Clin. exp. Immunol. (1966) **1**, 377–389.

AN IATROGENIC AUTOANTIBODY: IMMUNOLOGICAL RESPONSES TO 'PITUITARY SNUFF' IN PATIENTS WITH DIABETES INSIPIDUS

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(*Received* 12 *January* 1966; *accepted* 1 *April* 1966)

SUMMARY

In the treatment of patients with diabetes insipidus, due to a variety of causes, by nasal insufflation of 'pituitary snuff' (porcine or bovine, acetone-dried posterior pituitary), asthma may develop and immediate reactions are given on skin testing with the treatment material, In addition, in patients with or without asthma, precipitating antibodies have been found in the serum capable of reacting with heterologous serum protein constituents of the pituitary snuff, and also with antigens of homologous and heterologous pituitary gland. Immunofluorescence studies have shown that antibodies are present not only to heterologous tissues (bovine and porcine) but also to human tissue—in particular to antigens in the pituitary. Inhalation of the heterologous pituitary antigens has thus led to the appearance of 'hetero-stimulated', 'autoreactive' antibodies against the homologous pituitary. These antibodies are related to the treatment and not to the original causation of the disease.

The entry of antigens through the respiratory tract may result not only in the well-known appearance of reaginic antibody responsible for bronchial asthmatic reactions, but also for the appearance of precipitating antibody. These precipitins appear to be responsible for reactions in the peripheral gas-exchanging tissues of the lungs, resulting in diseases such as farmer's lung (Pepys & Jenkins, 1965) and bird breeder's lung (Barboriak, Sosman & Reed, 1965; Reed, Sosman & Barbee, 1965; Hargreave *et al.*, 1966). Inhaled vegetable dusts also stimulate the production of specific precipitins (Pepys, Longbottom & Jenkins, 1964).

The present report on patients with diabetes insipidus under treatment by nasal insufflation of porcine and bovine pituitary snuff shows that the inhalation of these animal antigens may also stimulate the production of precipitins which react with heterologous serum proteins and with antigens in both heterologous and homologous pituitary glands.

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MATERIALS AND METHODS

Patients under investigation

The sera of nine patients treated with pituitary snuff were examined (Table 1). A diagnosis of diabetes indipidus due to a variety of different causes was made in eight cases, and of

TABLE 1. Clinical features of patients under observation

Patient No.	Sex	Age (yr)	Cause of diabetes insipidus	Duration of disease (yr)	Nature and duration of treatment	Respiratory tract and other reactions
1	F	17	Tuberculous meningitis	14	Porcine pituitary snuff; 14 years	Nil
2	F	60	Not known	?	Porcine and bovine pituitary snuff	Asthma, faintness ½ hr after each of three injections of vasopressin
3	F	44	Not known	6	Porcine and bovine pituitary snuff; 6 years	Rhinitis, asthma, itching of eyes and face, and miliary infiltration of lungs, appearing 5 years after the start of treatment
4	M	56	Not known	16	Porcine pituitary snuff and pitressin tannate injections	Asthma and miliary infiltration of lungs, appearing 9 years after the start of treatment
5	M	20	Suprasellar syst	13	Pituary snuff and pitressin tannate injections	Nil
6	F	47	Hypophysectomy for Cushing's syndrome	2	Porcine pituitary snuff and pitressin tannate injections; 2 years	Itching of face after injections of pitressin
7	M	43	Idiopathic hypopituitarism	1½	Porcine pituitary snuff and pitressin tannate injections	Occasional sneezing 3 weeks after starting the snuff
8	F	29	Eosinophilic granuloma of lungs, thyroid and pituitary	6	Porcine pituitary snuff and pitressin tannate injections	Nil
9	M	34	Psychogenic polyuria	5	Porcine pituitary snuff and pitressin tannate injections; 5 years, intermittent treatment	Nil

psychogenic polyuria in one case. All had been treated with porcine pituitary snuff, patients Nos. 2 and 3 had had both porcine and bovine pituitary snuff, and five had also been given pitressin tannate by injection. Patient No. 3 was investigated in more detail in the Brompton Hospital.

Test materials

(1) The predominantly porcine pituitary snuff (Disipidin) and bovine pituitary snuff (5281) used for testing were made by Messrs Paines & Byrne Ltd. They consisted of finely ground acetone-dried posterior pituitary gland. A comparable preparation of human pituitary was also tested. The pituitary snuff was dissolved in physiological saline containing 0·5% phenol for skin tests and 0·1% sodium azide for precipitin tests. The insoluble material was removed by centrifugation.

(2) Porcine and bovine sera were used for skin and serological tests.

(3) Freeze-pressed, freeze-dried, ground, whole tissue of human, bovine, porcine and bovine thyroid gland was used.

(4) Synthetic lysine vasopressin (Lypressin B.P. Syntopressin, Sandoz Ltd, 50 units/ml) containing 9 amino-acid residues (M.W. 1056) was used for skin and nasal tests. The vehicle was provided by Sandoz Ltd for use as a control.

TABLE 2. Skin (prick) tests

Patient No.	Respiratory and other reactions to pituitary snuff	Immediate reactions to:				
		Pituitary snuff		Sera		
		Porcine	Bovine	Human	Porcine	Bovine
1	Nil	0	0	0	0	0
3	Rhinitis, asthma and itching of eyes and face	+	+	0	+	+
6	Itching of face	+	+	0	0	0
7	Occasional sneezing	0	0	0	0	0
8	Nil	0	0	0	0	0

Skin tests

Prick tests were made in five subjects (Table 2) with the pituitary snuff (10 mg/ml); with the porcine and bovine sera, and with the lysine vasopressin (50 units/ml) and its vehicle.

In patient No. 3 intracutaneous tests were made with porcine pituitary snuff at concentrations of 1–0·001 mg/ml and with undiluted porcine and bovine sera. The pituitary snuff and lysine vasopressin and its vehicle were also tested by nasal insufflation.

Serological tests

Double diffusion tests by the Ouchterlony method were made with the pituitary snuffs in a concentration of 10 mg/ml. Immunoelectrophoresis tests were made with a concentration of 30 mg/ml using a modification (Longbottom & Pepys, 1964) of the micro-method of Scheidegger (1955). The sera were tested undiluted and concentrated two-fold, and were absorbed by adding 10 mg of the pituitary snuffs or thyroid extracts, or 0·1 ml of the animal sera, to 1·0 ml of the test serum.

Fluorescent antibody tests

Human tissues were obtained within 12 hr of death. Animal tissues were obtained at the slaughterhouse. All tissues were cut in blocks and frozen at −196°C until use. Sections 6 μ thickness cut on a Harris International Cryostat were fixed in acetone at 4°C for 10 min, and were then washed in saline and treated for 30 min at room temperature with antiserum. After one wash they were incubated for 30 min at 37°C with a 1:5 dilution of human complement. After three further washes the sections were stained with a fluorescent conjugate of an anti-human β_{1C}-globulin (C'_{3a}) serum. The fluorescein conjugate had been fractionated on DEAE-cellulose and the fraction used had a fluorescein–protein ratio of around 1 molar. The conjugate was used at a protein concentration of 1·0 mg/ml.

RESULTS

Skin tests

The reactions to prick tests with the pituitary snuff were modified by their vasoconstrictor effects. In twenty control subjects only a round blanched area, 3–5 mm in diameter, which was not accompanied by itching or erythema, was produced. In patients Nos. 3 and 6 reactions were obtained consisting of a blanched area surrounded by mottled erythema of approximately 10–15 mm diameter accompanied by itching. In some instances swelling, but not definite urticarial weals, was perceptible over the blanched area. Prick tests with porcine and bovine sera gave typical weals with erythema and itching in patient No. 3. Patient No. 6 gave negative reactions to the sera, and positive reactions to the pituitary snuffs.

Prick tests with the synthetic lysine vasopressin solution gave reactions which appeared to be more itchy and erythematous than the control vehicle in patients Nos. 3, 6 and 9. The reactions to the lysine vasopressin could not be accepted as unequivocally positive because of the irritant effect of the vehicle.

The reactions to the intracutaneous tests with the porcine pituitary snuff in patient No. 3 showed the marked effect of the vasopressin. Tests with 1·0 mg/ml gave only an area of blanching with no wealing, erythema or itching. With lower concentrations the wealing became more obvious, so that at 0·01 mg/ml a 5-mm weal was present with erythema 30 mm diameter and marked itching.

Intracutaneous tests with 0·02 ml of porcine and bovine serum were made in patient No. 3 and large wealing reactions 10–15 mm in diameter were produced. Within 3–4 hr of the test—after resolution of the weal —a second reaction appeared, consisting of an egg-shaped swelling protruding well above the skin. The swelling had a small central area of induration lying deep in the dermis, accompanied by extensive soft oedema and itching. The reaction was maximal within 7–8 hr, and resolved within 24–36 hr.

Nasal tests

Nasal insufflation of both porcine and bovine pituitary snuffs provoked rhinitis and asthma in patient No. 3. The porcine snuff was more finely powdered and provoked more symptoms than the coarser bovine material.

Nasal tests were made on separate days and four times each with both the synthetic lysine vasopressin solution and its vehicle in the patient No. 3. The patient was not aware of the nature of the material being tested on each occasion. No reactions were obtained to

the vehicle. The lysine vasopressin solution, however, regularly provoked rhinitis, asthmatic symptoms and itching of the nose, eyes and face and of the body, which appeared within ½ to 1½–2 hr. Eosinophil cells predominated in the watery nasal discharge.

Serological tests

The sera of five out of the nine patients gave precipitin reactions against the pituitary extracts and the heterologous sera. The reactions could be divided into two groups. The first consisted of reactions against serum protein antigens (SPA) of porcine and bovine origin, these antigens being present in the respective pituitary snuffs. The second consisted of reactions in two regions, against antigens of pituitary origin (PA), at the origin around

TABLE 3. Immunoelectrophoresis tests

Patient No.	Precipitation reactions to sera and pituitary snuffs				
	Sera		Pituitary snuffs (reactions attributed to serum protein antigens SPA, and pituitary antigens PA(a) and (b))		
	Porcine	Bovine	Porcine	Bovine	Human
1	+	+	*SPA/PA(a)(b)	SPA/0	0
2	+	+	*SPA/PA(a)(b)	SPA/0	PA(b)†
3	+	+	SPA/PA(b)	PA(b)	PA(b)‡
4	+	+	*SPA/PA(a)	SPA/0	0
5	+	+	SPA	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0

* Sera concentration two-fold.

† Shown by absorption tests in which addition of human pituitary to the serum inhibited the (b) arc produced by the heterologous pituitary snuffs.

‡ Positive precipitation reaction against human pituitary antigen also shown in double-diffusion test by direct tests and absorption tests.

the serum well and termed (a), and against negatively charged antigens, and termed (b). In four patients both SPA and PA precipitins were present, and in one, No. 5, only SPA precipitins were present (Table 3).

Double-diffusion tests

The precipitation reactions of the serum of patient No. 13 to porcine pituitary snuff are shown in Fig. 1. Attention is drawn to the strongest, innermost, arc lying nearest the serum well. Absorption of the serum with human pituitary snuff led to inhibition of this arc. Absorption of the patient's serum with porcine serum had no effect on this innermost arc,

but inhibited all of the other arcs. Thus, precipitins were present against antigens present in human as well as porcine and bovine pituitary snuff, in addition to the precipitins against the porcine serum proteins.

Tests on the sera of patients Nos. 2 and 3 with pituitrin (B.P.) (Parke-Davies) and corticotrophin (B.P.) (Organon) gave reactions of identity with arcs produced by the porcine pituitary extracts. All of these arcs were inhibited by absorption of the sera with the corticotrophin.

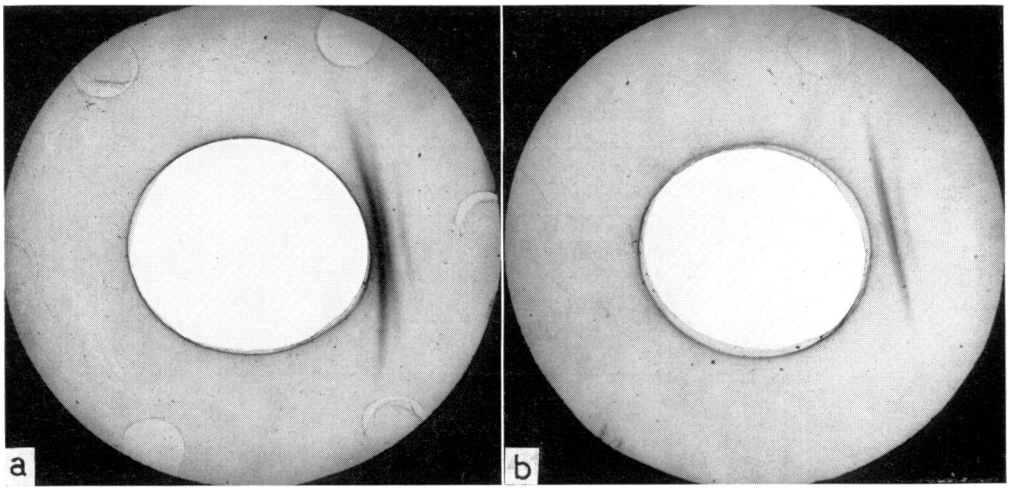


FIG. 1. Double-diffusion test with serum of patient No. 3 against porcine pituitary extract. Before (a) and after (b) absorption of serum with human pituitary. Absorption has resulted in the inhibition of the arc nearest the central serum well.

Immunoelectrophoresis tests

The immunoelectrophoresis test between porcine serum and pituitary snuff and the serum of patient No. 1 shows strong precipitation reactions against porcine serum proteins (Fig. 2). Absorption with the porcine serum led to the inhibition of all of the reactions to the porcine serum, and of certain of the reactions to the porcine pituitary snuff. The reactions which remained in regions (a) and (b) against the pituitary snuff were not inhibited by absorption of the serum with both porcine serum and porcine thyroid—thus showing that the reactions were not due to general tissue antigens. The arc in region (b) was inhibited by absorption with human pituitary snuff and all of the reactions were inhibited by porcine pituitary snuff. This confirmed the finding that the precipitins shown in the double-diffusion tests were reacting not only with porcine serum proteins but also pituitary antigens. Thus, the arcs in region (a) were due to porcine pituitary antigen, and in region (b) to related antigens in human and porcine pituitary.

Table 4 gives the results of absorption tests with the four sera which contained precipitins against both serum proteins and pituitary antigens. No inhibition was obtained by absorption of the sera with human thyroid. Absorption with porcine thyroid inhibited only the

reactions to the serum proteins in the porcine pituitary snuff. Absorption with bovine thyroid and with sheep thyroid inhibited the serum protein reactions of the porcine pituitary snuff in patient No. 3, but not in the other two patients tested. Whilst all the reactions to

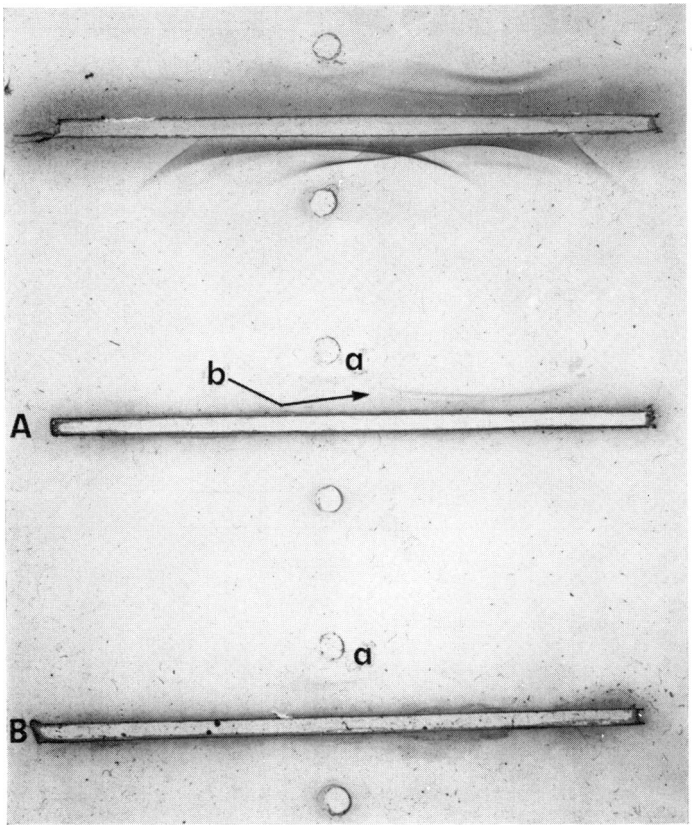


FIG. 2. Immunoelectrophoresis tests on serum of patient No. 1 against porcine pituitary extract above and porcine serum below.

(A) Absorption of serum with porcine serum and porcine thyroid has led to complete inhibition of reactions to porcine serum and inhibition of arcs attributable to porcine serum protein antigens contained in the pituitary extract, leaving arcs in regions (a) and (b).

(B) Absorption of serum with porcine serum and thyroid and human pituitary. This has led to the further inhibition of arcs in region (b) attributed to common pituitary antigens in the human and bovine pituitary, leaving the arcs in region (a) which were inhibited by antigens in the porcine pituitary only.

porcine pituitary snuff were inhibited by absorption with it, only the pituitary antigen PA(b) arc in patients Nos. 1 and 3 was inhibited by absorption with human pituitary. Absorption of the sera with both porcine serum and porcine thyroid inhibited only the serum reactions. When followed by absorption with human pituitary, the pituitary arc PA(b) was also

inhibited in patients Nos. 1 and 3, whilst the pituitary arc PA(a) given by the sera of patients Nos. 1 and 2 was not affected.

TABLE 4. Inhibition of serum protein antigen (SPA) and/or pituitary antigen (PA(a) and (b)) precipitation arcs of absorbed sera, in tests against porcine pituitary snuff

	Patient No. 1	Patient No. 2	Patient No. 3	Patient No. 4
Precipitation arcs present against SPA and PA antigens:	SPA/PA(a)(b)	SPA/PA(a)(b)	SPA/PA(b)	SPA/PA(a)
Absorption of serum with:	Inhibition of following precipitation arcs:			
Porcine serum	SPA	SPA	SPA	SPA
Human thyroid	0	NA	0	0
Porcine thyroid	SPA	NA	SPA	SPA
Bovine thyroid	0	NA	SPA	0
Sheep thyroid	0	NA	SPA	0
Human pituitary	PA(b)	NA	PA(b)	0
Porcine pituitary	SPA/PA(a)(b)	NA	SPA/PA(b)	SPA/PA(a)
Porcine serum and porcine thyroid	SPA	SPA	SPA	SPA
Porcine serum and porcine thyroid and human pituitary	SPA/PA(b)	SPA/PA(b)	SPA/PA(b)	SPA/0

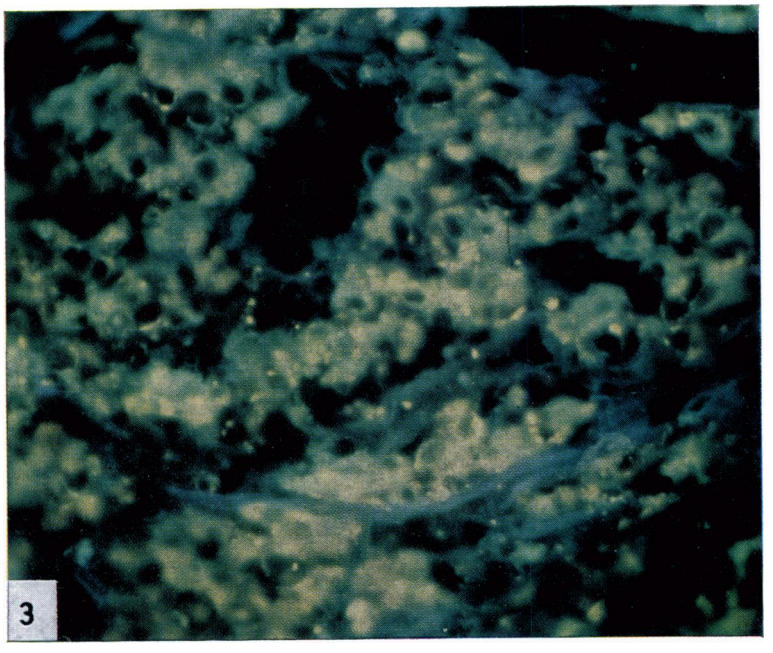
NA = Not available.

Fluorescent antibody tests

Detailed fluorescent antibody studies were performed on two sera, Nos. 1 and 3, which came to hand early in this study. Both sera gave universal staining of bovine and porcine pituitary sections in immunofluorescence complement fixation tests. Absorption with acetone powder of bovine and porcine liver weakened the staining slightly but no discrimination among different cellular elements could be detected. Distinct patterns of staining were, however, observed, in tests on human pituitary sections. With serum No. 1 bright staining was seen in the posterior pituitary of the nerve fibre elements. In addition, in the anterior pituitary, there was bright staining of certain groups of cells, and weaker staining of numerous other cells but a considerable number of the cell population showed no staining at all (Fig. 3). Serum No. 3 showed similar staining to No. 1 in the posterior pituitary but there was no staining of anterior pituitary elements (Fig. 4).

Comparison of the pattern of anterior pituitary staining given by the serum No. 1 with parallel sections stained by the Mallory trichrome method showed that the cells staining strongly belonged to the eosinophilic series. However, more cells were staining by the fluorescent antibody method than showed eosinophilic staining by the Mallory stain.

Absorption experiments were carried out to see whether the antibodies reacting with the human pituitary antigens were true autostimulated antibodies, or were cross-reacting antibodies stimulated by the heterologous pituitary antigens (Table 5). The sera, unabsorbed and after absorption with the various antigenic preparations shown, were tested both on human and on pig pituitary sections. The staining of human pituitary was abolished not



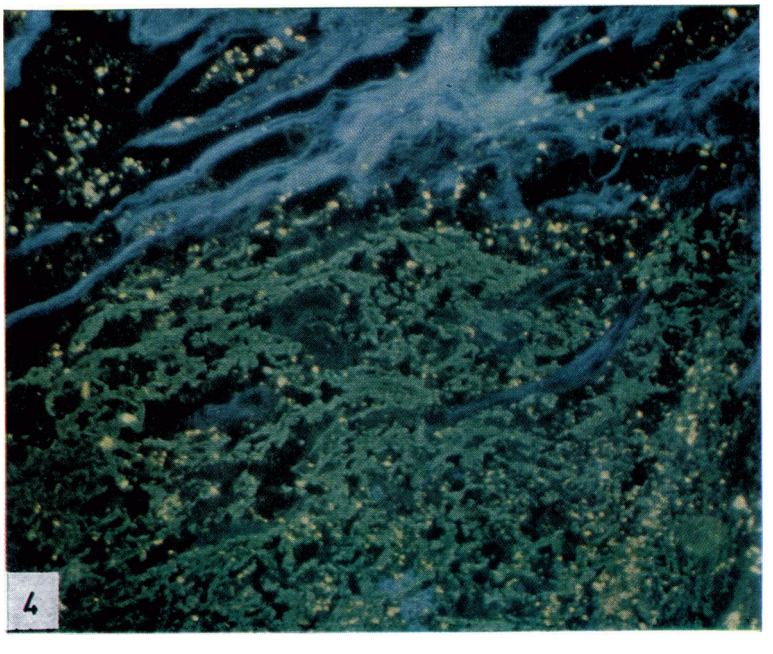


FIG. 3. Staining of human anterior pituitary by serum of patient No. 1. ×400. Mallory stain showed that the strongly immunofluorescent cells belonged chiefly to the eosinophilic series, though other cells were also immunofluorescent.

FIG. 4. Staining of human posterior pituitary by serum of patient No. 3. ×100. Bright staining of nerve fibre elements.

(*Facing p.* 384)

only by absorption by the human pituitary but also by absorption with pig pituitary. On the other hand, staining of pig pituitary was weakened by absorption with pig pituitary powder but was unaffected by the absorption with human pituitary. This suggests strongly that the staining of human pituitary is a cross-reacting activity, the stimulating antigen being the pig pituitary. This conclusion is reinforced by the failure of absorption with a mixture of human pituitary with pig thyroid to affect the staining of pig pituitary. This control

TABLE 5. Specificity of pituitary staining by Nos. 1 and 3

Serum	Absorbed with	Human pituitary		Pig pituitary	
		Posterior	Anterior	Posterior	Anterior
No. 3	—	++	0	+++	+++
	Human pituitary	0	0	+++	+++
	Pig pituitary	0	0	(+)	++
	Pig serum	++	0	+++	+++
	Lysine vasopressin	++	0	+++	+++
No. 1	—	+++	+++	+++	+++
	Human pituitary	0	0	+++	+++
	Human thyroid	+++	+++	+++	+++
	Pig pituitary	0	0	++	(+)
	Pig thyroid	++	+++	+++	+++
	Pig thryoid + human pituitary	0	0	+++	++
	Pig serum	+++	+++	+++	+++
	Lysine vasopressin	+++	+++	+++	+++

Absorptions with pituitary powders were performed at 2°C in the presence of 1/1000 sodium azide using 50 mg of the powder per ml serum.

Absorptions with the various 'whole thyroid' preparations were performed similarly except that 200 mg thyroid preparation per ml serum was used and that the absorption was carried out during dialysis against saline.

Absorption with pig serum was performed by addition 1/5 volume undiluted pig serum; with lysine vasopressin by addition of 1 volume of this substance to 1 volume serum.

Assessment of staining: +++, Very strong staining; ++, strong staining; +, moderate staining; (+), weak staining; 0, no staining.

was necessary since it could not be excluded that there was staining of non-organ-specific pig antigens in addition to specific pituitary antigens. Pig thyroid alone as well as pig serum and purified lysine vasopressin had no effect on the staining of either species of pituitary section. The staining of human posterior pituitary by serum No. 3 can be seen similarly to be a cross-reacting staining, the homologous antigen again being the pig pituitary.

It would thus appear that these autoreactive anti-pituitary antibodies were, in fact, stimulated by the heterologous immunization by the pig pituitary powder.

A variety of other human tissues were stained with these sera in an attempt to see if

antigenic material similar to that found in the pituitary was also found elsewhere (Table 6). To make sure that any staining found was indeed due to the same antibodies giving rise to the pituitary staining the sera were also tested after absorption with pig pituitary. It was seen that staining apparently equivalent to that found in the posterior pituitary was present in the adrenal medulla and in the brain. The staining in both cases was of nerve fibre elements morphologically similar to that seen in the posterior pituitary. Both of these patterns of staining were abolished by the pig pituitary powder. It may be noticed in passing that all three of these organs are of neuroectodermal origin.

Serum No. 1, however, also showed bright staining of the cytoplasm of thyroid cells as well as of thyroid colloid. This staining resisted absorption with pig pituitary. This made it appear unlikely that this pattern was in any way related to the pattern of staining found in the anterior pituitary; a conclusion that was reinforced by the failure of a typical Hashimoto serum, L.G., to show any staining of human pituitary.

TABLE 6. Staining of various human tissues

Serum	Absorbed with:	Pituitary		Adrenal medulla	Brain	Thyroid	
		Posterior	Anterior			Cytoplasm	Colloid
No. 3	—	++	0	++	++	0	0
	Pig pituitary	0	0	0	0	0	0
No. 1	—	+++	+++	+++	+++	+++	++
	Pig pituitary	0	0	0	0	+++	++
L.G. (Hashimoto's disease)		0	0	—	—	+++	+++

Absorption procedures and assessment of staining are as in Table 5.

Patient No. 1 was known to suffer from hypothyroidism and to have been treated for several years with dried thyroid. It therefore appeared not unlikely that the thyroid antibodies might have arisen from heterologous stimulation in a way similar to the antibodies to pituitary. Absorption experiments (Table 7) showed that the staining of human thyroid was abolished only by absorption with human thyroid itself. Neither pig thyroid nor a mixture of pig, sheep or ox thyroid had any effect on the staining of human thyroid. Furthermore, even a mixture of pig thyroid and human pituitary was without effect. These findings suggest strongly that the human thyroid was the homologous antigen in this case. The staining of pig thyroid is abolished not only by pig thyroid but also by pig pituitary showing that the staining of pig thyroid is not entirely due to organ-specific antibodies. This is presumably the reason that absorption with human thyroid fails to abolish the staining of pig thyroid. Absorption experiments on the Hashimoto serum, L.G., showed that this staining too was homologous with human thyroid, the staining of pig thyroid being cross-reacting. In this case absorption with human thyroid powder abolished the staining on

TABLE 7. Specificity of thyroid staining

Serum	Absorbed with	Human thyroid		Pig thyroid	
		Cytoplasm	Colloid	Cytoplasm	Colloid
No. 1	—	+++	++	+++	+++
	Human thyroid	0	0	+++	++
	Pig thyroid	+++	++	0	0
	Pig, sheep and ox thyroid	+++	++	0	0
	Pig pituitary	+++	++	(+)	0
	Pig thyroid + human pituitary	+++	++	—	—
L.G. (Hashimoto's disease)	—	+++	+++	+++	++
	Human thyroid	0	+	0	0
	Pig thyroid	+++	+++	0	0
	Human pituitary	+++	+++	—	—

Absorption procedures and assessment of staining are as in Table 5.

the pig thyroid sections. It would appear that the anti-thyroid antibodies were entirely comparable with those found in Hashimoto's disease.

Of the remaining sera which were tested on sections of human pituitary (Table 8), Nos. 2 and 4 showed staining patterns similar to those given by No. 1. One further serum, No. 5, gave moderate staining of occasional cells in the anterior pituitary without other staining. This serum contained demonstrable precipitins only against the serum protein antigens of the pituitary extracts, unlike the other four sera which contained precipitins against both serum protein and pituitary antigens.

TABLE 8. Complement fixing antibodies to human pituitary found by the fluorescent antibody technique

Patient	Posterior pituitary	Anterior pituitary	
No. 1	+++	+++	Mainly eosinophilic cell series
No. 2	+++	+++	Similar pattern to serum 1
No. 3	++	0	
No. 4	++	++	Similar pattern to serum 1
No. 5	0	++	Occasional cells only
No. 6	0	0	
No. 7	0	0	
No. 8	0	0	
No. 9	0	0	

Assessment of staining as in Table 5.

DISCUSSION

The inhalation of porcine and bovine pituitary snuffs in the treatment of diabetes insipidus leads to the appearance of reaginic antibody, of precipitating antibody, and of antibodies capable of combining with pituitary antigens as shown by immunofluorescence tests.

In one patient, No. 3, reaginic antibodies against the heterologous serum proteins were demonstrated by direct tests and by passive transfer tests on one of the authors (J.P.). The presence of reagins against synthetic lysine vasopressin was suggested by the rhinitis, asthma and generalized itching which this material provoked on repeated testing. It was only possible to obtain the lysine vasopressin in solution and, since the vehicle was itself irritant, the skin test reactions could not be interpreted with confidence. It should be pointed out that lysine vasopressin is found in the pig, whilst in man arginine–vasopressin is present. The skin test reactions to the pituitary snuffs were also complicated by the vaso-constrictor effect of the vasopressin, though in this case there was no doubt about the modified immediate reactions. It seems likely that in practice the vaso-constrictor effect of pituitary snuff would tend to mitigate the severity of immediate, anaphylactic reactions to this potentially antigenic material. By contrast such reactions are not uncommon in treatment with ACTH which does not contain vasopressin.

The production of an immediate wealing reaction followed several hours later by a second oedematous reaction, in response to intracutaneous tests with porcine and bovine serum in patient No. 3, is similar to the dual skin test reaction in patients with the hypersensitive form of pulmonary aspergillosis (Longbottom & Pepys, 1964). The second reaction was regarded as an Arthus-type reaction because of its association with the presence of precipitins. Similar dual reactions have also been elicited in patients with bird breeder's lung, in whom specific precipitins are also present (Hargreave *et al.*, 1966).

Miliary infiltrations of the lungs were present in patients Nos. 3 and 4 (Mahon *et al.*, 1966), in whom precipitins were present. The immunological findings, radiographic appearances and clinical features are like those of farmer's lung and bird breeder's lung. It is clear that inhaled antigens of microbial, animal and avian origin are potent stimuli for the appearance of precipitins in man and it seems that these antibodies may be related to the characteristic lung disorder described, although the participation of Type IV, delayed, hypersensitivity cannot be excluded.

The capacity of the precipitins stimulated by the heterologous pituitary snuff to react with the homologous pituitary antigens of man raises other problems. It should be noted that whilst precipitins against both pituitary and serum protein antigens were present in four out of five sera in which precipitins were found, the fifth serum contained demonstrable precipitins only against the serum protein antigens of the pituitary snuff. In the immunofluorescence tests, however, this serum, like the other four, was found to contain antibodies against human pituitary antigens. The stimulation of autoreactive antibodies by the injection of heterologous antigens or altered homologous antigens, usually together with adjuvants, is well known. In the present case the route of entry of the antigens has been through the apparently normal respiratory tract. The question arises whether a similar process may result from the entry of antigens through the gut. The production of autoreactive antibodies in this way would imply a breakdown of tolerance by exposure to cross-reacting antigenic material.

The antibodies to pituitary demonstrated by the fluorescent antibody technique were primarily directed against the heterologous pituitary antigens in the pituitary snuff and their reaction with human pituitary was a cross-reaction, indicating that their appearance is not related causally to the diabetes insipidus. Nor, in the absence of functioning pituitary tissue in these patients, can they be considered to be of any pathological significance. It is, in fact, not impossible that the absence of homologous antigen in the patients is one factor making their immunization by heterologous antigen easier. Loss of tolerance due to elimination of the relevant antigen from the host is compatible with most forms of the elective theory of antibody formation. Perhaps the most interesting feature of these antibodies is that they can provide reagents for tracing specific antigens in various organs. Thus it would appear that antigenically similar material occurs in the adrenal medulla and in certain elements in the brain as well as in the posterior pituitary.

Anti-thyroid antibodies were found in one patient, No. 1, in this series, who had been treated for prolonged periods with dried thyroid. However, the hypothesis that these antibodies too were cross-reactive antibodies stimulated by heterologous antigen was found to be untrue and these antibodies appeared to be similar to those commonly found in Hashimoto's disease.

ACKNOWLEDGMENTS

We wish to thank Dr A. C. Crooke, Dr A. G. Davies, Professor Russell Frazer and Dr C. S. Treip for their generous clinical co-operation and advice; Mr K. M. Ferguson of the Endocrine Unit, Chelsea Hospital for Women, for providing the human pituitary extract, and Sandoz Products Limited, for providing the vehicle for syntopressin.

The work was supported in part by Grant No. 5872 from the Institute of Arthritis and Metabolic Disease, N.I.H., Bethesda, Maryland.

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