cross react with PGE effectively, we have recently measured PGE-9-keto-reductase in organs of several species.[1] The relative activities of PGE-9-keto-reductase in various tissues of the rat are shown in figure 10. What is striking is the presence of the enzyme in cardiac muscle extracts and its relative absence in both smooth and skeletal muscle extracts.

A transplantable mouse fibrosarcoma which enhances the resorption of bone in tissue culture has been described (9). Fragments of this tumor ($HSDM_1$) cultured alone, and the medium from such explants also stimulated bone resorption when it was added to calvaria in organ culture. The stimulating factor could be extracted from the tumor tissue and recovered from the medium of $HSDM_1$ cells grown in monolayer culture (44). Of particular interest was the finding that the $HSDM_1$ factor could be extracted into organic solvents, and that it had several chemical and biological properties of a prostaglandin. Earlier it had been demonstrated that prostaglandins can stimulate bone resorption in tissue culture (18).

Previously we had failed to detect PGF, PGA, or PGB in several functional cell lines that were examined. However, in tissue culture media of five clonal strains of mouse tumor cells established from the transplantable mouse fibrosarcoma which produces the bone-resorption factor, high concentrations of prostaglandin were found. This prostaglandin was identified to be PGE by assaying the material in the serum-free tissue culture fluid before and after NaOH treatment. $NaBH_4$ reduction of the serum-free culture fluid and subsequent assay with anti-$PGF_{2\alpha}$ and anti-$PGF_{1\alpha}$ confirmed its PGE class and identified the PGE to be PGE_2 (23).

Since it has been reported that aspirin and related drugs inhibit the synthesis and release of prostaglandins in several systems (6, 32, 39), the effects of aspirin (acetylsalicylic acid), sodium salicylate, and indomethacin on PGE_2 production by $HSDM_1C_1$ cells were studied. Indomethacin was found to be a potent inhibitor of PGE_2 production, causing a 50% reduction in the rate of PGE_2 synthesis at a dose level of 1 ng/ml; whereas aspirin caused a 50% decrease at a dose level of 10 μg/ml (fig. 11). Sodium salicylate caused < 20% decrease in the rate of PGE_2 synthesis at a dose level of 50 μg/ml. In cells treated with up to 50 ng/ml of indomethacin or 50 μg/ml of aspirin, intracellular levels of PGE_2 did not differ from those in untreated cultures. Therefore, the drugs did not act on $HSDM_1C_1$ cells merely by inhibiting the secretion of PGE_2 into the culture medium. The five clonal strains of mouse tumor cells also secrete the bone-resorption stimulating factor into the tissue culture medium. Indomethacin also inhibits production by the cells of this bone-resorption stimulating factor. PGE_2 and the bone-resorption stimulating factor appeared to be identical. Mice bearing the tumor had elevated blood levels

[1] L. Levine: Unpublished observations.

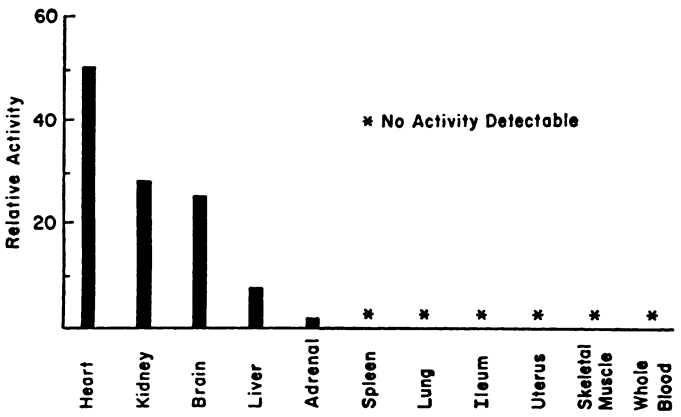

FIG. 10. PGE-9-keto-reductase distribution in various tissues of rat.

of Ca, and as shown in table 4, also had high levels of PGE_2. It was suggested that secretion of PGE_2 by the tumor *in vivo* caused the relative hypercalcemia in the tumor-bearing animals (34). When indomethacin was administered to the mice bearing the $HSDM_1$ fibrosarcoma, the elevated Ca levels and the elevated PGE_2 levels in the blood, and both the bone-resorption stimulating factor and PGE_2 levels in the tumor extracts were reduced (35). Some of these data are shown in table 5.

We have assayed recently some selected human synovial fluids for PGB (28). Our preliminary results show that: 1) Nineteen patients with inflammatory effusions [> 1000 polymorphonuclear leukocytes (pmn)/mm^3] who had not received significant anti-inflammatory therapy gave a mean value of 247 pg/ml of PGB. Only 3 of the 19 patients had values < 100 pg/ml. Most of these patients had definite or probably rheumatoid arthritis, three had pseudogout, one gout, and one infectious arthritis. 2) Sixteen patients with inflammatory effusions who had received anti-inflammatory therapy (salicylates or indomethacin) had a mean PGB value of 80 pg/ml. All of these patients had rheumatoid arthritis. 3) Sixteen patients with non-inflammatory effusions (< 1000 pmn/mm^3), who also had not received anti-inflammatory therapy, had a mean PGB value of 80 pg/ml. Only one of these values was > 100 pg/ml. Within each group

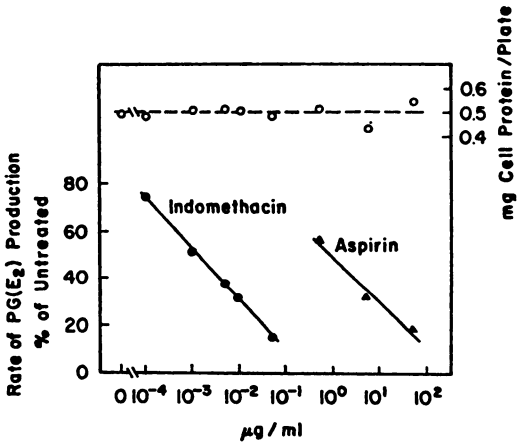

FIG. 11. Inhibition of PGE_2 production of $HSDM_1C_1$ cells by indomethacin and aspirin.

there was little or no correlation between the total leukocyte or pmn count and the prostaglandin levels. It was concluded that many untreated patients with inflammatory arthritis have higher levels of PGB in synovial fluids than in treated patients or patients with non-inflammatory joint diseases.

Culture fluids of human synovia have also been analyzed for prostaglandins. Several of the synovial fluids had measurable levels of $PGF_{2\alpha}$ and PGB. The effect of colchicine and vinblastin on prostaglandin production by these synovia is striking. Synovial cultures incubated with colchicine (0.1 µg/ml = 0.25 µM) produced larger amounts of

TABLE 4

Levels of PGE_2 in the serum of control and $HSDM_1$ tumor-bearing mice (84)

Experiment No.	Treatment	Serum PGE_2
		pg/ml
I*	Control	245 ± 35
II†	Control	185
	Tumor-bearing 1	680
	Tumor-bearing 2	520
	Tumor-bearing 3	500
	Tumor-bearing 4	500
III†	Control	275
	Tumor-bearing 1	720
	Tumor-bearing 2	840

* Mean value ± SE; six separate pools of control sera; each pool was derived from 5 to 10 mice.

† Each value is the mean of duplicate determinations of a separate pool of serum from 6 to 20 control or tumor-bearing mice.

PGB and $PGF_{2\alpha}$ than controls over the entire duration of the experiments. Maximum stimulation regularly exceeded 10-fold and considerable stimulation was also observed with as little as 0.01 μg/ml (fig. 12). Indomethacin (5 μg/ml) reduced prostaglandin levels to < 2% of both control and colchicine stimulated cultures. The prostaglandin levels in the media at varying time intervals of culture were a reflection of rates of prostaglandin synthesis. Vinblastin (2.5 μM) had a similar but smaller stimulating effect over the subsequent 7-day incubation.

Summary

Because of the low concentrations of these prostaglandin compounds in tissues and fluids and because of the large number of closely related structures, analytical procedures for their quantitative and qualitative estimation are not adequate or are restricted to only a few laboratories. Specific antibodies can now quantitate the following compounds: $PGF_{2\alpha}$, $PGF_{1\alpha}$, 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, and 13,14-dihydro-$PGF_{2\alpha}$. For some analyses, the samples can be assayed and identified with only one antiserum. For other analyses, a combination of antisera must be used for positive identification. Antisera directed toward PGB, which indirectly measure PGE or PGA or both, *i.e.*, PGE or PGA can be converted to PGB by treatment with NaOH, are also available; it is hoped that soon antisera that can measure the PGE metabolites will be produced.

Knowledge of the levels of prostaglandins, prostaglandin metabolites, and activities of the prostaglandin synthetic and metabolic enzymes in tissues and biological fluids would help to establish a physiological role(s) for the prostaglandin system.

Acknowledgments. We very much appreciate the gifts of prostaglandins and metabolites from the Upjohn Company of Kalamazoo, Michigan. Dr. E. J. Corey of the Department of Chemistry, Harvard University gave us the PGE_2 for immunization.

TABLE 5

Summary of tumor contents of bone resorption-stimulating activity and of PGE_2 concentrations in experimental animals (55)

Experiment Group	Tumor Content of Bone Resorption-stimulating Activity	Tumor Content of PGE_2*	Serum PGE_2†
	cpm × 10^{-4}/mg fresh wt	*ng/g fresh wt*	*pg/ml*
Control			270 ± 53
Indomethacin alone			117 ± 75
Tumor alone	24.1‡	136 ± 27	516 ± 53
Tumor + indomethacin	0.86§	4.9 ± 1.8	242 ± 53
Ratio of tumor/tumor + indomethacin	28	28	

* Mean values + S.E. for 7 separate experiments.

† Mean values ± S.E. for 6 separate experiments (except indomethacin alone, 3 experiments only).

‡ Mean value for 5 separate experiments (total range of values was 7 to 36 cpm × 10^{-4}/mg fresh wt).

§ Mean value for 5 separate experiments (total range of values was 0.39 to 1.2 cpm × 10^{-4}/mg fresh wt).

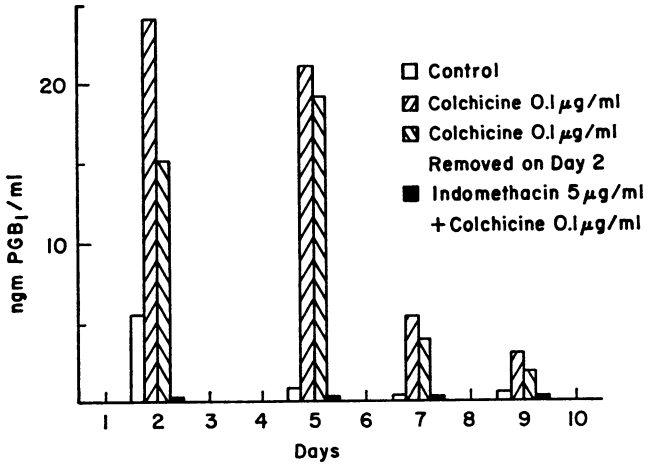

FIG. 12. Prostaglandin production by synovial membranes incubated in organ culture: effect of colchicine present throughout the organ culture, colchicine removed from the organ culture at day 2, and indomethacin and colchicine incubated throughout the organ culture.

REFERENCES

1. ANGGÅRD, E. AND SAMUELSSON, B.: Biosynthesis of prostaglandins from arachidonic acid in guinea pig lung. J. Biol Chem. 240: 3518-3521, 1965.
2. BERGSTRÖM, S., DANIELSSON, H. AND SAMUELSSON, B.: The enzymatic formation of prostaglandin E_2 from arachidonic acid. Biochim. Biophys. Acta 90: 207-210, 1964.
3. CALDWELL, B. V., BURSTEIN, S., BROCK, W. A. AND SPEROFF, L. Radioimmunoassay of the F prostaglandins. J. Clin. Endocrinol. Metab. 33: 171-175, 1971.
4. CAMMOCK, S.: Conversion of PGE_1 to a PGA_1-like compound by rat kidney homogenates. *In* Advance Abstracts, International Conference on Prostaglandins, Pergamon Press, Vieweg, 1972.
5. ERLANGER, B.: Principles and methods for the preparation of drug protein conjugates for immunological studies. Pharmacol. Rev. 25: 271-280, 1973.
6. FERREIRA, S. H., MONCADA, S. AND VANE, J. R.: Indomethacin aspirin abolish prostaglandin release from the spleen. Nature New Biol. 231: 237-239, 1971.
7. GERSHMAN, H., NATHANSON, N., ABELES, R. H. AND LEVINE, L. Production and specificity of antibodies to B_{12} derivatives. Arch. Biochem. Biophys. 153: 407-409, 1972.
8. GERSHMAN, H., POWERS, E., LEVINE, L. AND VAN VUNAKIS, H.: Radioimmunoassay of prostaglandins, angiotensin, digoxin, morphine and adenosine-3,5-cyclic monophosphate with nitrocellulose membranes. Prostaglandins 1: 407-423, 1972.
9. GOLDHABER, P.: Enhancement of bone resorption in tissue culture by mouse fibrosarcoma. Proc. Amer. Ass. Cancer Res. 3: 113, 1960.
10. GOODFRIEND, T. L., LEVINE, L. AND FASMAN, G. D.: Antibodies to bradykinin and angiotensin: a use of carbodiimides in immunology. Science 144: 1344-1346, 1964.
11. GRANSTRÖM, E.: On the metabolism of prostaglandin $F_{2\alpha}$ in female subjects. Structures of two metabolites in blood. Eur. J. Biochem. 27: 462-469, 1972.
12. GRANSTRÖM, E. AND SAMUELSSON, B.: Development and mass spectrometric evaluation of a radioimmunoassay for 9α,11α-dihydroxy-15-ketoprost-5-enoic acid. Fed. Eur. Biochem. Soc. Lett. 26: 211-214, 1972.
13. GUTIERREZ-CERNOSEK, R. M., MORRILL, L. M. AND LEVINE, L.: Prostaglandin $F_{2\alpha}$ levels in peripheral sera of man. Prostaglandins 1: 71-80, 1972.
14. HORTON, E. W.: Prostaglandins. Monogr. Endocrinol. 7: 1-197, 1972.
15. JAFFE, B. M., SMITH, J. W., NEWTON, W. T. AND PARKER, C. W.: Radioimmunoassay for prostaglandins. Science 171: 494-496, 1971.
16. JONES, R. L.: 15-Hydroxy-9-oxoprosta-11,13-dienoic acid as the product of a prostaglandin isomerase. J. Lipid Res. 13: 511-518, 1972.
17. KIRTON, K. T. CORNETTE, J. C. AND BARR, K. L.: Characterization of antibody to prostaglandin $F_{2\alpha}$. Biochem. Biophys. Res. Commun. 47: 903-909, 1972.
18. KLEIN, D. C. AND BAISZ, L. G.: Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology 86: 1436-1440, 1970.
19. LANDSTEINER, K.: The Specificity of Serological Reactions, 2nd ed, Harvard Univ. Press, Cambridge, Mass., 1945.
20. LEVINE, L. AND VAN VUNAKIS, H.: Antigenic activity of prostaglandins. Biochem. Biophys. Res. Commun. 41: 1171-1177, 1970.
21. LEVINE, L., GUTIERREZ-CERNOSEK, R. M. AND VAN VUNAKIS, H.: Specificities of prostaglandins B_1, $F_{1\alpha}$ and $F_{2\alpha}$ antigen-antibody reactions. J. Biol. Chem. 246: 6782-6785, 1971.
22. LEVINE, L. AND GUTIERREZ-CERNOSEK, R. M.: Preparation and specificity of antibodies to 15-keto-prostaglandin $F_{2\alpha}$. Prostaglandins 2: 281-294, 1972.
23. LEVINE, L., HINKLE, P. M., VOELKEL, E. F. AND TASHJIAN, A. H., JR.: Prostaglandin production by mouse fibrosarcoma cells in culture: inhibition by indomethacin and aspirin. Biochem. Biophys. Res. Commun. 47: 888-896, 1972.
24. LEVINE, L., GUTIERREZ-CERNOSEK, R. M. AND VAN VUNAKIS, H.: Specific antibodies: reagents for quantitative analysis of prostaglandins. Advan. Biosci. 9: 1973, in press.
25. ORCZYK, G. P. AND BEHRMAN, H. B.: Ovulation blockade by aspirin or indomethacin. *In vivo* evidence for a role of prostaglandin in gonadotropin secretions. Prostaglandins 1: 3-20, 1972.
26. PESKAR, B. A., PESKAR, B. M. AND LEVINE, L.: Specificities of antibodies to normetanephrine. Eur. J. Biochem. 26: 191-195, 1972.
27. POLET, H. AND LEVINE, L.: Serum prostaglandin A_1 isomerase. Biochem. Biophys. Res. Commun. 45: 1169-1176, 1971.
28. ROBINSON, D. R., SMITH, H. AND LEVINE, L.: Prostaglandin synthesis by rheumatoid synovial cultures and its stimulation by colchicine. Arthritis Rheum. 16: 129, 1973.
29. SAMUELSSON, B.: Biosynthesis of prostaglandins. Fed. Proc. 31: 1442-145 , 1972.

30. Samuelsson, B.: Endogenous synthesis of prostaglandins. *In* Third Conference on Prostaglandins in Fertility Control, WHO Research and Training Centre on Human Reproduction, pp. 1-17., Karolinska Institutet, Stockholm, 1972.

31. Samuelsson, B., Granström, E., Green, K. and Hornberg, M.: Metabolism of prostaglandins. Ann. N. Y. Acad. Sci. 180: 138-163, 1971.

32. Smith, J. B. and Willis, A. L.: Aspirin selectively inhibits prostaglandin production in human platelets. Nature New Biol. 231: 235-237, 1971.

33. Stylos, W. A. and Rivetz, B.: Preparation of specific antiserum to prostaglandin A. Prostaglandins 2: 103-113, 1972.

34. Tashjian, A. H., Jr., Voelkel, E. F., Levine, L. and Goldhaber, P.: Evidence that the bone resorption-stimulating factor produced by mouse fibrosarcoma cells is prostaglandin E_2. A new model for the hypercalcemia of cancer. J. Exp. Med. 136: 1329-1343, 1972.

35. Tashjian, A. H., Jr., Voelkel, E. F., Goldhaber, P. and Levine, L.: Successful treatment of hypercalcemia by indomethacin in mice bearing a prostaglandin-producing fibrosarcoma. Prostaglandins, April, 1973.

36. Van Dorp, D. A., Beerthius, R. K., Nugteren, D. H. and Vonkeman, H.: The biosynthesis of prostaglandins. Biochim. Biophys. Acta 90: 204-207, 1964.

37. Van Dorp, D. A., Beerthius, R. K., Nugteren, D. H. and Vonkeman, H.: Enzymatic conversion of all-cis-polyunsaturated fatty acids into prostaglandins. Nature (London) 203: 839-841, 1964.

38. Van Dorp, D. A.: Aspects of the biosynthesis of prostaglandins. Progr. Biochem. Pharmacol. 3: 71-82, 1967.

39. Vane, J. R.: Inhibition of prostaglandin synthesis as a mech anism of action for aspirin like drugs. Nature New Biol. 231: 232-235, 1971.

40. Van Vunakis, H., Kaplan, J., Lehrer, H. and Levine, L.: Immunogenicity of polylysine and polyornithine when complexed to phosphorylated bovine serum albumin. Immunochemistry 3: 393-402, 1966.

41. Van Vunakis, H., Bradvica, H., Benda, P. and Levine, L.: Production and specificity of antibodies directed toward 3,4,5-trimethoxyphenylethylamine, 3,4-dimethoxyphenylethylamine, and 2,5-dimethoxy-4-methyl amphetamine. Biochem. Pharmacol. 18: 393-404, 1969.

42. Van Vunakis, H., Farrow, J. T., Gjika, H. and Levine, L.: Specificity of the antibody receptor site to D-lysergamide: model of a physiological receptor for lysergic acid diethylamide. Proc. Nat. Acad. Sci. U.S.A. 68: 1483-1487, 1971.

43. Van Vunakis, H., Wasserman, E. and Levine, L.: Specificities of antibodies to morphine. J. Pharmacol. Exp. Ther. 180: 514-521, 1972.

44. Voelkel, E. F., Tashjian, A. H., Jr. and Goldhaber, P.: A non-peptide factor produced by fibrosarcoma cells that stimulates bone resorption in organ culture. *In* Calcium, Parathyroid Hormone and the Calcitonins, ed. by R. V. Talmage, and P. L. Munson p. 478, Amsterdam, Excerpta Medica, 1972.

45. Yu, S. C. and Burk, G.: Antigenic activity of prostaglandins: specificities of prostaglandins E_1, A_1 and $F_{2\alpha}$ antigen-antibody reactions. Prostaglandins 2: 11-22, 1972.

46. Zusman, R. M., Caldwell, B. V., Speroff, L. and Behrman, H. R.: Radioimmunoassay of the A prostaglandins. Prostaglandins 2: 41-53, 1972.