Immunology, 1971, **21**, 101.

In Vitro Synthesis of Immunoglobulin-A by Salivary Glands from Animals of Different Species

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(*Received 1st October* 1970)

Summary. The *in vitro* synthesis of immunoglobulins by the salivary glands from eight different species was studied. It has been demonstrated that salivary glands from the cow, horse, sheep, pig, rat and guinea-pig preferentially synthesize a fast migrating immunoglobulin which seems to be analogous to IgA. In three of the species, the cow, sheep and pig, the IgA-like component cross-reacts with human IgA. The IgA synthesized by the salivary glands from the rat cross-reacts with the mouse IgA. When one compares the salivary IgA from the cow, horse, sheep, pig and rat with the IgA synthesized by the lymph nodes, there is evidence of additional antigenic determinants in the salivary IgA. These additional determinants could very possibly correspond to a secretory component. Thus, it appears that in most mammalian species there exists a mechanism for the synthesis of secretory IgA analogous to the one which exists in humans.

INTRODUCTION

The immunoglobulins of human secretions are well characterized. It has been demonstrated that immunoglobulin-A (IgA) is the most concentrated immunoglobulin found in secretions and that it is different from serum-IgA (Tomasi and Bienenstock, 1968). One molecule of secretory IgA consists of a dimer of serum-IgA covalently bound to a protein specific for secretions, the so-called secretory component (Tomasi, Tan, Solomon and Prendergast, 1965; Newcomb, Normansell and Stanworth, 1968; Hurlimann, Waldesbühl and Zuber, 1969). The large amount of secretory IgA found in secretions is to a great extent due to local synthesis in the mucosal tissues (Heremans and Crabbé, 1967; Hurlimann and Zuber, 1968; Tourville, Adler, Bienenstock and Tomasi, 1969).

Although the immunoglobulins of animal secretions are well documented (Vaerman, 1970), the results are sometimes conflicting. Studies on secretions from the cow (Mach, Pahud and Isliker, 1969; Porter and Noakes, 1970), the rabbit (Cebra and Small, 1967), the sheep (Heimer, Jones and Maurer, 1969) and the mouse (Nash, Vaerman, Bazin and Heremans, 1970) have established that there exists an immunoglobulin analogous to human secretory IgA. Data from work on pig secretions confirm the presence of γA; the presence of a secretory component remains in question (Bourne, 1969; Richardson and Kelleher, 1970; Vaerman and Heremans, 1970). Work on rat secretions and also serum suggests the presence of an immunoglobulin analogous to human IgA, but lacking a secretory component (Nash, Vaerman, Bazin and Heremans, 1969; Bistany and Tomasi,

1970; Stechschulte and Austen, 1970). In the guinea-pig the data only suggest that there may exist an immunoglobulin analogous to human secretory IgA (Hathaway and Peters, 1969). Recently, Vaerman, Heremans and Van Kerckhoven (1969) have demonstrated an immunoglobulin in the dog, cat, cow, goat, sheep, pig and horse that cross-reacts with human IgA. Vaerman (1970) has also shown that the concentration of this IgA is highest in secretions. The results concerning the horse conflict with those of Genco, Yecies and Karush (1969) who were unable to find an immunoglobulin analogous to human IgA in saliva.

Studying various species, Vaerman (1970) has shown, by immunofluorescent techniques, that IgA is synthesized in numerous plasmocytes of the digestive mucosa. Except for the work of Lawton, Asofsky and Mage (1970) on rabbit mammary glands, there are no reports concerning local synthesis of immunoglobulins in any other mucosae.

The present experiments were undertaken to study and compare the *in vitro* synthesis of immunoglobulins by the salivary glands of various species. The purpose was to see whether or not immunoglobulin synthesis by one or several species studied was analogous to that in man and if it could be used in experiments concerning antibody production by the mucosae. The salivary glands were selected because we had previously studied the immunoglobulin synthesis by human salivary glands and could benefit by comparison.

We have given particular attention to three points. First, is there an immunoglobulin which is preferentially synthesized by the salivary glands as compared with the lymph nodes? Second, do the immunoglobulins synthesized by the salivary glands cross-react with any human immunoglobulins? Third, do any immunoglobulins possess a component analogous to the human secretory component?

MATERIALS AND METHODS

Serum

The serum of each species was obtained from normal animals and pooled. Serum of horse immunized against diphtheria toxin was obtained from the Swiss Serum Institute, Berne.

Saliva

Saliva from cow, horse, pig and sheep was obtained from the Veterinary Institute, Berne. Saliva from rabbit, guinea-pig, rat and mouse, was aspirated from the mouth with a polyethylene tube, after the animal had received a subcutaneous injection of pilocarpin (5 mg/kg of weight). The samples were centrifuged to remove cellular debris and lyophilized.

Submandibular extract

The submandibular glands of these animals were homogenized in 0·15 M sodium phosphate buffer, pH 7·4, at 4° in a Silverson mixer (Silverson, London, England). After centrifugation at 3000 ***g*** for 20 minutes, the supernatant was lyophilized.

Antisera

The antisera against saliva, serum and submandibular extract from rabbits were obtained by immunization of guinea-pig and rats. The antisera against saliva, submandibular extract, serum and the purified fractions from serum and saliva of the other

animals and man were obtained by immunization of rabbits. The technique of immunization has been previously described (Hurlimann and Zuber, 1968).

The fractions from serum and saliva which were used for immunization were obtained as follows: Immunoglobulins (Ig) were precipitated from serum by making the sample 33 per cent saturated with ammonium sulfate. Immunoglobulin-G (IgG) from serum of the various species was obtained by chromatography on DEAE-cellulose with 0·01 M phosphate buffer pH 7·4.

Bovine γ-globulin (BGG) was the fraction II from bovine plasma, commercially available (Armour, Eastbourne, Sussex). Human salivary and ascitic IgA were obtained according to the technique previously described (Hurlimann and Zuber, 1968). A fraction rich in horse γG(T) was obtained by chromatography of horse serum on DEAE-cellulose with a 0·02 M phosphate buffer + 0·075 M NaCl pH 7·4. This fraction was passed through a Sephadex G-200 column and the ascending portion of the second peak was used for immunization.

Antisera to human IgA were also obtained commercially: from Cappel, Downington, PA. goat lot 3730; from Nordic, Tilburg, Holland rabbit 7-468 and swine 5-169; from Dutch Red Cross, Amsterdam, Holland horse PH14-2-P15; from Hyland, Los Angeles, Calif. U.S.A. goat 8212HOO3B1; from Behringwerke AG, Marburg, Germany goat 1583G and rabbit 1564 U.

An antiserum to rat IgA was obtained from Dr Bazin, Louvain. An antihorse immunoglobulins serum was a gift of Dr Karush, Philadelphia. An antiserum to mouse myeloma IgA was given to us by Dr Jaquet, Lausanne.

In certain experiments, antisera to saliva and submandibular extracts were absorbed with a pool of the corresponding normal serum. After absorption, they were no longer able to precipitate any serum constituents.

Submandibular glands and lymph nodes

The submandibular glands and lymph nodes of the cervical region of the animals were removed immediately after death and cultured. The horse, cow, pig and sheep material was obtained through the courtesy of Mr Pahud, slaughterhouse, Lausanne.

Tissue cultures and analysis of culture fluids

The submandibular glands and lymph nodes of the animals were cultured in a medium containing [^{14}C]amino acids, according to the technique described by Hochwald, Thorbecke and Asofsky (1961). The culture fluids were analyzed by autoradiograph of immunoelectrophoresis. A minimum of five glands was cultured for each species.

Immunoelectrophoresis

The micromethod of Scheidegger (1955) was used with slight modifications for the LKB apparatus 6800 (LKB products, Stockholm, Sweden).

Chromatography on DEAE-cellulose

Serum and saliva from the animals were chromatographed on DEAE-cellulose using a stepwise gradient of seven buffers: (1) 0·01 M phosphate pH 7·4; (2) 0·02 M phosphate pH 7·4, (3) 0·02 M phosphate pH 7·4 + 0·05 M NaCl, (4) 0·02 M phosphate pH 7·4 + 0·075 M NaCl, (5) 0·02 M phosphate pH 7·4 + 0·1 M NaCl, (6) 0·02 M phosphate pH 7·4 + 0·15 M NaCl, (7) 0·02 M phosphate pH 7·4 + 0·2 M NaCl. The fractions were pooled and lyophilized

RESULTS

Cow

The submandibular glands of the cow synthesize a fast migrating gammaglobulin which can be seen on autoradiographs of immunoelectrophoresis with rabbit antisera to submandibular extract, saliva, serum or bovine γ-globulin. This radioactive line is detectable if the cold carrier used is bovine saliva or a fraction of bovine serum eluted on DEAE-cellulose chromatography with 0·02 M phosphate pH 7·4+0·075 M NaCl. If the cold carrier is the complete bovine serum, no radioactive line is visible.

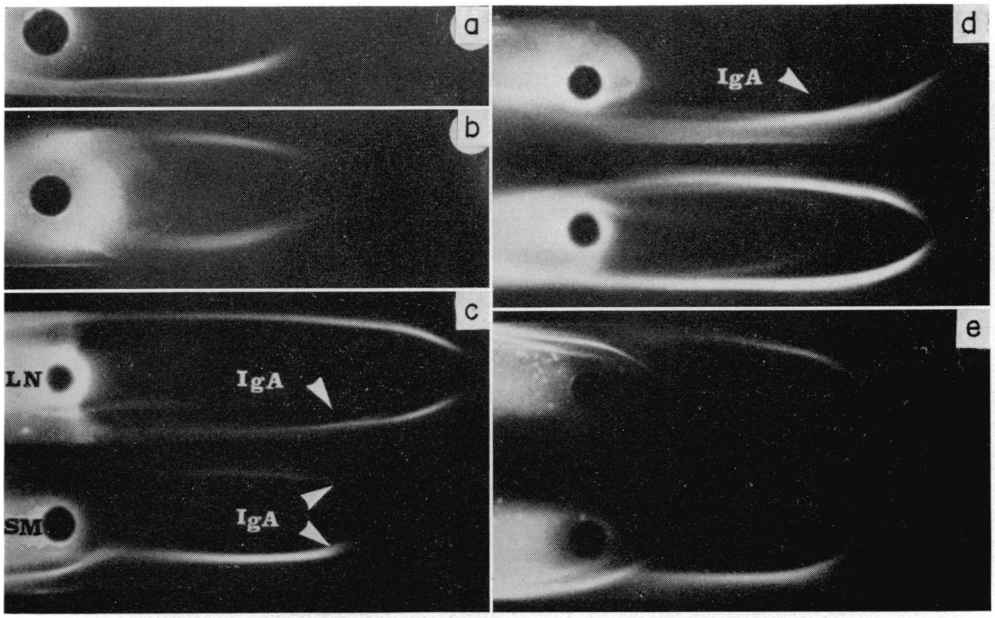

FIG. 1. Autoradiographs of culture fluids from cow lymph nodes and submandibular glands. (a) Culture fluid from a submandibular gland revealed by an antihuman IgA serum. (b) The same culture fluid as in (A) revealed in parallel by an antihuman IgA (top) and an antibovine γ-globulins (bottom). (c) Comparison of a culture fluid from lymph node (LN) and from submandibular gland (SM) revealed by an anti-bovine serum (middle) and an antibovine saliva (top and bottom). (d) Culture fluid from lymph node revealed by an antibovine serum (middle), an antibovine saliva (bottom) and an antibovine saliva absorbed with serum (top). (e) Culture fluid from submandibular gland. Same arrangement as in (d). The IgA line is revealed with an antibovine saliva absorbed with serum (top). In this and the following figures the anode is to the left.

The submandibular glands synthesize only this fast migrating immunoglobulin, while the lymph nodes synthesize four additional immunoglobulins (Fig. 1c).

The immunoglobulin synthesized by the submandibular glands can be precipitated by antihuman IgA sera even if no cold carrier is used (Fig. 1a). Immunoelectrophoresis with an antibovine γ-globulin and an antihuman IgA used in parallel on the same slide, shows that the immunoglobulin revealed by the antihuman IgA corresponds in shape and mobility to that seen with antibovine saliva (Fig. 1b).

This immunoglobulin can be precipitated by antisera to saliva and submandibular extract absorbed with bovine serum (Fig. 1e), but these antisera are unable to reveal the five immunoglobulins synthesized by the lymph nodes (Fig. 1d).

Horse

The submandibular glands of the horse synthesize a fast migrating γ-globulin which can be seen on autoradiographs of immunoelectrophoreses using rabbit antisera to horse submandibular extract, saliva, serum or γ-globulins (Fig. 2a). A heavy radioactive line is visible if the cold carrier is horse saliva or serum. This line does not correspond to the

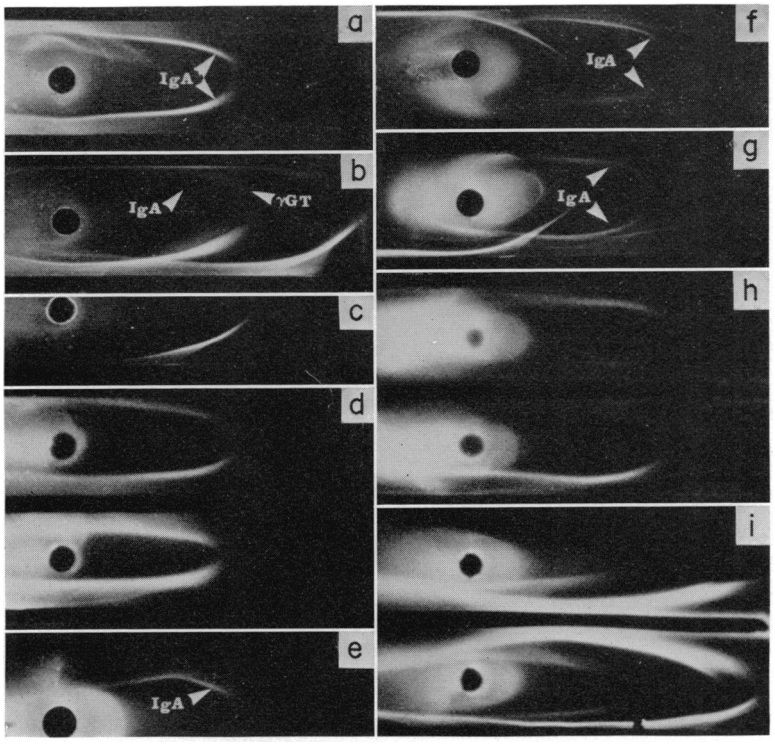

FIG. 2. Autoradiographs of culture fluids from horse and sheep. (a) Culture fluid from horse submandibular gland revealed by an antihorse saliva serum (top) and an antiserum against horse serum (bottom). (b) Culture fluid from horse lymph node. Same arrangement as in (a). (c) Culture fluid from horse lymph node revealed by an anti-γG(T) serum. (d) Culture fluid from horse submandibular gland revealed by an antihorse serum (middle), an antihorse saliva (bottom) and an antihorse saliva absorbed with serum (top). (e) Culture fluid from sheep submandibular gland revealed by an antihuman serum. (f) The same culture fluid as in (e) revealed by an antihuman IgA (bottom) and an antisheep submandibular extract (top). (g) The same culture fluid as in (e) revealed by an antihuman IgA (top) and an antisheep serum (bottom). (h) The same culture fluid as in (e) revealed by an antisheep serum (middle), an antisheep saliva (bottom) and an antisheep saliva absorbed with serum (top). (i) Culture fluid from sheep lymph node. Same arrangement as in (h). The IgA line is not revealed by the antisaliva absorbed with serum.

γG(T); the radioactive line cannot be superimposed on the γG(T) precipitation line obtained with a specific antiserum to horse γG(T). Moreover, using as carrier the serum from a horse immunized with diphtheria toxin, which contains large amounts of γG(T), the γG(T) precipitation line is closer to the antibody trough and less curved. In this case the position and shape of the radioactive line remain unchanged. The immunoglobulin synthesized by the submandibular glands is not precipitated by any of the antihuman IgA sera.

Antisera to horse submandibular extract or saliva, absorbed with horse serum, still reveal the immunoglobulin synthesized by the submandibular glands (Fig. 2d), but are unable to reveal the corresponding immunoglobulin synthesized by the lymph nodes.

The lymph nodes synthesize five immunoglobulins. The radioactive line which corresponds to the immunoglobulin synthesized by the submandibular glands is very fine and at times reaches the limit of visibility (Fig. 2b and c).

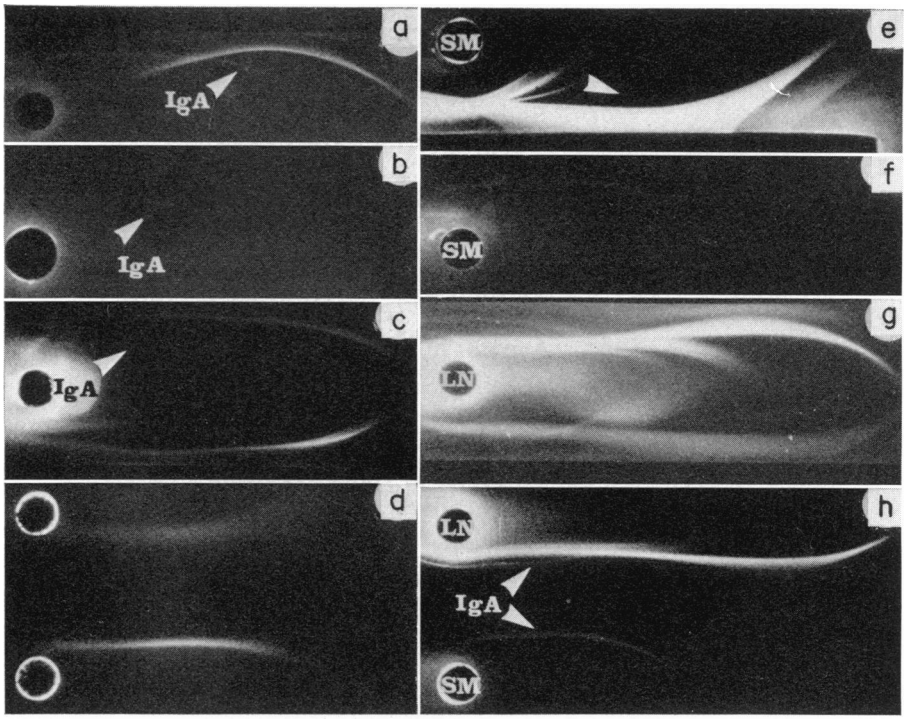

FIG. 3. Autoradiographs of culture fluids from pig, mouse and guinea-pig (a) Culture fluid from pig submandibular gland revealed by an antipig saliva. (b) The same culture fluid as in (a) revealed by an antihuman IgA. (c) Culture fluid from pig lymph node revealed by an antipig serum (top) and an antipig saliva (bottom). (d) Culture fluid from mouse intestinal mucosa (top) and mouse mesenteric lymph node (bottom) revealed by an antimouse IgA. (e) Culture fluid from guinea-pig submandibular gland revealed by an antiguinea-pig saliva. The arrow indicates the IgA line. (f) The same culture fluid as in (e) revealed with an antiguinea-pig serum. (g) Culture fluid from guinea-pig lymph node (LN) revealed by an antiguinea-pig serum (top) and an antiguinea-pig saliva (bottom). (h) Comparison of a culture fluid from guinea-pig lymph node (LN) and submandibular gland (SM) revealed by an antiguinea-pig serum.

Sheep

Autoradiographs on the culture fluids from submandibular glands show a fine radioactive line corresponding to γG2 and a second heavier line which is closer to the anode. The latter can be seen not only with antisera to sheep serum, submandibular gland and saliva, but also with antiserum to human IgA and to human serum (Fig. 2e–g). This line is visible when the antisera to saliva and submandibular extract are absorbed with sheep serum (Fig. 2h).

Autoradiographs on the culture fluids from lymph nodes show heavy γG2 and γG1 lines, a fine γM line and a weak line which corresponds to the heavy one of the sub-

mandibular glands. The last line is no longer visible when antisera to saliva and submandibular extract are absorbed with sheep serum (Fig. 2i).

Pig

In the culture fluids from submandibular glands, a fast migrating γ-globulin is revealed with antisera to serum, saliva (Fig. 3a) and submandibular extract of pig, to human IgA (Fig. 3b) and with antisera to saliva and submandibular extract of pig absorbed with serum.

In lymph node culture fluids, three immunoglobulins can be seen, one of which corresponds to that synthesized by submandibular glands (Fig. 3c). When antisera to submandibular extract and saliva absorbed with pig serum are used, this line is no longer apparent.

Mouse

The submandibular glands synthesize a small amount of IgG which is seen as a fine line when antisera to serum and immunoglobulins are used. There is no synthesis of IgA as no line is visible with antiserum specific to IgA, while it is demonstrable in culture fluids from the intestinal wall and mesenteric lymph nodes (Fig. 3d). There is no cross-reactivity between mouse IgA, from intestinal wall and mesenteric lymph nodes, and human IgA. Antisera to submandibular extract and saliva reveal the IgA produced by the intestinal wall and mesenteric lymph nodes. After these antisera were absorbed with mouse serum, γA was undetectable.

The lymph nodes synthesize IgA and four additional immunoglobulins, IgG2a IgG2b, IgGl and IgM.

Rat

The submandibular glands synthesize immunoglobulin-A which can be precipitated by an antiserum specific to rat IgA and with antisera to saliva and submandibular extract (Fig. 4a). This immunoglobulin is also revealed by an antiserum specific to mouse IgA when mouse serum is used as carrier (Fig. 4c). The IgA synthesized by submandibular glands can be seen even if the antisera to saliva and submandibular extract are absorbed by rat serum (Fig. 4d). None of the antisera to human IgA are able to reveal rat IgA.

The lymph nodes synthesize IgGa, IgGb, IgM and IgA (Fig. 4b). This IgA is not revealed with antisera to saliva and submandibular extract absorbed with rat serum (Fig. 4e).

Guinea-pig

The submandibular glands synthesize a fast migrating γ-globulin which is precipitated by antisera to whole serum (Fig. 3f), submandibular extract and saliva (Fig. 3e). This immunoglobulin does not cross-react with human, rat or mouse IgA. After absorption with guinea-pig serum, antisera to submandibular extract and saliva, no longer reveal this line; owing to the superposition of several precipitation lines in this region an interpretation is difficult. The immunoglobulin synthesized is not γl but it does cross-react with γl and γ212.

The lymph nodes synthesize the fast migrating γ-globulin and γG1, γG2 and γM (Fig. 3g and h).

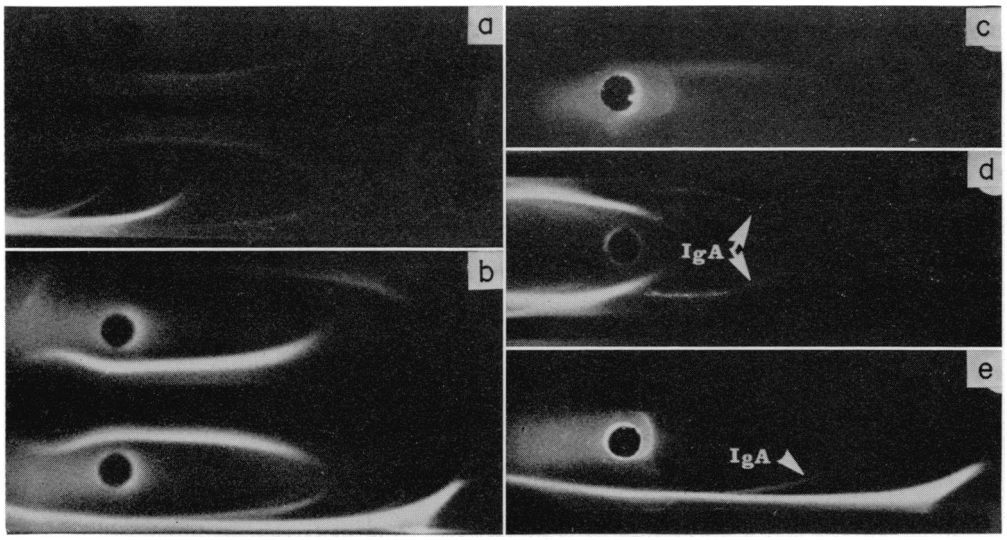

FIG. 4. Autoradiographs of culture fluids from rat (a) Culture fluid from submandibular gland revealed by an antirat immunoglobulins (top), an antirat IgA (middle) and an antirat saliva (bottom). (b) Culture fluid from lymph node. Same arrangement as in (a). (c) Culture fluid from submandibular gland revealed by an antimouse IgA. (d) Culture fluid from rat submandibular gland revealed by an antirat saliva (bottom) and an antirat saliva absorbed with serum (top). (e) Culture fluid from lymph node. Same arrangement as in (d). The IgA line is not revealed by the absorbed antiserum.

TABLE 1

Species	Immunoglobulins synthesized	Detected by	Cross-reacts with	Secretory component
Cow	IgA	Anticow serum Anticow saliva Anti-BGG Anti-SM extract	Human IgA	+
Horse	IgA	Antihorse serum Antihorse saliva Antihorse Ig Antihorse SM extract	0	+
Sheep	γG2 IgA	Antisheep serum Antisheep serum Antisheep saliva Antisheep SM extract	Human IgA	+
Pig	IgA	Antipig serum Antipig saliva Antipig SM extract	Human IgA	+
Mouse	IgG	Antimouse serum Antimouse Ig	0	0
Rat	IgA	Antirat IgA Antirat saliva Antirat SM extract	Mouse IgA	+
Guinea-pig	IgA	Anti-GP serum Anti-GP saliva Anti-GP SM extract	0	?
Rabbit	IgG	Antirabbit IgG Antirabbit serum Antirabbit Ig	Human IgG	0

In vitro synthesis of immunoglobulins by salivary glands of different species. BGG, Bovine gammaglobulin; SM extract, submandibular extract; GP, guinea-pig.

Rabbit

The submandibular glands synthesize γG; the radioactive line can be seen with antisera to rabbit serum and rabbit immunoglobulins, using rabbit serum as cold carrier. It is also evident with an antihuman IgG serum used with human serum as carrier. There is no synthesis of an immunoglobulin which cross-reacts with human IgA. With various antisera to submandibular extract and saliva, it is not possible to detect the synthesis of any immunoglobulins.

The lymph nodes synthesize γG and γM.

The demonstration of cross-reactivity between the IgA-like component from some species and the human IgA was possible with only two antisera: a rabbit antihuman ascitic IgA and a horse antihuman IgA from the Dutch Red Cross, Amsterdam. The results are summarized in Table 1.

DISCUSSION

Analysis of the culture fluids from submandibular glands by autoradiograph of immunoelectrophoresis shows one and in some cases two radioactive lines. It has been said that this technique does not prove that IgA is synthesized by human salivary glands because [^{14}C] amino acids could be incorporated into the secretory component only (Tomasi and Bienenstock, 1968; Lawton *et al.*, 1970). The labelled secretory component, thereafter, would fix to the cold IgA used as carrier or to the IgA remaining in the tissues. This hypothesis seems improbable: first, it is possible to obtain a radioactive line without carrier; second, after having washed and cut the tissues, there remains an insufficient amount of IgA that could fix to the component. Thus, we can consider that the radioactive line obtained with this method corresponds to an immunoglobulin synthesized *in vitro*.

The synthesis of the immunoglobulins by the salivary glands is selective. In all species but the mouse and the rabbit, which will be discussed later, the submandibular glands synthesize a fast migrating γ-globulin while the lymph nodes synthesize from two to four additional immunoglobulins.

In the rat, the immunoglobulin from submandibular glands is IgA which cross-reacts with mouse IgA but not with human IgA. In the cow, sheep and pig, the fast migrating immunoglobulin synthesized by the submandibular glands cross-reacts with human IgA. Such cross-reactivity is a good criterion for the existence of homology between immunoglobulins. Thus, we can consider that the immunoglobulin from the submandibular glands in these three species is IgA. In the horse and the guinea-pig, the immunoglobulin from submandibular glands does not cross-react with human, mouse or rat IgA, but the fact that it is a fast migrating γ-globulin, which guinea-pig γl and horse γG(T) are not, and that it is selectively synthesized by the submandibular glands, strongly suggests that it is IgA.

The results show that the immunoglobulin selectively synthesized by the submandibular glands is analogous to human IgA, and confirm the data obtained with immunofluorescent techniques on other mucosae (Vaerman, 1970).

In the cow, horse, sheep, pig and rat, the IgA synthesized by the submandibular glands possesses antigenic determinants which do not exist on IgA synthesized by the lymph nodes. This is probably true for guinea-pig as well, but it is difficult to be certain. One can hypothesize that IgA synthesized by the submandibular glands possesses a component analogous to the secretory component present in human salivary IgA. The secretory component

found in the secretions of several species favours this hypothesis. We have not isolated the various salivary IgAs and, therefore, cannot exclude other possibilities. It is feasible that the submandibular gland could synthesize IgA as dimers without the secretory component, while the lymph nodes synthesize IgA as a monomer (Lawton and Mage, 1969; Bienenstock and Strauss, 1970). The dimers could have specific antigenic properties as those described for man (Apicella and Allen, 1970). This hypothesis, however, seems less plausible because a large part of the IgA present in the serum of the species studied is in the dimeric form (Vaerman, 1970). Another possible explanation is that submandibular gland selectively synthesize a subclass of IgA which occurs in the serum in small amounts. In such a case, one should find some sign of synthesis in lymph node cultures and we have found no such evidence.

In the mouse the salivary glands do not synthesize IgA, but the mesenteric lymph nodes and intestinal mucosa do. This particular finding could be explained by the smaller number of plasmocytes which are found in submandibular glands of mouse than those found in other species. This emphasizes the notion that in the same animal the synthesis of IgA can differ greatly from one mucosal tissue to the other.

In the case of the rabbit, IgA synthesis is undetectable. This is probably due to the antisera to submandibular extract and saliva which lack antibodies against rabbit IgA. The great synthesis of IgG which cross-reacts with human IgG is surprising and must be compared with the synthesis of other immunoglobulins by using antisera specific to IgG, IgM and IgA.

Except in the mouse and rabbit, the mammals studied have salivary glands that are able to selectively synthesize IgA, which in some cases cross-reacts with human IgA. The synthesized IgA has antigenic determinants which could correspond to a secretory component. These mammals, thus, present a situation analogous to man and could be used for studying the local synthesis of antibodies and their transport into external secretions.

ACKNOWLEDGMENTS

We are grateful to Professor H. Gerber, Veterinary Institute, Berne, for providing the saliva from various species. We wish to thank all the donors of Antisera.

This work was supported by the Swiss National Foundation for Scientific Research.

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