Antiserum against a partially purified growth hormone receptor derived from rabbit liver were generated in guinea pigs. The antisera specifically inhibited the binding of 125I-ovine growth hormone (oGH) to liver membranes but had no effect on the binding of 125I-ovine prolactin to rabbit mammary gland receptors. These antisera did not bind or destroy 125I-oGH. Moreover, the binding of labeled growth hormone to membrane particles derived from liver of several species was also inhibited by the antiserum, thus suggesting that immunological determinants of the growth hormone receptor of several species are similar. γ-Globulin fractions derived from the antiserum were responsible for the inhibition. In addition 125I-γ-globulin derived from one antiserum bound to membrane pellets with a corresponding decline in 125I-oGH binding. Kinetic analysis of inhibition of 125I-oGH binding suggested a hyperbolic competitive inhibition, a point of view which is favored by the demonstration of a hormone receptor-antibody complex. The availability of the antireceptor sera confirmed previous data that differential affinity chromatography separated growth hormone and prolactin receptors in solubilized rabbit liver membrane preparations. The antireceptor sera will be useful probes in further characterization of the growth hormone receptor.

A number of aspects of receptor structure, function, and turnover can be probed with specific antireceptor antisera, and for this reason efforts have been made to generate antisera against a number of partially or totally purified receptor preparations (1-5). In some pathological states such antisera occur naturally as a result of an autoimmune disorder, and this provides a rather more convenient source of such antibodies (1, 6, 7). These antireceptor antisera may act as agonists (3, 7-9) or as antagonists (1, 4, 10) of hormone action and provide useful tools for studying the mechanisms of hormone-receptor interaction (1, 7, 10). Indeed, if these antibodies are generated against a purified putative receptor, their effectiveness in blocking hormone action in vitro can be taken as evidence that a receptor molecule and not a hormone-degrading enzyme has been isolated (10, 11). Furthermore, antibodies raised against the individual polypeptide chains of a purified receptor are of great utility in defining the role of these chains in overall receptor function (12).

These antibodies can also be used as structural probes to study the species specificity of structural determinants on the receptor molecule, including those determinants not directly at the hormone-binding site (1, 13). Similarly, pathological structural variants of receptors may be detected with antireceptor antisera of defined specificity. Such antibodies are also valuable in electron microscopic studies of receptor distribution (14). Immune precipitation of labeled, newly synthesized receptors with antireceptor antibodies (15) is also likely to provide a major use for these antibodies in the future. Finally, active immunization with purified receptors (1, 11) or passive immunization with antireceptor antibodies (16, 17) provides important information about the in vivo role of these receptors and can provide useful experimental models for specific autoimmune diseases such as myasthenia gravis, where divalent anti-acetylcholine receptor antibodies induce disappearance of acetylcholine receptors (18), both by "modulation" and by directing macrophage attack (1).

We have recently developed a method for the more than 8000-fold purification of rabbit liver growth hormone receptors by a combination of differential affinity chromatography, isoelectric focusing, and gel filtration (19). The work presented here describes the characterization of antiserum generated against this preparation and the use of these antisera for structural analysis of the receptor. This study provides a starting point for further studies of the growth hormone receptor structure, function, and turnover based on the use of these antisera.

MATERIALS AND METHODS

Ovine growth hormone (NIH oGH1 (88)), bovine growth hormone (NIH bGH 1003A), and ovine prolactin (NIH oPrl S12) were gifts of the National Institutes of Health. Insulin (crystalline pork insulin, 24 IU/mg) was donated by Connaught Laboratories, Toronto, and highly purified bovine follitropin was a gift from Dr. K. W. Cheng, University of Manitoba. All hormones except insulin were iodinated by the lactoperoxidase method of Thorell and Johannson (20) as described previously (19). Insulin was iodinated with chloramine-T, using a 2 μl excess of chloramine-T reacted for 25 s at 20°C. All hormones were fractionated on Sephadex (G-100 or G-50) columns after iodination. γ-Globulin fractions (5 μl) were iodinated as described with lactoperoxidase, and the resulting iodination mixture fractionated on Sepharose CL6B.

Goat anti-guinea pig immunoglobulins was purchased from Miles Laboratories, Inc. Rabbit anti-human growth hormone (anti-hGH) and rabbit anti-ovine growth hormone (anti-oGH) antisera were generated against National Institutes of Health standard preparations in our laboratory using standard techniques.

Preparation of Receptor-containing Fractions—The liver receptor preparations used in this study represent three stages in the purification of the growth hormone (GH) receptor, as described elsewhere (19). For most of the basic studies on inhibition of 125I-hormone binding, crude microsomal (100,000 x g/60 min) fractions of late pregnant rabbit, sheep, mouse, and rat livers, or of human liver, were used. Similar fractions were also prepared from term human placenta, porcine testis, and late pregnant rabbit mammary tissue for the

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specificity studies. In all cases, initial homogenization of the tissue was obtained with a Polytron PT-10 homogenizer (Brinkmann) at a setting of 8 to 10 for 30 to 60 s, depending on the nature of the tissue. Protein content of the resuspended membranes was estimated by the method of Lowry et al. (21) using albumin standards.

Immunoprecipitation and some inhibition studies were carried out in some cases with crude Triton X-100 solubilized (1% v/v) extracts of pregnant rabbit liver membranes (300,000 g/90-min superna-
tants). In other cases, rabbit liver growth hormone and prolactin receptors purified by differential affinity chromatography on hGH affinity columns (19) were used.

Binding Assays—The membrane-binding assay was essentially that of Tsuchiya and Friesen (22) except that membranes (0.2 ml in 25 mm Tris-HCl, 10 mm MgCl₂, 0.1% albumin, pH 7.5, radioreceptor assay buffer) were preincubated for 16 h at 4°C with antisera dilutions (0.1 ml) before being washed three times with 10 volumes of assay buffer. Membranes were then resuspended in 0.3 ml of assay buffer, and the hormone binding reaction was initiated by the addition of [¹²⁵I]-hGH with or without excess unlabeled hormone, in order to determine specific binding. An incubation time of 3 to 5 h at 22°C was used for GH receptor assay, an incubation of 16 h at 22°C for prolactin and follicitropin receptors, and an incubation of 16 h at 4°C was used for insulin receptor assay. Incubations were terminated by the addition of 5 ml of ice-cold assay buffer, centrifuged, decanted, and the pellets counted. For soluble receptor assay inhibitory serum effects were generally minimized by the use of y-globulin fractions, and bound/free separation was achieved with polyethylene glycol (PEG) (2000) (23). Determinations were always at least in duplicate in all binding assays.

In some experiments an immunoprecipitation technique was used to obtain bound/free separations, and in this case the soluble receptors were prelabeled with [¹²⁵I]-hormone for the standard incubation time in assay buffer, and the hormone-receptor complex was then combined with antireceptor antibody at varying dilutions over a 16- h 4°C incubation. After addition of normal guinea pig serum to give a fixed final serum concentration, the ternary complex was immunoprecipitated by the addition of anti-guinea pig immunoglobulin anti-

In order to establish the specificity of this inhibition, we examined the effects of these antisera on the binding of a number of other [¹²⁵I]-labeled hormones to their receptors. Fig. 2A shows that these antisera, as well as control sera, had no effect on the binding of [¹²⁵I]-oPr1 to late pregnant rabbit mammary membranes, while antisera generated against partially purified (>1000-fold) rabbit mammary prolactin receptor inhibited [¹²⁵I]-oPr1 binding effectively. In Fig. 2B this experiment was repeated with rabbit liver membranes, and it can be seen that here the anti-prolactin receptor antisera was not as effective in inhibiting [¹²⁵I]-oPr1 binding as it was with the mammary prolactin receptor. This is presumably because the growth hormone receptor comprises a component of the [¹²⁵I]-oPr1 binding, and this component can be inhibited by anti-oGH receptor antisera as illustrated in the figure (see under "Discussion"). The binding of [¹²⁵I]-insulin to rabbit liver and human placental membranes was unaffected by antisera 1:4, as was the binding of [¹²⁵I]-bovine follicitropin to porcine testicular membranes (data not shown). Thus, it would seem that the inhibitory effect is specific for the oGH receptor among the polypeptide hormone receptors examined.

The binding of [¹²⁵I]-oGH binding as described could also result from the presence of anti-oGH or anti-hGH antibodies generated against hGH originating from hGH affinity columns by release of bound hormone. The hGH becomes bound to the

Fig. 1. Inhibition of [¹²⁵I]-oGH binding to rabbit liver membranes by antisera raised against purified oGH receptor. The assay procedure was described under "Materials and Methods" and involved preincubation of pregnant liver microsomal membranes X (114 µg of protein) for 16 h at 4°C with varying dilutions of antisera (AS) to oGH and control sera, followed by buffer wash of the membranes, addition of [¹²⁵I]-oGH (76,000 cpm), and incubation for 5 h at 22°C. Excess unlabeled oGH (1 µg/ml of final concentration) was added to determine specific binding in control ani serum tubes, and all data are expressed as a percentage relative to this control (15.3%). Normal guinea pig (G.P.) serum controls (3) also included antimammary prolactin receptor antisera.

**RESULTS**

**Inhibitory Action of Antisera Toward oGH Binding**

Assay of a number of antisera for inhibition of [¹²⁵I]-oGH binding to rabbit liver membranes as previously described

† Determined by the oGH radioimmunoassay using guinea pig anti-
oGH.

* Lindström et al. (12) have reported experimental myasthenia gravis in rats with doses as low as 0.3 µg of purified acetylcholine receptor, in accord with the highly immunogenic nature of complex glycoproteins.
soluble receptors and is carried through the remainder of the purification procedure. This can be demonstrated by coupling $^{125}$I-oGH to the affinity columns along with the hGH and then following the counts (19). Fig. 3 shows that no anti-oGH antibodies are present in the four antisera and that only a trace of anti-hGH antibody is present in antisera 3. In another experiment, incubation of $^{125}$I-oGH with antisera 3 at a 1:40 dilution for the standard assay time (5 h, 22°C) produced no change in the elution position of $^{125}$I-oGH (2 x void volume) on Sephadex G-100 column (1.5 x 50 cm), indicating the absence of hormone-binding proteins in the antisera. Since the membrane wash procedure prior to addition of $^{125}$I-oGH reduces serum proteins to a very low level, it is considered extremely unlikely that the inhibition of $^{125}$I-oGH binding shown here results from the presence of anti-oGH antibodies or other serum oGH-binding proteins.

**Immunoglobulin Nature of the Inhibitory Activity**

The inhibitory activity is an immunoglobulin, according to the following criteria. Firstly, inhibitory activity is only present in the 20 to 40% ammonium sulfate fraction and elutes in the same position as the y-globulin standard on Sepharose 6B (Fig. 4). Secondly, it is possible to immunoprecipitate the $^{125}$I-oGH-soluble receptor complex after it has been incubated with antisera 1 to 4 by the addition of goat anti-guinea pig immunoglobulin antisera. Fig. 5A shows that only the y-globulin fractions derived from antisera 1 to 4 were able to facilitate immunoprecipitation of hormone-receptor complexes in soluble rabbit liver extracts. The maximum extent of $^{125}$I-oGH specifically immunoprecipitated (12%, calculated as the percentage of counts immunoprecipitated after subtraction of the percentage precipitated in the presence of 1 µg/ml of oGH) approaches the amount of specific binding determined by polyethylene glycol precipitation (14%). The order of effectiveness of the antisera in immunoprecipitation also correlated with their effectiveness in inhibiting binding of $^{125}$I-oGH to liver membrane preparations. The specificity of the immunoprecipitation reaction was corroborated by the lack of immunoprecipitation of $^{125}$I-oPrl-prolactin receptor complexes in rabbit mammary gland-soluble extracts by these antisera, whereas anti-prolactin receptor antisera were able to immunoprecipitate all of the specifically bound $^{125}$I-oPrl (data not shown).

Immunoprecipitation of $^{125}$I-oGH was also observed when the highly (>8000-fold) purified receptor was incubated with y-globulin fractions from serum 3, and the extent of immunoprecipitation found correspond to the extent of specific binding of $^{125}$I-oGH determined by polyethylene glycol precipitation (6.2%) (Fig. 5B). Thus there is good evidence that the inhibitory activity is an antibody generated against the purified putative growth hormone receptor.
Studies with Anti-Growth Hormone Receptor Antibodies

Mechanism of Inhibition

Lineweaver-Burk analysis of the inhibition produced by different concentrations of the γ-globulins from antiserum 3, in the presence of varying 125I-oGH concentrations (Fig. 6A) shows the mechanism of inhibition to be competitive, resulting in a lowered affinity of receptor for its hormone. This observation in apparent disagreement with the observation that these antibodies can form an immunoprecipitable ternary complex with the 125I-oGH-soluble receptor complex. However, a Dixon plot of the Lineweaver-Burk data (Fig. 6B), along the lines suggested by Shiu and Friesen (2), reveals that, as with the prolactin receptor antiserum, the plot obtained is similar to that seen with hyperbolic competitive inhibition (24), which would allow for the existence of 125I-hormone-receptor-antibody complexes. This conclusion is supported by the results shown in Fig. 7, which demonstrate that the order of addition of trace and antibody is critical in facilitating ternary complex formation. Thus, if the normal order of first addition of trace followed 5 h later by antibody addition is reversed, producing a situation closer to the membrane assay, immunoprecipitation of ternary complexes by second antibody is markedly reduced. Thus it seems that at least one of
Studies with Anti-Growth Hormone Receptor Antibodies

The antigenic determinants overlap with the hormone-binding site in such a way as to partially block it. Further evidence for competition by these antibodies in the region of the binding site was provided by an experiment with

![Diagram](http://www.jbc.org/)

**Fig. 8.** Correspondence of displacement of specific $^{125}$I-$\gamma$-globulin binding and $^{125}$I-oGH binding by antiserum $\gamma$-globulins. $\gamma$-Globulin fractions derived from antiserum 3 by ammonium sulfate precipitation and Sepharose CL-6B gel filtration were lactoperoxidase-iodinated as described under "Materials and Methods." Rabbit liver membranes (160 $\mu$g of protein) were incubated with 0.1 ml of $\gamma$-globulin and $5 \times 10^7$ cpm of $^{125}$I-$\gamma$-globulins (60 $\mu$Ci/ug) in a final volume of 0.5 ml, using radioreceptor assay buffer with 1% albumin added. A parallel set of incubations was set up with $^{125}$I-oGH (80,000 cpm) and the same set of $\gamma$-globulin standards. Two parallel sets of tubes were also set up with normal serum $\gamma$-globulins. All tubes were then incubated for 6 h at 22°C, and the reaction was terminated by chilled buffer addition and centrifugation, as usual. A total binding of 2.2% was obtained using $^{125}$I-$\gamma$-globulins, and 40% of these counts were displaced by 500 ng/ml of antireceptor $\gamma$-globulin standard only. Addition of 1 $\mu$g/ml of oGH also displaced 25% of bound $^{125}$I-$\gamma$-globulins. A total of 14% of $^{125}$I-oGH added was specifically bound, and, as can be seen, displacement of the tracer by increasing concentrations of antireceptor $\gamma$-globulins coincided with the ability of these $\gamma$-globulins to displace $^{125}$I-$\gamma$-globulin binding.

**Fig. 9.** Effect of anti-GH receptor antisera on other species of GH receptors. The standard membrane competitive binding assay was set up with varying dilutions of antisera (AS1 to 4) and the following receptors: A, pregnant rat liver membranes (710 $\mu$g of protein); B, pregnant mouse liver membranes (440 $\mu$g of protein); C, female sheep liver membranes (733 $\mu$g of protein); D, female human liver membranes (312 $\mu$g of protein); E, female sheep liver membranes (314 $\mu$g of protein). In A, B, and C, $^{125}$I-oGH was used as tracer, in D, $^{125}$I-hGH, and in E, $^{125}$I-oP1 ($^{125}$I-oPL). Approximately 80,000 cpm of tracer was added in all cases, and incubation was for 6 h at 22°C. Specific binding was 8.1% for A, 10.2% for B, 9.2% for C, 10.7% for D, and 9.1% for E.

**Structural Studies**

**Species Specificity**—One of the uses of antireceptor antibodies is that of a probe for structural similarities between receptors from different species. Currently, growth hormone receptors have been identified in pregnant rabbit, rat, mouse, and sheep livers, and in human liver (see Ref. 19). Fig. 9 shows the inhibition patterns obtained with the four anti-GH receptor antisera when examining $^{125}$I-oGH, $^{125}$I-hGH, and $^{125}$I-oP1 binding to membranes prepared from these livers. It should be noted that control guinea pig sera were ineffective in all species. It is clear from this figure that while the antisera are considerably more effective against rabbit GH receptors than against the other species, nevertheless there are common antigenic determinants in the region of the hormone-binding site. The order of effectiveness of the different antisera varies with each species. Thus, antiserum 3 is more effective in rabbit and man, whereas antiserum 1 is more effective in the...
other species. That the inhibition of $^{125}\text{I}-\text{oPr1}$ binding to human liver was not caused by the minimal anti-hGH titer in antisera 3 referred to earlier was shown by the addition of anti-hGH antisera at concentrations (1:10' to 2:10') able to totally immunoprecipitate $^{125}\text{I}-\text{hGH}$ in the hGH radioimmunoassay, in place of the anti-GH receptor antisera. Subsequent washing of the membranes (as detailed under "Materials and Methods") prior to addition of tracer resulted in no inhibition of $^{125}\text{I}-\text{hGH}$ binding to the human liver membranes. Since the titer of anti-hGH antibodies found in antisera 3 represents only a minute portion of the anti-hGH antisera added here, it is extremely unlikely that the inhibition seen here is the result of anti-hGH antibody action.

One other relevant finding shown in Fig. 9 is the immunological similarity that exists between ovine placental lactogen and mouse growth hormone receptors in the sheep liver. Since there was no inhibition of $^{125}\text{I}-\text{oPr1}$ binding by anti-prolactin receptor antisera, even though these antisera have been shown to be effective in species as disparate as mouse and man (2), it appears that the oPr1 receptor closely resembles (or is) the GH receptor, as indicated by hormonal specificity studies (25).

The Shared Growth Hormone Receptor—The Scatchard analyses and displacement data presented elsewhere (19) provide strong support for the concept that rabbit liver contains a prolactin-specific receptor and a GH receptor which has a high affinity for oGH and 10-fold lower affinity for oPr1. Evidence was presented that differential affinity chromatography on hGH affinity gels separated these two receptors, specifically by means of 4 M urea elution (yielding the GH receptor) or 5 M MgCl2 elution (yielding the prolactin receptor).

Fig. 10, A and B, shows how we have been able to apply the anti-GH and anti-prolactin receptor antisera to test our hypothesis of a shared GH receptor and a specific prolactin receptor. Various concentrations of $\gamma$-globulins of the most inhibitory antisera were preincubated with the urea affinity fraction for 24 h at $4^\circ\text{C}$ before adding $^{125}\text{I}-\text{oGH}$ and performing the standard soluble receptor assay. Fig. 10A shows that this antisera was very effective in inhibiting $^{125}\text{I}-\text{oGH}$ binding to the receptor and that both mammary prolactin receptor-specific antisera $\gamma$-globulins and normal serum $\gamma$-globulins were ineffective in competing for $^{125}\text{I}-\text{oGH}$ binding. In accord with our hypothesis, $^{125}\text{I}-\text{oPr1}$ binding to the urea affinity fraction was also blocked by anti-GH receptor, but not by anti-prolactin receptor antisera $\gamma$-globulins. When the MgCl2 affinity fraction ("prolactin receptor") was assayed on the other hand, the anti-GH receptor antisera $\gamma$-globulins and normal serum $\gamma$-globulins failed to inhibit binding of $^{125}\text{I}-\text{oPr1}$ to the receptor, whereas anti-prolactin receptor $\gamma$-globulins were very effective inhibitors (Fig. 10B), as would be predicted if this were a prolactin-specific receptor.

DISCUSSION

Evidence has been presented here that we have produced specific antisera to the GH receptor by immunization of guinea pigs with a highly purified GH-binding fraction from rabbit livers. The fact that these antisera did not inhibit the binding of $^{125}\text{I}-\text{insulin}$ or $^{125}\text{I}-\text{oPr1}$ to their respective receptors is taken as evidence that the affinity chromatography step has selectively separated the putative GH receptor from other polypeptide hormone receptors in the solubilized membrane extracts. This complements the finding that anti-prolactin receptor antisera inhibit only the binding of $^{125}\text{I}-\text{oPr1}$ to its specific receptor and underlines the distinction between the two receptors. Tsushima (26) has recently presented a preliminary report on "anti-GH receptor antibodies" generated against a rabbit liver preparation purified by concanavalin A-Sepharose, DEAE-cellulose, and Sepharose 6B chromatography. No specificity studies were presented with this report, but in the light of the considerations detailed in the preceding paper, a hormone-specific affinity step would seem to be an essential prerequisite for the isolation of particular hormone receptor.

We are fortunate that our antisera allowed us to test the hypothesis proposed on the basis of our hormone specificity studies in rabbit liver membranes that the liver GH receptor will bind oPr1, albeit with considerably lower affinity than oGH. Thus the anti-GH receptor antisera was able to inhibit both $^{125}\text{I}-\text{oGH}$ and $^{125}\text{I}-\text{oPr1}$ binding to the GH receptor fraction, even though it was unable to inhibit $^{125}\text{I}-\text{oPr1}$ binding to the oPr1-specific receptor. Conversely, anti-prolactin receptor antisera was unable to inhibit the binding of either $^{125}\text{I}-\text{oGH}$ or $^{125}\text{I}-\text{oPr1}$ to the GH receptor fraction. This clear immunological separation of receptor type was only made possible by the use of a receptor-specific differential affinity chromatography procedure.

The data presented here also represent the first demonstration of antigenic similarities between the GH receptors of such differing species as mouse and man and represent the converse

**Fig. 10.** A, effect of antiserum $\gamma$-globulins on $^{125}\text{I}-\text{oGH}$ and $^{125}\text{I}-\text{oPr1}$ binding to GH receptor (urea) fraction purified by affinity chromatography. An aliquot (5 $\mu$g of protein) of dialyzed urea fraction (prepared as previously described) was incubated with increasing dilutions of $\gamma$-globulins (1.6 ng/ml) derived from anti-GH receptor 3, from anti-prolactin receptor antisera, or from normal guinea pig serum, for 24 h at $4^\circ\text{C}$ in a final volume of 0.3 ml of radioreceptor assay buffer. $^{125}\text{I}-\text{oGH}$ (66,000 cpm) or $^{125}\text{I}-\text{oPr1}$ (92,000 cpm) were then added, and the incubation carried on for a further 5 h at $22^\circ\text{C}$. The reaction was terminated with polyethylene glycol separation of bound and free. Triplicate tubes were included with excess cold oGH and $^{125}\text{I}-\text{oPr1}$ for the determination of specific binding, which was 6.0% for $^{125}\text{I}-\text{oGH}$ and 2.5% for $^{125}\text{I}-\text{oPr1}$. The inset figure shows the effect of these $\gamma$-globulins on $^{125}\text{I}-\text{oPr1}$ binding $\bullet$, anti-GH receptor $\gamma$-globulins; $\blacksquare$, anti-prolactin receptor $\gamma$-globulins; $\blacktriangle$, normal guinea pig serum $\gamma$-globulins. B, effect of antiserum $\gamma$-globulins on $^{125}\text{I}-\text{oPr1}$ binding to the rabbit liver prolactin receptor (MgCl2) fraction purified by affinity chromatography. Conditions are identical with A, except that only $^{125}\text{I}-\text{oPr1}$ was used, and the incubation time was 12 h at $22^\circ\text{C}$. Specific binding of the control was 7.0%. Excess cold oPr1, 1 $\mu$g/ml.
of studies on immunological specificity among the growth hormones. It is of interest that the extensive immunological differences between primate and nonprimate GH (27) are not reflected in the actions of antisera 3, which is quite effective against human and rabbit liver receptors. The variability in relative potency of the four antisera among the five species is reminiscent of that reported by Flier et al. (6) for the insulin receptor, although it is clear that these antisera are far more effective against rabbit receptors. An identity of antigenic determinants over a wide range of species has already been reported for the acetylcholine receptor (1) and the prolactin receptor (2) and presumably indicates the importance of these structures (either protein or carbohydrate) in the biological functioning of these receptors. We intend to pursue this problem further using the immunoprecipitative technique to examine antigenic determinants located away from the hormone-binding site.

The mechanistic studies reported here show that antisera 3 acts by decreasing the affinity of the receptor for its hormone, as do a number of other antireceptor antisera (2, 6, 7). The observation that it is possible to immunoprecipitate a ternary (hormone-receptor-antibody) complex if the receptor is prelabeled with $^{125}$I-oGH can be rationalized with this conclusion by the finding of hyperbolic competitive inhibition on replotting of the reciprocal plot data in the form of a Dixon plot. In this model the antibody would bind at a site partly overlapping the hormone-binding site, or it may induce a conformational change by binding to a distant antigenic site in such a way as to block hormone binding. If the hormone were to bind first, this would lock the hormone receptor together in a way that still allowed antibody binding. The alternative model, involving a heterogenous population of antibodies directed at other antigenic sites on the receptor in addition to the hormone-binding site, rather like the variability seen with acetylcholine receptor antibodies (1) and anti-(Na$^+$,K$^+$)-ATPase antibodies (28) is also very likely. In this model, binding of hormone to receptor would block access of the binding site-directed antibodies while still allowing other site-directed antibodies to interact in the immunoprecipitation mechanism. This would explain the fact that oGH is only partly effective in displacing $^{125}$I-labeled antireceptor $\gamma$-globulins from liver membranes compared to the $\gamma$-globulins themselves.

In view of the lack of homogeneity of the receptor preparation used for immunization (one major and two minor bands observed on sodium dodecyl sulfate gels), it is likely that these antisera contain antibodies directed against some nonreceptor determinants. It may be possible to remove these antibodies by absorption with nonreceptor-containing tissues, and this will be necessary before these antisera can be used to study receptor biosynthesis. It appears unlikely that the antibodies studied here are directed against a molecule closely associated with, but different from the receptor, for two reasons. Firstly, the antibodies were effective in totally immunoprecipitating a highly (>8000-fold) purified receptor preparation, as judged by comparison with polyethylene glycol-determined specific $^{125}$I-oGH binding. Secondly, the correlation of $^{125}$I-$\gamma$-globulin displacement with $^{125}$I-oGH displacement argues in favor of a receptor-directed antibody, as does the finding of competitive inhibition and lowered receptor affinity for its hormone.

As outlined in the introduction, there are a variety of valuable applications for these antibodies. At present we are examining the biological effects of these antisera both in vitro and in vivo, with the intention of establishing the biological significance of the GH binding fraction and of obtaining an estimate of "spare receptor" content.

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