

Studies Using Anterior Pituitary Hormones as Antigens.* (25279)

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(Introduced by R. V. Christie)

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Production of antibodies to anterior pituitary hormones became a subject of controversy following the report of Collip and Anderson that animals receiving repeated injections of TSH and gonadotrophin became refractory to these hormones(1,2). The results of subsequent work on this subject were conflicting; even where evidence of antibody formation was obtained, there remained the possibility that such antibodies were not to the hormone itself, but rather to impurities in the hormone preparations(3-7). More recently, Cruickshank and Currie reported on the production of precipitating antibodies to human growth hormone, ACTH and TSH, which were not, however, hormone specific(8). In the past year, reports have appeared which present evidence for antigenicity of growth hormone(9-12). Thus, Read has reported on production of hemagglutinating antibodies to human growth hormone, and applied anti-human growth hormone sera to detection of growth hormone in human body fluids(10). Hayashida and Li produced precipitating antibodies to bovine and human growth hormones which could inhibit the effects of growth hormone on the epiphyseal cartilage of the hypophysectomized rat(11,12).

Methods. In our laboratory, thyroidectomized rabbits were immunized against bovine TSH, adrenalectomized rabbits against porcine ACTH, and intact rabbits against bovine, porcine and human growth hormones. For immunization, these hormones were emulsified in Freund's adjuvant (complete), and also precipitated with alum. Total dosage of each hormone varied from 5 to 80 mg, and a

variety of immunization schedules and routes were used. At suitable intervals the rabbits were bled and sera tested for antibodies using a hemagglutination test in which antigen is coupled to red blood cells by means of bis-diazotized benzidine(13-16). In this test, hemagglutination occurs when antigen-sensitized red cells are suspended in antiserum, and hemagglutination is inhibited by pre-incubating the antiserum with specific antigen. Using this technic, the following determinations were carried out on each antiserum: 1) titre, 2) specificity, and 3) smallest quantity of specific antigen which could be detected in the system by means of hemagglutination inhibition (Table I).

Results. Antibodies in good titre were obtained to all substances used as antigens. With TSH, extensive cross reactions occurred, although much smaller quantities of TSH (0.1 μ g) were required to consistently produce hemagglutination-inhibition of TSH antiserum as compared with other pituitary hormones (10 μ g). These cross reactions are not surprising since the TSH which we used was relatively impure.

From the studies done, the antibodies to ACTH appeared to be hormone specific but not species specific. Thus either porcine or human ACTH in quantities as little as 0.01 μ g consistently produced hemagglutination inhibition.

ACTH preparations from a number of animal species are known to be biologically active in man and other animals. Lack of species specificity of ACTH antiserum which we have demonstrated is in keeping with the known biological activity of ACTH from various animal species.

Antibodies to human growth hormone were for the most part hormone specific, although some cross reactions with human ACTH did occur. Whereas as little as 0.01 μ g of human growth hormone consistently produced hemagglutination-inhibition, at least 1 μ g of human ACTH was required for this reaction.

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TABLE I.

Antigen*	Antibody titer†	Hemagglutination-inhibition (μ g)‡									
		BGH	PGH	HGH	P ACTH	H ACTH	PGT	BTSH	PROL	BGG	HGG
BGH	1/8000-1/256,000	1	0	0	0	ND	0	0	ND	1	ND
PGH	1/80 -1/1280	0	.01	0	0	"	0	0	"	ND	"
HGH	1/64 -1/12,800	0	0	.01	0	1	0	0	0	"	0
P ACTH	1/20 -1/8000	0	0	0	.01	.01	0	0	0	"	ND
BTSH	1/1000-1/8000	10	10	ND	10	ND	10	.1	ND	0	"

* BGH, bovine growth hormone (Frank W. Horner); PGH, porcine growth hormone (Nordic Biochemicals); HGH, human growth hormone (supplied by Dr. Phillip Henneman); all growth hormone prepared by Raben technique; P ACTH, porcine adrenocorticotrophic hormone (Nordic Biochemicals); H ACTH, human adrenocorticotrophic hormone (Nordic Biochemicals); PGT, porcine pituitary gonadotrophin (Ferring Ab. Malmo); BTSH, bovine thyroid stimulating hormone (Nordic Biochemicals); PROL, porcine prolactin (Ferring Ab. Malmo); BGG, bovine gamma globulin; HGG, human gamma globulin.

† Range of titer of antisera from a group of animals.

‡ Where a figure is given, this represents smallest quantity of antigen which would consistently inhibit hemagglutination.

0 = no hemagglutination inhibition; ND = not done.

Cross reactions between anti-human growth hormone sera and ACTH were also reported by Read(10).

Antibodies to bovine growth hormone showed cross reactions with bovine gamma globulin, which could be eliminated by prior absorption of the serum with bovine gamma globulin. This is consistent with the findings of Hayashida and Li(11). Antibodies to porcine growth hormone appeared from the studies done, to be hormone specific.

Growth hormone antisera showed no cross reactions with growth hormone preparations from other species. This may be interpreted as immunologic evidence for species specificity of growth hormone, a property which is well known biologically(17), and which is consistent with the report of Hayashida and Li (12).

Read reported on the use of anti-human growth hormone sera in assaying growth hormone in human body fluids(10). In our laboratory the application of human growth hormone and ACTH antisera to detection and measurement of these hormones in human body fluids is in progress.

Summary. Evidence is presented for antigenicity of TSH, ACTH, and growth hormone. Rabbit antisera to these hormones were prepared and then tested by means of a hemagglutination test in which antigen is coupled to red blood cells, by means of bis-diazotized benzidine. The antibodies to TSH showed extensive cross reactions with other pituitary hormones. The antibodies to ACTH appeared to be hormone specific but not species specific. Antibodies to bovine, porcine,

and human growth hormones appeared to be species specific, and for the most part, hormone specific. The application of human growth hormone and ACTH antisera to detection and measurement of these hormones in human body fluids is in progress.

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