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IMMUNOLOGICAL FEATURES OF IDIOPATHIC ADDISON'S DISEASE: CHARACTERIZATION OF THE ADRENOCORTICAL ANTIGENS

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SUMMARY

The antibodies in the sera of two patients with idiopathic Addison's disease, one reacting specifically with adrenocortical cells, the other with the various cell types which produce steroid hormones, have been found to be predominently IgG. The behaviour of the two antibodies in complement-fixation tests is in agreement with their reactivity, reported in the previous paper, in immunofluorescence tests.

Cell fractionation studies suggest that both adrenal antigens are associated with the microsomal fraction. The biochemical properties of the antigens are consistent with the view that they are lipoproteins, possibly with associated carbohydrate. While the properties of the adrenal antigens are generally similar to those of the thyroid and gastric parietal cell organ-specific autoantigens, they differ from these, and from each other, in their susceptibility to destruction by certain enzymes and chemical treatments.

INTRODUCTION

The literature on the immunological aspects of idiopathic Addison's disease provides little information on the nature of the adrenal antigen, and apart from an investigation reported by Blizzard & Kyle (1963) in which they detected it in both microsomal and mitochondrial fractions of adrenocortical cells, little has been written concerning its chemical properties.

In this paper, we describe briefly the characterization of the immunoglobulin type of the adrenal antibodies in two Addisonian sera, and, more fully, investigations on the properties of the adrenocortical antigen which reacts with the type of antibody commonly present in the serum in idiopathic Addison's disease, i.e. the antigen specific for the adrenal cortex, and also the adrenocortical antigen reacting with the steroid-cell antibody described in the previous paper (p. 107).

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MATERIALS AND METHODS

Sera

Sera were obtained from two patients, H and M, with idiopathic Addison's disease. Serum M contained an unusually high titre of antibody reacting specifically with the cytoplasm of adrenocortical cells, while serum H was known to react with the cytoplasm of all types of cells producing steroid hormones. The main clinical features of these two patients, and immunofluorescence tests upon their serum, are described in the previous paper. A third serum, containing strong thyroid microsomal antibody, was used for comparing the properties of the adrenal and thyroid cytoplasmic antigens: this serum was from a patient B, with histologically confirmed Hashimoto's disease (lymphadenoid goitre).

Immunoglobulin types of adrenal antibodies

This was investigated by indirect immunofluorescence tests, performed as in the previous paper, but using fluorescein-labelled antisera specific individually for human γ, μ and α chains. The antibodies were prepared in rabbits by the method of Goudie, Horne & Wilkinson (1966), and, where necessary, were absorbed with purified IgG. The specificity of the fluorescein-conjugated antibodies was confirmed by immunoelectrophoresis and by immunofluorescence tests with antinuclear antibodies of known immunoglobulin class.

Complement fixation (CF) technique

Tissues obtained from the same sources as those used for the immunofluorescence studies were freed from adherent fat and stored at $-70°C$ for up to 12 months without deterioration. Unless otherwise stated, tissue extracts were prepared by adding 10 volumes of CF buffer, pH 7·2 (Oxoid Ltd), and disrupting at full speed for 3 min in a small MSE homogenizer. Coarse tissue fragments and the lipid pellicle were removed following centrifugation at 500 ***g*** for 15 min. The turbid supernatant was stored at 4°C and used as the complement-fixing antigen, usually within 24 hr of preparation since it was found that freezing of such extracts caused aggregation of the particles and diminished antigenicity. For the purpose of description, tissue extracts prepared as above have been considered to represent 1:10 dilutions of tissue.

Complement preserved by Richardson's method (Stayne Laboratories Ltd), sheep erythrocytes in Alsever's solution (Oxoid Ltd) and rabbit anti-sheep red cell haemolytic serum (Stayne Laboratories Ltd) were used in all tests, and the reagents were accurately standardized and titrated as described by Osler, Strauss & Mayer (1952). Complement in test sera was inactivated by heating at 56°C for 30 min. Tests were performed in Perspex trays using doubling dilutions of antigen or antibody; each test consisted of 0·1 ml of serum, 0·1 ml of tissue extract and 0·2 ml of complement diluted to contain 5 $C'H_{50}$ units. After 1 hr at 37°C, 0·1 ml of 1·5% sensitized sheep cells was added to each cup and the trays were incubated for a further hour at 37°C; the trays were repeatedly shaken during the first and second stages of the test. Results were recorded after the trays had stood overnight at 4°C to allow the red cells to settle: 50% haemolysis as judged visually was taken as the end point.

Anti-complementary activities of test and normal control sera were compared by matching the size of the buttons of red cells obtained in control tests using 0·1 ml of doubling serum dilutions, 0·1 ml of CF buffer and 0·2 ml of complement diluted to contain 3 $C'H_{50}$ units.

Dilutions of tissue extracts were considered too anti-complementary for testing when they gave 50% haemolysis or less in the presence of normal serum with 5 $C'H_{50}$ units of complement (e.g. the adrenal extract diluted 1:40, Fig. 2). When tissue extracts gave more than 50% haemolysis with 5 $C'H_{50}$ complement and a dilution of normal serum of similar anti-complementary activity to the test serum, the tissue extract was considered suitable for use.

A unit of antigen was arbitrarily defined as the amount required to give 50% haemolysis with serum H diluted 1:8 or 1:16, or with serum M diluted 1:16 or 1:32 (Fig. 1) in the presence of 5 $C'H_{50}$ of complement, provided that the antigen was not anti-complementary.

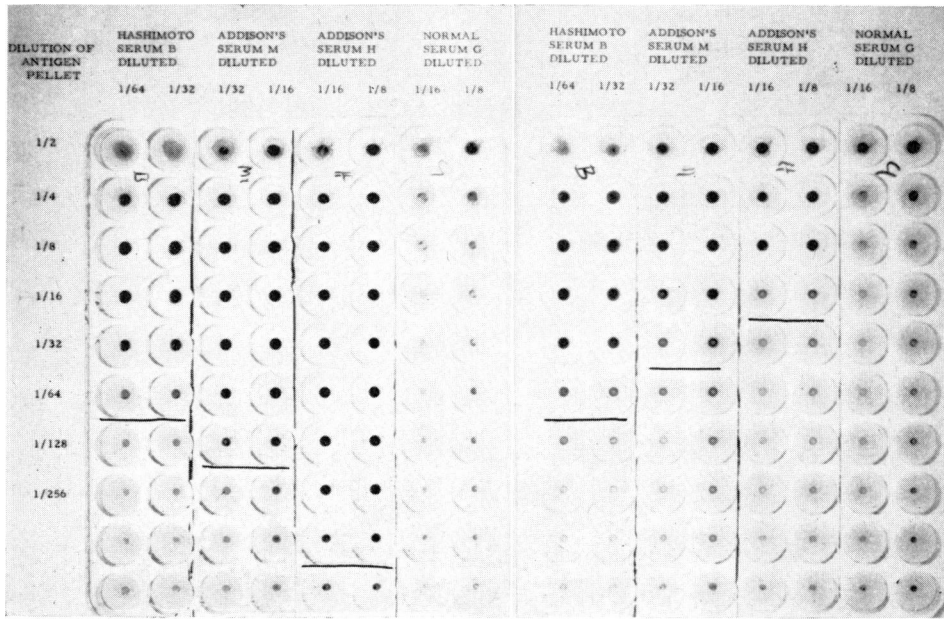

FIG. 1. Complement fixation titration of pooled thyroid and adrenal antigens with (right) and without (left) treatment by 0·05 M-pyrophosphate. The end points are indicated by horizontal pencilled lines and are easily read. Pyrophosphate has not affected thyroid microsomal antigen but only 25% of the adrenal antigen reacting with serum M and 3% of that reacting with serum H has survived pyrophosphate treatment.

Adrenal cell fractionation

Normal adrenal tissue was obtained surgically from a 31-year-old female undergoing bilateral adrenalectomy for carcinoma of the breast. The tissue was immediately cooled and forthwith fractionated at 4°C. After sieving, the tissue was homogenised with a teflon pestle in approximately 10 volumes of 0·25 M-sucrose solution until most of the cells had been ruptured. Cell fractions were then obtained by differential centrifugation as shown in Table 1. Since the adrenal antigen is cytoplasmic as shown by immunofluorescence, the deposit consisting of nuclei and coarse debris was discarded. After obtaining representative samples for electron microscopy, the pooled mitochondrial preparations I and II (washed) and pooled microsomal preparations I and II (unwashed) were resuspended in the original

volume of sucrose solution and kept at 4°C for assay of CF antigen on the following day.

The adrenal cell fractions were examined chemically as follows. Samples of pooled cell fractions were assayed for ribonucleic acid (RNA) measured relative to rat liver RNA, following the methods of Hutchison & Munro (1961), Fleck & Munro (1962) and Hallinan, Fleck & Munro (1963) and using the correction factor given by Fleck & Begg (1965) for extinction due to protein. Protein estimations were carried out by the method of Lowry *et al.* (1951) and succinate dehydrogenase was measured by a modification of the technique of Jardetzky & Glick (1956).

Effects of enzymes, solvents and chemical treatment

Attempts to destroy the antigen were made using the following enzymes: trypsin (crystallized, Armour Pharmaceutical Co. Ltd), papain (British Drug Houses Ltd), α-chymotrypsin (Worthington Biochemical Corp.), wheat germ lipase (British Drug Houses Ltd), lecithinase C (from *Cl. welchii*), lecithinase D (from cabbage, Koch Light Laboratories Ltd), takadias-

TABLE 1. Preparation of adrenal cell fractions

Preparation	Centrifugation		Products	
	Time	Maximum RCF (*g*)	Deposit	Supernatant
Crude homogenate	10 min	5×10^2	Nuclei and coarse debris	A
Supernatant A	20 min	5×10^3	Mitochondria I	B
Supernatant B	20 min	5×10^3	Mitochondria II	C
Supernatant C	60 min	$1{\cdot}04\times10^5$	Microsomes I	D
Supernatant D	60 min	$1{\cdot}04\times10^5$	Microsomes II	E
Supernatant E*	15 hr	$1{\cdot}26\times10^5$	Post-microsomal button	F

* This procedure was performed on supernatant E after it had been kept at −70°C for 5 days.

tase (Parke Davis and Co.), ribonuclease (Worthington Biochemical Corp.) and hyaluronidase ('Hyalase', Benger Laboratories Ltd). Similar experiments were performed with the detergents Lubrol W (Imperial Chemical Industries Ltd) Triton X-100 (Lennig and Co.) and Tween 20 (G.T. Gurr Ltd) and with other chemical treatments.

For these experiments the antigen was prepared from supernatants of tissue extracts (made in CF buffer as described above) by centrifugation at 24,000 ***g*** for 1 hr to obtain a particulate fraction (presumably mitochondria and microsomes). The deposit, resuspended in CF buffer, was stored at 4°C while the antigenic activity of a sample was tested. The following day 200–640 units of antigen were subjected to the appropriate treatment with enzyme, etc., in a total reaction volume of 3·5 ml and at the end of the treatment the tubes were centrifuged at 24,000 ***g*** for 1 hr at 4°C. The deposit was resuspended in 0·8 ml of CF buffer and doubling dilutions were titrated by CF testing with appropriately diluted normal serum, serum M and serum H, the results being expressed finally as a percentage of the titre of untreated antigen which had been processed concurrently as a control. A parallel series of separate experiments was conducted in an identical manner using extracts of surgical

specimens of human thyrotoxic thyroid tissue and serum B, the Hashimoto's serum containing high titre CF thyroid microsomal antibody. In some experiments (marked with an asterisk in Tables 4, 6 and 7) the treatment was applied to mixtures of thyroid and adrenal antigen and the deposit was tested with sera M, H and B (Fig. 1).

The enzymes were dissolved in CF buffer and their final concentration in the antigen mixture is shown in Table 4: the mixtures were incubated for 2 hr at 37°C and in all cases the reaction was stopped by cooling to 4°C and centrifuging at once to remove the supernatant. The papain was activated by 0·02 M-cysteine hydrochloride (Greenberg, 1955). Deoxycholate formed a precipitate with CF buffer and was used in physiological saline solution. Extraction with hexane was carried out thrice for 5 min at 20°C using equal volumes of hexane and of antigen suspended in CF buffer. The experiments with other detergents, chemicals and solvents followed exactly the methods described by Roitt *et al.* (1964) in their investigations on the thyroid cytoplasmic autoantigen.

RESULTS

Immunoglobulin typing of the adrenal antibodies

Immunofluorescent staining of the cytoplasm of adrenocortical cells was observed in indirect tests with serum H and serum M using fluorescein-conjugated antibodies to IgG and to γ chains, but not with those specific for μ or α chains.

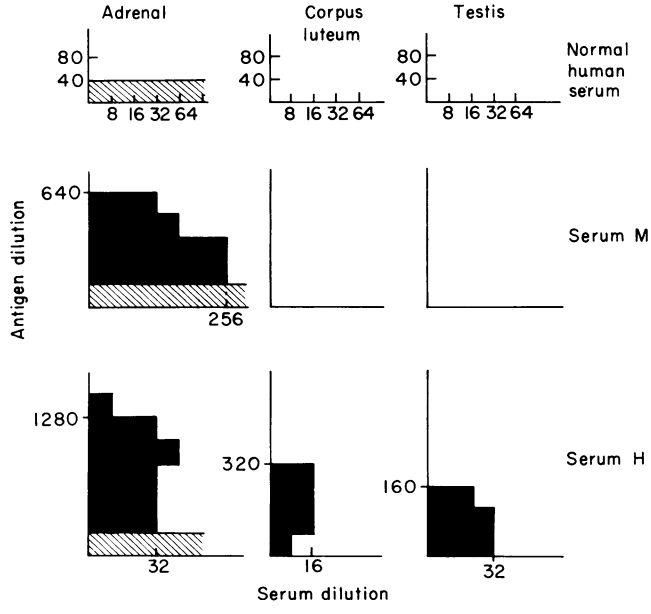

FIG. 2. Checkerboard complement fixation titrations of extracts of adrenal, corpus luteum and testis with serum M and serum H. The black areas indicate reactions between serum and organ extract in which complement is fixed. The tests with normal serum show the dilutions of the organ extracts at which specific complement fixation cannot be detected due to anti-complementary activity of the antigen (cross-hatched).

Complement fixation tests with crude tissue extracts

Serum M reacted specifically in CF tests with adrenal tissue extracts, whereas serum H reacted also with extracts of testis and corpus luteum (Fig. 2). Attempts to investigate the specificities of the antibodies in sera H and M by absorption with tissue homogenates could

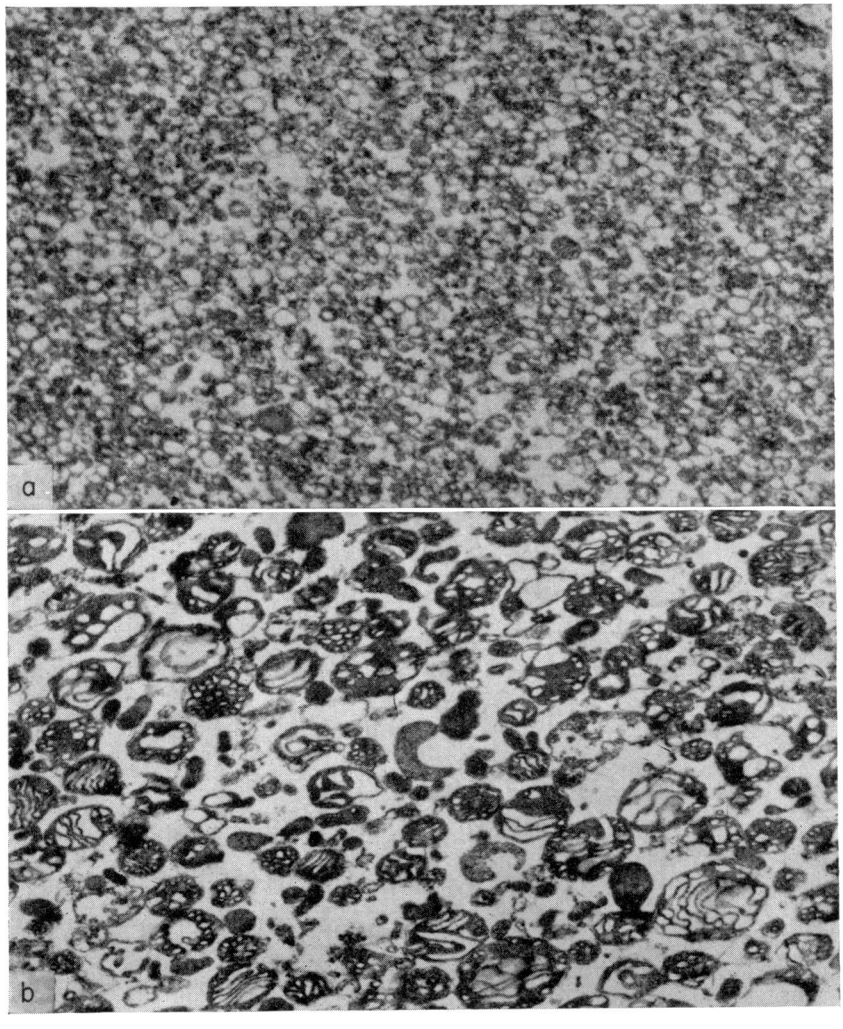

FIG. 3. Electron micrograph of: (a) 'microsomes' and (b) mitochondria prepared from human adrenal by differential centrifugation. ×21,600.

not be performed by the CF technique as the absorbed sera were found to be strongly anti-complementary. Addition of hydrocortisone, cortisone acetate, fluorocortisone, prednisone and prednisolone to sera M and H did not interfere with their reactions in immunofluorescence tests with tissue extracts.

Cell fractionation

Electron micrographs of microsomal and mitochondrial fractions obtained by differential centrifugation of homogenized fresh human adrenal (Fig. 3a and b) show remarkably good separation of these structures, though some microsomal vesicles are present among the mitochondria. The chemical analyses recorded in Table 2 confirm that satisfactory separation of microsomes and mitochondria has been achieved.

TABLE 2. Chemical composition of adrenal cell fractions

	Protein (mg/ml)	Ribonucleic acid (μg/ml)	Succinate dehydrogenase extinction (units/ml)
Mitochondria	4·6	93	2·7
Microsomes	9·0	595	0·6
Post-microsomal fraction	13·5	316	–
Supernatant F	26·7	212	–

Antigenicity of cell fractions

Table 3 shows that the microsomes contain 4–32 times the amount of antigen present in the mitochondrial fraction. The wide range of results is attributable to the use of doubling

TABLE 3. Antigenicity of adrenal cell fractions (units per gram of whole tissue)

	Serum H diluted		Serum M diluted	
	1:8	1:16	1:16	1:32
Whole extract	⩾12800	⩾12800	>12800	>12800
Mitochondria	1600	1600	1600 (800)	1600 (800)
Microsomes	6400 (6400)	6400 (6400)	12800 (25600)	6400 (12800)
Supernatant E	⩾1600 (800)	⩾1600 (800)	⩾1600 (1600)	1600 (1600)
Post-microsomal fraction	(400)	(400)	(1600)	(800)
Supernatant F	(<400)	(<400)	(400)	(<400)

Figures in parentheses refer to tests performed after storage of fractions for 6 days at −70°C.

dilutions in the antigen titrations, and to the performance of titrations on different days. It is likely that the microsomes really contain 4–8 times more antigen than the mitochondria, a finding which cannot be explained by the protein content of the two fractions. A significant amount of antigen was present in supernatant E (104,000 ***g*** for 2 hr) which contained enough particulate material to cause definite turbidity. The turbidity and antigen in supernatant E

were removed in the post-microsomal fraction deposited by centrifugation at 126,000 ***g*** for 15 hr. There is thus no evidence for the presence of a soluble CF adrenal antigen which reacts with serum H or M.

Immunofluorescent staining of adrenal was given by serum M absorbed by concentrated mitochondrial fraction, whereas the staining was abolished by absorption with a similar preparation of microsomes. Unfortunately in the corresponding experiment with serum H, the diluted unabsorbed specimen used did not give fluorescent staining of adrenal and the effects of absorption could not be determined. CF studies of the absorbed sera were prevented by their anti-complementary activity.

Biochemical characteristics of microsomal autoantigen

In view of the limited supply of serum M and H which fixed complement well, it was not

TABLE 4. Antigenicity of adrenal and thyroid extracts treated with enzymes

Enzyme	mg/ml	Antigenicity (percentage of untreated control)				
		Adrenal		Thyroid serum B	Thyroid Roitt *et al.* (1964)	Gastric Baur *et al.* (1965)
		Serum H	Serum M			
Trypsin	0·2	12	18	12	<25	35
Papain	0·24	<12	<25	<25	8	19
Chymotrypsin	0·3	6*	<12*	100*	57	100
		18	25			
Lipase	7·2	50	100	100	100	100
Lecithinase C	0·8	100*	50*	100*	73	100
	0·8	25	<25	100		
Lecithinase D	0·4	100	100	100	–	–
Takadiastase	3	25*	100*	100*	100	100
		18				
Ribonuclease	1	100*	100*	100*	–	–
Hyaluronidase	300 units	100	100	100	100	–

* Indicates tests on mixtures of adrenal and thyroid antigen.

possible to duplicate all the experiments shown below nor was it possible to test the supernatants for solubilized or fragmented antigen. The findings given below must therefore be regarded as provisional.

(*a*) *Effects of enzymes.* These are shown in Table 4. The adrenal antigens with which sera H and M react are largely destroyed by trypsin, papain and chymotrypsin, and little affected by wheat germ lipase, lecithinase D, ribonuclease and hyaluronidase. The effect of lecithinase C is uncertain due to the conflicting results obtained in repeated tests. Takadiastase damages the antigen with which serum H reacts, but not that reacting with serum M. The results of the control studies with thyroid microsomal antigen correspond closely to those described by Roitt *et al.* (1964). The properties of the adrenal antigens are similar to those of the thyroid microsomal antigen apart from the increased sensitivity of the former to α-chymotrypsin and the susceptibility of the H antigen to takadiastase.

(*b*) *Effects of organic solvents.* Table 5 shows that the adrenal antigens were largely destroyed by all the organic solvents tested. The results obtained in control tests with thyroid microsomal antigen are similar to those described by Roitt *et al.* (1964) though inconsistent results were obtained with ether treatment. The adrenal antigens are probably more severely damaged by n-hexane than is the thyroid antigen.

TABLE 5. Antigenicity of adrenal and thyroid extracts treated with organic solvents

Solvent	Concentration (%)	Antigenicity (percentage of untreated control)				
		Adrenal		Thyroid serum B	Thyroid Roitt *et al.* (1964)	Gastric Baur *et al.* (1965)
		Serum H	Serum M			
Ethanol	75	<12	<25	3	<5	0
n-Butanol	30	<12	<25	12	<17	0
Acetone	80	<12	<25	3	<9	–
Ether	50	<12	<25	6	31	–
n-Hexane	See text	<12	<25	50	83	31–64
		<12	<25	50		

(*c*) *Effects of surface active agents* (Table 6). Deoxycholate and Triton X-100 severely damaged both the adrenal antigens and the thyroid microsomal antigen. Lubrol W and Tween 20 caused approximately 80% reduction in the antigenicity of the adrenal preparation, and in the case of Tween 20 this is probably a more severe effect than that obtained with

TABLE 6. Antigenicity of adrenal and thyroid extracts treated with detergents

Treatment	Antigenicity (percentage of untreated control)				
	Adrenal		Thyroid serum B	Thyroid Roitt *et al.* (1964)	Gastric Baur *et al.* (1965)
	Serum H	Serum M			
Deoxycholate (0·1%)	<6	<50	<3	7	0
Lubrol W (0·2%)	18	25	<50	<17	28
Triton X-100 (0·1%)	6*	6*	12*	10	36
Tween 20 (0·1%)	18	37	100	100	100
	9*	18*	37*		

* Indicates tests on mixtures of adrenal and thyroid antigen.

thyroid microsomal antigen. Apart from the inconsistent results obtained with Tween 20 treatment, the findings with thyroid microsomal antigen are similar to those obtained by Roitt *et al.* (1964).

(*d*) *Effects of other chemical treatments.* The results are shown in Table 7. While those obtained with the control tests on thyroid are consistent among themselves and (with the

exception of the effect of strong alkali) are similar to those obtained by Roitt *et al.* (1964), the results of tests with the adrenal antigens fluctuate widely in replicate tests. This is particularly so with serum M in the tests with alkali- and pyrophosphate-treated adrenal antigen. It appears that ethylene diamine tetra-acetic acid (EDTA) does not substantially damage the adrenal antigens whereas their activity is reduced markedly by acid (like thyroid), and by alkali, bicarbonate and periodate (unlike thyroid). The effects of periodate on the adrenal antigen reacting with serum M is partial; there is a 90% reduction of the titre of antigen giving strong complement fixation, but a weak antigenic component giving a trace of complement fixation persists after the main antigen has been destroyed by periodate

TABLE 7. Antigenicity of adrenal and thyroid extracts treated with chemicals

	Antigenicity (percentage of untreated control)				
	Adrenal		Thyroid	Thyroid Roitt *et al.* (1964)	Gastric Baur *et al.* (1965)
	Serum H	Serum M	serum B		
Acid (0·005 N-HCl)	<25	<50	<3	<2	–
Alkali (0·005 N-NaOH)	<25	37	25	70–100	78–93
	<3*	6*	50*		
Bicarbonate (0·125 M)	12	25	100	100	–
	3*	12*	50*		
EDTA (0·05 M)	50	100	100	100	100
Periodate (0·005 M)	<1·5	<12†	50	47	–
	<1·5*	<6*†	25*		
Pyrophosphate (0·05 M)	3*	25*	100*	100	–
	0·7	6	100		
	18	100	100		
	9*	100*	100*		

* Indicates tests on mixtures of adrenal and thyroid antigen.
† A component of antigen M was not destroyed by periodate (see text).

treatment. The adrenal antigen with which serum H reacts is probably more susceptible to periodate and to pyrophosphate than that reacting with serum M.

DISCUSSION

The demonstration, by the indirect immunofluorescence technique, that the adrenal antibodies in sera H and M are predominantly of IgG type, is in keeping with the findings of Irvine, Stewart & Scarth (1967). The specificity of the CF reactions with the two sera parallel their behaviour in immunofluorescence tests, serum M showing specificity for adrenal tissue and serum H reacting with extracts of adrenal, testis and corpus luteum. No CF tests were attempted with placental tissue as antigen. The CF titre of serum H for adrenal tissue (1:32) is less than that of serum M (1:256) and, therefore, its reactivity with corpus luteum and testis cannot be attributed to a quantitative difference from serum M.

Fractionation of human adrenal tissue

Observations by immunofluorescence show that the adrenal antigen is cytoplasmic, the nuclei of the adrenal cells remaining unstained. Blizzard & Kyle (1963) absorbed Addisonian sera with cell fractions of adrenal and found that the antigen was present in both mitochondria and microsomes, particularly the latter; no evidence was given of the effectiveness of their separation of these cell fractions. We found approximately 4–8 times as much antigen in the adrenal microsomes as in the mitochondrial preparation. Although electron micrographs suggest that our preparations are remarkably pure, the RNA content of the mitochondrial fraction (one-sixth that of the microsomes) indicates that the adrenal antigen may be entirely microsomal and that apparent mitochondrial antigenicity may merely be the result of microsomal contamination. It seems that this is a likely explanation and that the adrenal antigens are microsomal, in keeping with the observations of Roitt *et al.* (1964) on thyroid cytoplasmic antigen and of Baur, Roitt & Doniach (1965) on that of the gastric parietal cells.

Biochemical characters of the adrenal antigens

The investigation of the thyroid microsomal autoantigen by Roitt *et al.* (1964) provided a model for the study of the biochemical properties of an unknown insoluble tissue antigen, and the method has since been successfully applied to the antigen of gastric parietal cells. In the present study their methods have been largely followed, but the simpler and less accurate method of titrating the antigen by doubling dilutions in Perspex trays has been adopted. The remarkably close agreement between our control experiments with thyroid microsomal antigen and those of Roitt *et al.* (1964) show the validity of the method. When inconsistent results were obtained on repeating the same experiment, this was more readily attributable to variation in the effect of the enzyme or chemical treatment under study than in the technical matter of assay of the antigen by complement fixation. Thus in Fig. 1 there is no difficulty in determining the end points in the CF test and errors of antigen assay exceeding one doubling dilution are unlikely. The problem of recovering a small deposit of antigen following centrifugation in these tests was considered a possible source of error. To overcome this, tests on mixtures of thyroid and adrenal antigens (marked with an asterisk in Tables 4, 6 and 7) were performed when differences in the susceptibility of thyroid and adrenal antigen had been found in separate experiments. Like the thyroid and gastric parietal cell autoantigens, the adrenal antigens are very susceptible to the effects of the proteolytic enzymes trypsin and papain, to the polar organic solvents, to strong acid and to the surface active agents, but due to an inadequate supply of strong CF adrenal antibody to test the supernatants, it has not been possible to attribute these findings to destruction or to fragmentation or solubilization of the adrenal antigens. Sodium hydroxide (0·005 N), sodium bicarbonate (0·125 M) and pyrophosphate (0·05 M) all probably affect the adrenal antigens (particularly that reacting with serum H) more than the thyroid antigen, but these effects are unlikely to be due to an action on ribosomal RNA since the adrenal antigens are not altered by EDTA or by ribonuclease. Possibly the action of alkali and sodium bicarbonate on the adrenal antigens is dependent on alteration of electrostatic forces binding protein to lipid or polysaccharide. The presence of a carbohydrate component in the adrenal antigen with which serum H reacts is suggested by the disappearance of antigenicity following treatment with periodate and takadiastase, and this may also be true of the antigen for serum M which is weakened by periodate. Unfortunately the takadiastase used was a crude

commercial preparation and periodate, a strong oxidizing agent, is likely to have effects on tissue constituents other than carbohydrates. Compared with thyroid and gastric parietal cell autoantigens, the adrenal antigens are more severely affected by Tween 20, and the non-polar solvent n-hexane, but these substances are less damaging to adrenal than the other detergents and polar solvents studied. The significance of the findings with organic solvents and detergents is uncertain; they probably point to an effect on lipid rather than protein (though the use of polar solvents as histological fixatives depends on their ability to denature protein). The above observations are consistent with the possibility that the adrenal antigens, like the thyroid and gastric autoantigens, are non-ribosomal insoluble microsomal lipoproteins, and this view, according to Roitt *et al.* (1964), is not ruled out by the lack of effect of lipase and lecithinase D and the uncertain results obtained with lecithinase C. The adrenal antigens differ from the thyroid and gastric autoantigens in being more severely altered by a variety of agents (α-chymotrypsin, alkali, bicarbonate, Lubrol W, n-hexane, periodate and pyrophosphate) and differ from each other in that the antigen reacting with serum H is in general more readily destroyed and is more likely to have an associated carbohydrate component.

In the previous paper, reference was made to common metabolic pathways in the biosynthesis of steroid hormones, and Δ^5-3β-hydroxysteroid dehydrogenase was instanced as an enzyme detectable in appreciable amounts in all cells considered to produce steroid hormones. It is of interest, in relation to the fractionation and chemical studies described above, that this enzyme has been localized predominantly in the microsomal fraction of adrenal tissue, and that attempts to obtain it in active form in solution were unsuccessful (Beyer & Samuels, 1956).

The possibility that the reaction of the antibody in serum H with steroid-producing cells is the result of immunization by therapeutically administered steroids which could conceivably act as haptens (Beiser *et al.*, 1959) appears unlikely; the antibody is not absorbed by any of the steroid hormones with which the patient has been treated.

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REFERENCES

BAUR, S., ROITT, I.M. & DONIACH, D. (1965) Characterization of the human gastric parietal cell autoantigen. *Immunology*, **8**, 62.

BEISER, S.M., ERLANGER, B.F., AGATE, F.J. & LIEBERMAN, S. (1959) Antigenicity of steroid-protein conjugates. *Science*, **129**, 564.

BEYER, K.F. & SAMUELS, L.T. (1956) Distribution of steroid-3-ol-dehydrogenase in cellular structures of the adrenal gland. *J. biol. Chem.* **219**, 69.

BLIZZARD, R.M. & KYLE, M. (1963) Studies of the adrenal antigens and antibodies in Addison's disease. *J. clin. Invest.* **42**, 1653.

FLECK, A. & BEGG, D.J. (1965) The estimation of ribonucleic acid using ultraviolet absorption measurements. *Biochim. biophys. Acta (Amst.)*, **108**, 333.

FLECK, A. & MUNRO, H.N. (1962) The precision of ultraviolet absorption measurements in the Schmidt-Thannhauser procedure for nucleic acid estimation. *Biochim. biophys. Acta (Amst.)*, **55,** 571.

GOUDIE, R.B., HORNE, C.H.W. & WILKINSON, P.C. (1966) A simple method for producing antibody specific to a single selected diffusible antigen. *Lancet*, **ii,** 1224.

GREENBERG, D.M. (1955) Plant proteolytic enzymes. *Methods in Enzymology* (Ed. by S. P. Colowick and N. O. Kaplan), Vol. II, p. 59. Academic Press, New York.

HALLINAN, T., FLECK, A. & MUNRO, H.N. (1963) Loss of ribonucleic acid into lipid solvents after acid precipitation. *Biochim. biophys. Acta (Amst.)*, **68,** 131.

HUTCHISON, W.C. & MUNRO, H.N. (1961) The determination of nucleic acids in biological materials; a review. *Analyst*, **86,** 768.

IRVINE, W.J., STEWART, A.G. & SCARTH, L. (1967) A clinical and immunological study of adrenocortical insufficiency (Addison's disease). *Clin. exp. Immunol.* **2,** 31.

JARDETZKY, C.D. & GLICK, D. (1956) Studies in histochemistry. XXXVII. Determination of succinic dehydrogenase in microgram amounts of tissue and its distribution in rat adrenal. *J. biol. Chem.* **218,** 283.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

OSLER, A.G., STRAUSS, J. H. & MAYER, M.M. (1952) Diagnostic complement fixation. I. A method. *Amer. J. Syph.* **36,** 140.

ROITT, I.M., LING, N.R., DONIACH, D. & COUCHMAN, K.G. (1964) The cytoplasmic autoantigen of the human thyroid. I. Immunological and biochemical characteristics. *Immunology*, **7,** 375.