A STUDY OF DISEASES OF THE SKIN OF DOMESTIC ANIMALS

by

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A summary of a thesis submitted for the degree of Doctor of Philosophy in the University of Glasgow, September, 1966.

Section I A study of the histology and histochemistry of skin samples from sixteen body areas of the cat gave results which in general were in agreement with those of previous workers. However, phospholipids were not detected in the sebaceous glands of the cat and cholesterol was absent from the epidermis. In addition, contrary to previous reports, the guard hair was found to erupt from the epidermis through a single opening. The structure and staining affinities of the sweat glands conformed closely to that described by Montagna (1962) and Munger (1962).

An investigation of the histamine-mast cell content of feline skin showed that the mast cells were numerous in the upper part of the dermis and that there was an associated high level of histamine in the tissue. The histamine-mast cell values were similar to those of Riley (1959). The staining characteristics of the mast cell in the cat differed from those of the dog in that adult cells of this type were PAS-positive and metachromatic when stained by toluidine blue. In contrast, canine mast cells were PAS-negative and metachromatic with toluidine blue. The results of differential staining of mast-cell granules by other techniques is included in the text.

In the third part of this section the response of normal skin and its histamine-mast cell component to parenteral administration of corticosteroids was studied. It was found that administration of cortisone acetate produced easily recognised changes in the epithelial components viz. thinning of the epidermis and atrophy of the sweat and sebaceous glands. In addition a few mast cells showed evidence of degranulation and vacuolation of the cytoplasm but there was no significant change in histamine-mast cell values between treated and control animals. However, administration of betamethasone over a period of fourteen days at a rate of 3 mg. daily caused more severe degranulation and vacuolation of the mast cells and a significant fall in the histamine content of the skin.

Section II In this section miliary eczema, a common skin condition of cats, was described. The aetiology of the disease is obscure and in a series of fifty cats no precise correlation could be made between the incidence of the disease, the sex of the animal and the presence or absence of ectoparasites. Vesiculation was not a feature of eczema in the cat as it is in man. The predominant changes on histological examination in the acute phase were fibrinous exudation, a heavy neutrophil infiltration and ulceration and erosion of the epidermis. Intercellular oedema and thickening of the epidermis were also present. In the dermis there was separation of collagen fibres by oedematous fluid and a perivascular mononuclear reaction.

In chronic eczema the main changes in the epidermis were acanthosis and parakeratosis.

The mast cell population of the upper dermis at this stage was markedly increased and there was a four-fold rise in skin-histamine values.

The effect of betamethasone therapy on miliary eczema was investigated. While a pronounced clinical improvement was noted in nine of ten animals there was no appreciable alteration in the mast cell population of the skin. A decrease in the amount of histamine in the skin of the cats in the group did occur but was of doubtful significance.

Section III A series of experiments was carried out in order to study the precipitin response of cats immunized by various methods with bovine serum albumin, bovine gamma globulin and heterologous sera. Procedures used included antigen elimination, immunodiffusion, immunoelectrophoresis and quantitative precipitation. The half-lives of I^{131}-BSA and I^{131}-BGG in the cat were 3.8 and 2.8 days respectively. The precipitin response to bovine serum albumin was only feeble while that to bovine gamma globulin, although stronger, was directed only to a minor β-globulin component of the preparation. The general characteristics of the quantitative precipitin reaction between bovine β-globulin and specific cat antiserum were similar to previously described systems but cat antiserum apparently contains some non-precipitating antibody.

Cutaneous hypersensitivity to bovine serum proteins was elicited in the cat by appropriate immunization procedures. In time course and histological features the reactions took the form of either local cutaneous anaphylaxis or an Arthus reaction. The best results were obtained by the use of bovine normal serum and bovine gamma globulin as antigens while the

results were negative with bovine serum albumin.

Passive cutaneous anaphylaxis reactions were demonstrated in the guinea-pig using cat antisera to bovine normal serum and bovine gamma globulin but in normal cats only with antiserum to bovine gamma globulin could sensitivity be transferred.

General anaphylaxis was produced in two cats in response to bovine serum proteins the main clinical features of which were severe respiratory embarrassment and general collapse. Pulmonary emphysema, haemorrhage and oedema were the principal post-mortem findings; histological examination showed the arterioles to be packed with leucocytes and the lung tissue to be devoid of mast cells. These observations suggest that the lung is the "shock organ" in the cat.

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INTRODUCTION

One of the commonest problems current in veterinary practice is that of the animal afflicted by a disease of the skin. Many of those conditions respond well to chemotherapy, for instance, demodectic mange in the dog may be cured by application of Trolene to affected areas, sarcoptic mange in the pig, cat, and dog yields to therapy with gammexane and in recent years ringworm in small animals has been found to be curable by the parenteral administration of griseofulvin (O'Sullivan, 1961). However, there remain a hard core of skin diseases which resist treatment and in this group the eczemas are probably those which are most perplexing to both the veterinary surgeon and the owner. In this thesis, attention has been concentrated on one condition, viz., miliary eczema of the cat, a disease which is of particular importance in small-animal practice.

At the start of this study it was found that the amount of information on the response of feline skin to injurious stimuli was extremely sparse as was also basic knowledge on the structure of normal skin in that animal. Thus, it was considered necessary initially to investigate the integument of the normal cat and then to make use of the information so obtained as a basis for comparison with the changes encountered in both naturally-occurring and experimentally-produced lesions.

Furthermore, since many skin diseases in man are accompanied by an increased dermal mast cell population and raised histamine content these factors have been studied in both normal and eczematous cat skin. Corticosteroids have been found of value in the treatment of eczema in the human and in dogs and cats. However, as little is known of the mode of action of the above drugs on the skin of domestic animals an investigation has been carried out on the effect of parenterally administered corticosteroids on both normal and eczematous skin.

There is much evidence that in man eczematous reactions are largely allergic in nature e.g., contact hypersensitivity to chemicals has been shown to be an immunological response of the delayed type which can only be transferred by cells from affected animals. In the dog eczematous reactions occur following flea bites (Schwartzman and Orkin, 1962) and in animals sensitized to ragweed (Patterson, 1959). The final part of the thesis has therefore been devoted to a study of immunological responses in the cat. In this regard an initial search of the literature yielded the information that cats are extremely refractory to anaphylaxis (Wilson and Miles, 1964) and that they cannot be sensitized to foreign proteins because they either break them down or excrete them rapidly (Akcasu, 1963). Experiments were therefore designed to investigate the serological responses of the cat to heterologous serum and to some defined

foreign proteins. In addition attempts were made to induce both immediate and delayed-type hypersensitivity in the feline skin.

THE SKIN OF THE NORMAL CAT

Introduction and Review of Literature

Apart from a few detailed papers published within recent years, such as that of Strickland and Calhoun (1963), there is comparatively little information on the skin of the cat that is to be found in the available literature. In this review existing data on the histology and histochemistry of feline skin will be considered under the headings of the major structural components, viz., epidermis, dermis, sweat and sebaceous glands and hair.

Epidermis

One of the first investigators to include the cat in a discussion of the histology of the skin of domestic animals was Stoss (1906) who reported six epidermal strata to be present at the external border of the nasal septum. A well-developed epidermis with a thick stratum corneum penetrated in some parts by the wavy excretory ducts of sweat glands was noted in the case of the digital pads (Frei, 1928). Varicak (1941) considered that the epidermis of the dog and cat resembled that of man and usually contained a stratum lucidum, while Montagna (1952) found all the layers of epidermis to be present in the paw and digital pad of the cat. Creed (1958) noted that many similarities exist between the skin of the dog and cat although there was considerable intraspecies variation. The

same author also observed that the epidermis reached its greatest thickness in the nose and in the digital pad and was thinnest over the ventral surface of the body. The epidermis of the digital pad was not as thick as that of the dog and the corrugations of the stratum corneum were less pronounced. Investigation by Strickland and Calhoun (1963) showed the epidermis in hair-covered areas to consist of four distinct layers with the stratum lucidum usually absent. The epidermis varied markedly in thickness from that of the digital pad and planum nasale, where it measured as much as 900 microns, down to an average of 25 microns thickness in the instance of areas of hairy skin. Again ridging of the epidermis was found to be most prominent in the planum nasale but to be absent from the digital pad of the cat in contrast with the horny papillae that are characteristic of the dog.

Pigmentation of skin

Strickland and Calhoun (1963) reported that the melanin concentration of feline skin varied with the area of the body and that the epidermis of hair-covered skin was devoid of that pigment. The colour of the coat depended on the amount of pigment contained by individual hairs. Large amounts of melanin were observed in the epidermis of the lip, the digital pads and planum nasale and also in specialized areas of the skin, such as that of the prepuce, scrotum, anal sac and teat. Montagna (1962) has stated that "seals, dogs, monkeys and cats

have both a thick coat of hair and a rich pigmentary melanocyte system in the epidermis and are 'dopa' positive."

Dermis

The dermis was described by Stoss (1906) to be dense and to impart to the skin a very firm texture although in the case of the foot the corium was declared by Frei (1928) to be poorly developed. Creed (1958) found it difficult to distinguish the stratum reticulare from the stratum papillare, the dermis consisting of bundles of collagen together with elastic and reticular fibres. Strickland and Calhoun (1963) described the dermis to be composed of collagen, elastic and reticular fibres, nerves and blood-vessels, which components vary in amount in different areas. In the instance of hair-covered skin the stratum papillare consisted mainly of fine collagenous fibres running parallel with the epidermis together with a fine network of elastic fibres, which structures appeared to interlace near the dermo-epidermal junction. The stratum reticulare was distinguished by dense fibres of collagen that were approximately three times thicker than those of the stratum papillare while elastic fibres were particularly numerous around the hair-follicles. Montagna (1962) considered fibroblasts to be ubiquitous in mammalian dermis and to vary in shape from long, thin, compressed cells distinctive of the reticular layer to broader mesenchymal-like cells in the papillary layer. Histiocytes, although present, were difficult to find in normal skin.

In the cat, mast cells are particularly common in the dermis and are related to the histamine content of that tissue, (Riley and West, 1953).

Sweat glands

Apocrine glands are present in all parts of the skin (Stoss, 1906) and reach up to the bulb of the cover hair. They consist of a secretory tube for about one-third their total length succeeded by a much narrower efferent duct which has a funnel-shaped opening into the hair-follicle. Backmund (1904) described extensively coiled apocrine glands to occur in the upper and lower lip. Trautman and Fiebiger (1952) found a poorly developed secretory tubular gland to be present only in the oral region, anus, lower jaw and digital pads. According to Strickland and Calhoun (1963), apocrine glands occurred in all areas of the body but the digital pads contained only those of merocrine type. Those authors further distinguished two forms of apocrine gland in feline skin, viz., the saccular (small and large) present in areas of hairy skin and the coiled encountered in the upper and lower lips, eyelids, anal sac and prepuce. Munger (1961) described the eccrine glands to be slightly undulating tubes that extend through the dermis and epidermis of the foot pad and the duct to be composed of two layers of cuboidal cells, one of which lined the lumen and the other rested on the basement membrane.

In the secretory segment of an eccrine sweat gland two types of

cells are present (Montagna et al. 1953). Thus, some small superficial cells are located largely towards the lumen and contain granules that stain avidly with basic dyes, for which reason they have been called "dark cells". The larger basal cells have small, sparse and slightly acidophilic granules in the cytoplasm and are known as "clear cells". Between the latter are to be found intercellular canaliculi which run from the lumen to the base of the gland. As a result of an electron and light microscopic study of the apocrine sweat glands of the cat and monkey, Munger (1965) came to the conclusion that those structures were also merocrine in nature and differed only from the eccrine glands of the digital pad in that they lacked "clear cells". He further stated that as revealed by electron microscopy "the secretory vacuoles form in the region of the Golgi apparatus, are bounded by an agranular limiting membrane, and are frequently observed approaching the luminal plasma membrane, fusing with it, and liberating the contents of the vacuole into the gland lumen after rupture of the fused membrane. This process of release is clearly merocrine."

Sebaceous glands

Stoss (1906) described the sebaceous glands as generally small and of hemispherical shape over most of the body but indicated that large sebaceous glands were present in the upper lip, the prepuce, the mental angle and on the dorsum of the tail. His findings agree with those of Creed (1950) and of Strickland and Calhoun (1963).

The sebaceous glands of the skin of the mental angle are referred to as the circumoral gland of the cat by von der Ohe (1927) who found an accumulation of large glands to fill the entire triangle of the lower jaw. Krolling and Grau (1960) allude to those glands as the "submental organ". Extending almost the entire length of the tail is the supracaudal organ (Mathis, 1935), a collection of large sebaceous and sweat glands, which latter become more active in cats that are in oestrus.

Hair

Domestic animals may be divided into two classes according to whether, or not, grouping of the hair-follicles is to be found in the skin (Jenkinson, 1965). Animals which do not exhibit that characteristic include the cow, the horse and the buffalo whereas the cat, dog, goat, sheep and camel have hair-follicles arranged in clusters. Trautman and Fiebiger (1952) describe the coat of the cat to be arranged in groups usually of three hairs, of which latter one is a main or guard hair and is larger than the other two. In carnivores each of those three guard or, as they are sometimes called, cover hairs is surrounded by six to twelve wool hairs, the follicles of which branch off from that of the cover hair at the opening of the sebaceous glands. A complete bundle of hairs projects from a common follicular opening. Strickland and Calhoun (1963) stated that the hair of the cat was arranged in clusters of two to five

groups around a large central guard hair, that double and triple clusters are more common on the dorsal part of the body and collections of 4 and 5 are frequent on the ventral and lower aspects. In each group there are usually three primary hairs surrounded by 6 to 12 lanugo hairs. From 12 to 20 hairs emerge from a common opening. The above authors describe follicular folds, similar to those reported in the cow (Goldsberry, 1959), to occur in the upper portion of the guard hair follicle only. Five to ten folds are present in the follicle of the cat compared with 15 to 20 in the case of the cow.

Tactile hairs which are found on the upper lip and eyelid are characterized by a blood sinus, lined by endothelium, that is interposed between the external sheath of the hair-follicle and an outer capsule (Trautman and Fiebiger, 1952). In the cat, that structure is divided into a superior non-trabecular ring or annular sinus and an inferior cavernous or trabecular cavity. A cushion-like thickening of mesenchyme, termed the "sinus pad" or "ringwulst", projects into the annular sinus (Strickland and Calhoun, 1963) and is continuous with the mesenchymal sheath. Above the annular sinus the capsule is thickened and contains a sebaceous glandular mass.

Histochemistry

Montagna (1949), in a study of the glands of the feline external auditory meatus, found that the lipids of the ceruminous fluid are secreted by the sebaceous glands whereas the protein and pigment of

cerumen is derived from the apocrine sweat glands. The sebaceous glands proved to contain triglycerides, cholesterol esters, some plasmal and phospholipids while marked alkaline phosphatase activity was demonstrable in the peripheral acinar cells. Ito (1943) applied the descriptions, superficial and deep, to the two types of cell which he encountered in the digital pad. Kamamura (1957) described both acid and alkaline phosphatase activity to be present in eccrine sweat glands and showed that more alkaline phosphatase was present in the "clear" cells while acid phosphatase was detectable to larger amount in the "dark" cells.

In the epidermis of the cat Montagna (1962) noted that an intense acid phosphatase reaction prevailed but that the response to alkaline phosphatase was negative.

Materials and Methods

Animals

Twenty adult male and ten adult female cats of mixed origin were obtained from local dealers and kept in single cages and fed on a diet of tinned meat, milk and water until required. Before use, each cat was examined to ensure that it was in good health and free from any disease of the skin whereupon it was killed by the intraperitoneal injection of nembutal (May & Baker, Ltd.).

Histology and Histochemistry

The hair was clipped from the sixteen sampling areas shown in Fig. 1 and from each part a piece of skin, approximately 2 cm. x 2 cm., was removed. The specimens destined for routine histological examination were flattened on a glass tray in pools of picro-formol for fifteen minutes after which they were placed in bottles of the fixative for 24 hours. Such a procedure helped to minimize curling of the tissue which tended to occur when the material was directly consigned to bottles of fixative.

After fixation, the skin was processed in the following sequence.

(1)	8% Phenol-Methanol	24 hours
(2)	Absolute alcohol I	4 hours
	[illegible] II	3 hours
(1)	8% Phenol-Methanol	1 hour
(2)	Absolute alcohol I	
(3)	Absolute alcohol II on 0^{o}C	48 hours
(4)	Benzene	24 hours
(5)	Benzene/paraffin on [illegible]	48 hours
(8)	Trimmed blocks placed in Mollifex (B.D.H.) at 4^{o}C	48 hours

Sections were cut at a thickness of 6 microns mainly perpendicular to the surface although some were cut in a parallel plane to subserve study of the distribution and arrangement of the different types of hair. The staining methods used were Mayer's haemalum and eosin, van Gieson, periodic acid-Schiff (Hotchkiss, 1948) with and without diastase to detect mucopolysaccharide and glycogen, respectively,

Best's carmine (Culling, 1957) for glycogen, aldehyde-fuchsin for elastica (Gomori, 1950) and the reticulin stain of Gordon and Sweet (1936). As required, sections were stained by Perl's method for ferric iron (Pearse, 1960), alcian blue and chlorantine fast red for acid mucopolysaccharide (Lison, 1954) and toluidine blue in citrate buffer at pH = 4.0 (Gomori, 1952). In addition, samples fixed in 10% formol-saline at 4°C. for 24 hours were cut on a Leitz freezing microtome at a thickness of 10 microns and stained by Sudan IV and by Oil red O in order to demonstrate neutral fats. Frozen sections of skin fixed in formol-calcium were stained by the acid-haematin technique (Baker, 1946) and the Nile blue sulphate method of Menschik (1953) for demonstration of phospholipids. Unstained mounted frozen sections were examined under polarized light for birefringence attributable to cholesterol and additional sections were treated by the Schultz method as described by Pearse (1960). Skin samples fixed in cold acetone (4°C.) were used for the demonstration of alkaline phosphatase by the calcium cobalt method of Gomori (Pearse, 1960) and of acid phosphatase by the lead nitrate method of Gomori (1950).

Results

Epidermis

In the cat, hairy skin was found to possess an epidermis only three or four cells in thickness, in which all the usual layers were

detectable excepting the stratum lucidum, which latter was limited to the digital pad and the nasal region. (Fig. 2). The stratum corneum was thin as was, too, the stratum granulosum which in places was reduced to a rudimentary and at times discontinuous layer of granules. In hair-covered areas, the stratum germinativum consisted of a single layer of large cuboidal cells, above which lay the stratum spinosum which was 2-3 cells in thickness. Scattered irregularly over the epidermis were considerable numbers of papillae, each made up of small projections of epithelium resting on highly vascular connective-tissue (Fig. 3). Ridging of the epidermis, which is so remarkable a feature of human skin, proved to be absent over the surface of the body but occurred in hairless parts of skin, e.g. over the nose and on the pads of the feet. In the latter situation (Fig. 4) the epidermis had a very thick stratum corneum and stratum lucidum and its other layers were also much developed. The ducts of the eccrine sweat glands of the digital pad were seen to pass through the epidermis in the form of spiral coils.

In hairy skin, pigmented cells were very few in number and, where present, occurred as occasional "clear" cells or melanocytes (Rothman, 1955) that were located in the basal layer of the epidermis. By contrast, large numbers of melanin-containing cells were encountered in the hair-bulbs. In the epidermis of the nose and in that of the pad of the foot, melanophores were prominent in both the stratum germinativum and the stratum spinosum.

Histochemical techniques revealed that the epidermis was very rich in acid phosphatase (Fig. 5), the reaction being most intense in the case of the stratum spinosum and the stratum granulosum. Sections stained by Sudan IV and by Oil red O disclosed the presence of lipids in epidermal cells in the form of intra-cytoplasmic globules. The stratum corneum had a uniformly positive reaction with the above stains. The acid haematin technique proved positive in the case of the epidermis but the Nile blue sulphate method for phospholipid was consistently negative in all areas examined. Glycogen and cholesterol were not detected in the epidermis of hairy skin but the former substance was present in that of the foot pad particularly in the stratum corneum. Both neutral and acid mucopolysaccharide were absent in sections stained by PAS and alcian blue respectively (Table 1).

Dermis

The dermis was found to be composed of collagenous, elastic and reticular fibres together with fibroblasts, mast cells, blood vessels and nerve-trunks. In areas of hairy skin there was not any definite demarcation between the stratum papillare and the stratum reticulare although the collagen fibres of the former layer appeared to be of much finer structure and more densely arranged (Fig. 6). There, too, a delicate network of elastic fibres was present and in the case of hairy skin those fibres formed a dense mesh-work investing the hair-follicles and the arrector pili muscles. The reticular

layer proved to have much thicker and more loosely arranged bundles of collagen which encircled the hair-follicles and were situated above the cutaneous adipose tissue.

Reticulin fibres were prominent at the dermo-epidermal junction where they not only formed a fine reticulum which interdigitated with the cells of the stratum germinativum (Fig. 7) but were also numerous around the hair-follicles and the sweat-glands. The dermis was provided with a rich blood-supply which was mostly of capillary type in the upper dermis and the underlying fatty tissue. In the digital pad pacinian corpuscles were present in the adipose tissue.

The dermis was much thinner due to displacement of collagen by larger sebaceous glands in the case of the supracaudal organ and lower lip and by large tactile hairs in the instance of the upper lip. Mast cells, fibroblasts and occasional histiocytes were the main types of cells found to be present.

Hair

In hair-covered areas of the body three types of hair were found to occur, namely (a) primary guard or cover hairs, (b) secondary guard hair and (c) lanugo or wool hair. The two latter forms were encountered in groups of 4-5 hairs distributed around a primary guard hair (Fig. 8) and each aggregation usually contained a hair, slightly larger than the rest, that was regarded as a secondary guard hair. The primary hair erupts from the skin through a single orifice (Fig. 9)

while the surrounding groups emerge via a common opening. Associated with each hair-follicle is a sweat gland situated at the base, and a sebaceous gland that is located just below the epidermis. Arrector pili muscles originate from the papillary layer of the dermis and are inserted into the follicular connective-tissue sheath at a point just above the bulb of the hair-follicle. The histological structure of the cover hair conformed exactly with that described by Montagna (1962). Histochemical studies of the hair-follicle showed it to be rich in glycogen in PAS-positive material which was also diastase-resistant, and in alkaline phosphatase.

Sweat glands

In the case of hair-covered skin, small saccular sweat glands, present at the base of the hair-follicle, were lined by cuboidal epithelium, rather flattened in actively secreting glands, that rested on a bed of myoepithelial cells, outside which was a thick basement-membrane. The excretory duct ran alongside the hair-follicle into which it opened just above the sebaceous glands. Large saccular glands were conspicuous in the supracaudal area of the body as well as in the upper and lower lips. Coiled apocrine glands lined by tall columnar epithelium were to be seen in the anal sac, the prepuce, the upper lip (Fig. 10) and in the hairy skin bordering the digital pad. In all of the above glands, surface blebs were noted on the epithelium which hitherto have been regarded as evidence of apocrine secretion.

All glands of the apocrine type manifested similar staining properties. Thus, they were PAS-positive but proved negative for glycogen and for ferric iron. Neutral fats, cholesterol and phospholipids were not detectable but phosphatase of both acid and alkaline type was found to be present.

In the subcutaneous adipose tissue of the digital pad, the eccrine glands occurred as long, undulating tubes composed of a secretory portion which, at the base of the dermal collagen, passed into a duct lined by a double layer of cuboidal cells. Where they entered the epidermis, the ducts were seen to lose their epithelial lining and to continue to the cutaneous surface covered only by keratin-like material. In the secretory portion of the duct, and resting on a myoepithelial layer borne by a basement membrane, were two types of cell differentiable into (a) superficial dark cells which stained deeply with PAS or with Best's carmine and (b) basal clear cells which gave a more diffuse and less intense reaction with these methods. In a number of cases, large secretory vacuoles were prominent in the clear cells (Fig. 11). Both acid and alkaline phosphatase were present in these glands which, however, proved to be devoid of neutral fats and phospholipids.

Sebaceous glands

In areas of hairy skin, small sebaceous glands of simple alveolar structure were found as appendages of the hair-follicles into which

Table 1

HISTOCHEMISTRY OF NORMAL SKIN

Technique	Chemical Substance	Epidermis	Apocrine Glands	Eccrine Glands	Sebaceous Glands
PAS	Polysaccharide	-	+	+	-
PAS + diatase	Glycogen	+*	-	+	-
Best's carmine	Glycogen	+*	-	+	-
Alcian blue	Acid mucopoly-saccharide	-	-	-	-
Sudan IV	Neutral fat	+	-	-	+
Nile blue sulphate	Phospholipid	-	-	-	-
Acid-haematin	Phospholipid	+	-	-	+
Schultz	Cholesterol	-	-	-	+
Birefringence under polarized light	Cholesterol	-	-	-	+
Gomori (lead nitrate)	Acid phosphatase	+	+	+	+
Gomori (calcium cobalt)	Alkaline phosphatase	-	+	+	+
Perl's Prussian blue	Ferric iron	-	-	-	-

* Digital pad only

they opened. Larger glands of the same type were present in the upper lip in association with the tactile hairs, ventral to the lower lip, in the mental foramina and in the dorsum of the tail where they formed the supracaudal organ. Other sebaceous glands to be encountered in the cat include the Meibomian gland of the upper eyelid and those of the anal sac (Fig. 12). Sections of skin stained by Sudan IV and by Oil red O revealed that sebaceous glands contained large amounts of lipid and examination for birefringence by means of polarized light and use of the Schultz technique disclosed that they were also rich in cholesterol (Fig. 13). Glands stained by the acid-haematin technique were coloured dark blue-grey but staining by Nile-blue sulphate was negative. Both acid and alkaline phosphatase (Fig. 14) were present in considerable amount. Glycogen or other polysaccharides were not detected in sections stained by Best's carmine, PAS and alcian blue.

Discussion

The structure of the epidermis investigated in this study conformed closely with the description given by Strickland and Calhoun (1963) although the stratum lucidum was not to be seen in the epidermal lining of the anal sac nor was it constantly present in the epidermis of the planum nasale. It may be that the presence of a translucent layer in skin depends on the total epidermal thickness. Histochemical findings were mainly in agreement with those of

Montagna (1962) but phospholipids were not demonstrable in sections stained by the Nile-blue sulphate method of Menschik, although positive reactions were obtained with the less specific acid-haematin technique. In addition, cholesterol was not to be found in the stratum corneum either by use of the Schultz method or by means of examination under polarized light for birefringence.

The dermo-epidermal junction in the cat was found to be similar to that described for man (Dick, 1947) and was characterized by accumulation of reticulin fibres which was strongly PAS positive and in which the fibrils interdigitated with the basal cells of the epidermis. Elastic fibres were also numerous in this area. Ridging of the undersurface of the epidermis was absent in hirsute feline skin, probably because the large numbers of hair-follicles effect satisfactory adhesion between the epidermis and dermis.

In the dermis few, if any, differences were to be noted from the descriptions already given by Creed (1958) and by Strickland and Calhoun (1963). The presence of numerous mast cells in the papillary layer of the feline dermis, as recorded by Riley and West (1953), was confirmed.

The arrangement and distribution of hairs in the cat closely conformed with that reported by Hofer (1912), namely, that of a single large guard, or cover hair surrounded by numerous bundles or groups of woolly hairs. The histological structure of guard and tactile hairs was found to be identical with that of mammalian hair

described by Montagna (1962) and by Strickland and Calhoun (1963). The observation that the cover hair emerges from the epidermis through a single opening is at variance with the experience of Strickland and Calhoun (1963) and Creed (1958) who stated that both cover and wool hairs leave the epidermis by way of a common orifice. However, not infrequently, there were to be seen single large follicles containing only one hair which, throughout its length, appeared to be entirely separate from the adjacent wool hairs with their obvious common opening. That finding was confirmed when shaved areas of cat skin were examined by means of a dissecting microscope and guard hairs were observed to issue singly from the epidermis, whereas wool hairs emerge via a common orifice.

The distribution of the apocrine glands was established to be similar to that described by Strickland and Calhoun (1963) in that those structures were found to be present throughout feline skin apart from that of the digital pad where only eccrine glands were encountered. The large saccular apocrine glands appeared to be much more active than were the small ones and their lumina frequently contained material that was strongly PAS-positive. In general, the histochemical results were similar to those reported by Montagna (1949) in respect of the glands of the feline external auditory meatus. Not without interest is a recent study, made of the large apocrine sweat glands existing in the hairy skin between the toe and the digital pad and

in the external auditory meatus of adult cats (Munger, 1965), which has shown those glands to be of merocrine type and to differ from the eccrine sweat glands of the digital pad only in that they lack the "clear" cells distinctive of the latter structures. Doubt has also been cast on the method of secretion of the bovine sweat glands by Findlay and Jenkinson (1960) who concluded that, if a sweat gland in the calf is stimulated as a result of thermal stress, it must function either by a secretory process that does not involve degeneration of glandular epithelium or by means of simple diffusion through the wall of the gland. In structure, eccrine glands of the digital pad conformed closely with the description given by Munger (1962) but coiling of those glands as described by Strickland and Calhoun (1963) was not observed. The dark and clear types of coil reported by Kamamura (1957) and by Munger (1961) were distinguishable in sections stained by PAS and by Best's carmine method for glycogen.

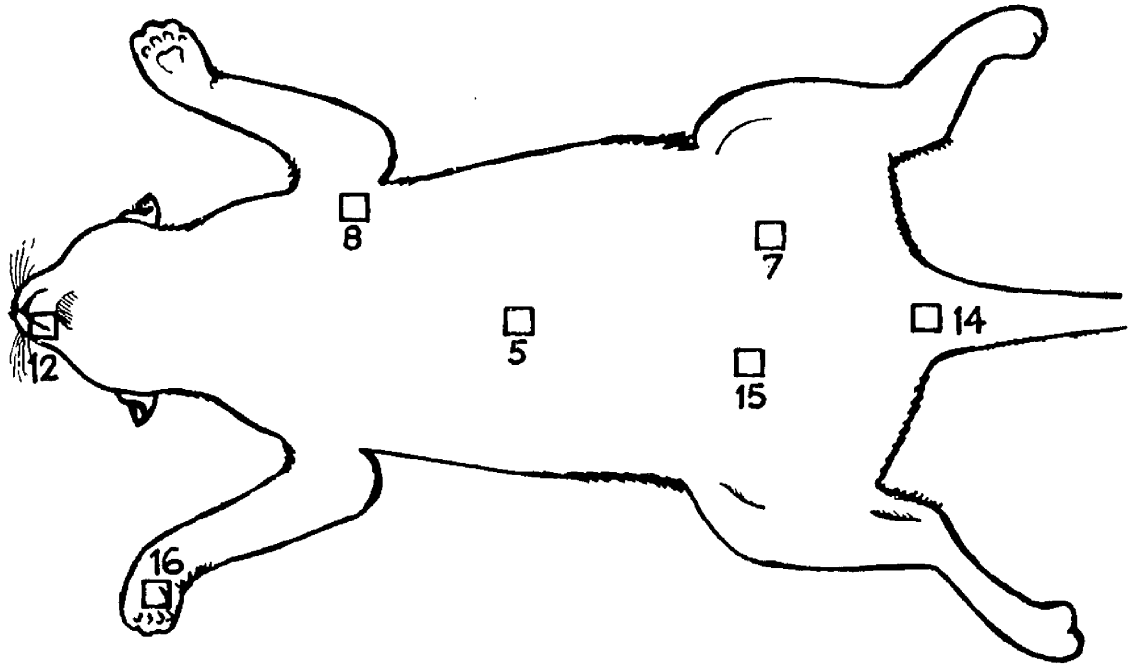
The sebaceous glands of the cat occurred either as small structures associated with the hair-follicles over the surface of the body or as large sebaceous glands in the case of the supracaudal organ, the lower lip and in association with the tactile hairs of the upper lip. Moreover, large sebaceous glands were found in the anal sac as well as in the eyelid where they form the Meibomian (or tarsal) gland. Generally, the staining reactions complied with the findings of Montagna (1949) in reference to the sebaceous glands of the feline

external auditory meatus. Sections stained by the acid-haematin technique gave a positive reaction but Dunnigan (1964) considered such a method insufficient to differentiate between hydrophobic lipids and phospholipids and recommended the use of Nile blue-sulphate (Monschik, 1953) at 60°C instead. By means of the latter technique, phospholipids proved never to be demonstrable in numerous sections of cat sebaceous glands although control sections of human arterial atherosclerosis were invariably positive. Sebaceous glands from all parts of the body were ascertained to contain large amounts of neutral fat, cholesterol as well as acid and alkaline phosphatases.

In general, feline skin resembles that of other mammals both in structure and in chemical composition but minor differences among species do occur for instance, the presence in the cat of a single guard hair-follicle which is not found in the dog (Creed, 1958) and the absence from the feline digital pad of the conical papillae which exist in dogs (Lovell and Getty, 1957). Further research into the histology and histochemistry of the feline skin is necessary to enable the veterinary dermatologist to understand its structure and function. Pertinently, it may here be mentioned that the available literature on feline anatomy has been found not to contain any reference either to the blood supply of the skin or to the mode of growth and manner of replacement of hair.

Fig. 1 Key to Skin Sampling Areas in the Cat

1. Dorsal neck
2. Dorsal thorax
3. Supracaudal organ
4. Frontal area - head
5. Mid-abdomen
6. Lateral chest
7. Inguinal area
8. Axilla
9. Lateral aspect - fore-leg
10. Planum nasale
11. Upper lip
12. Lower lip
13. Eyelid
14. Anal sac
15. Prepuce or teat
16. Digital pad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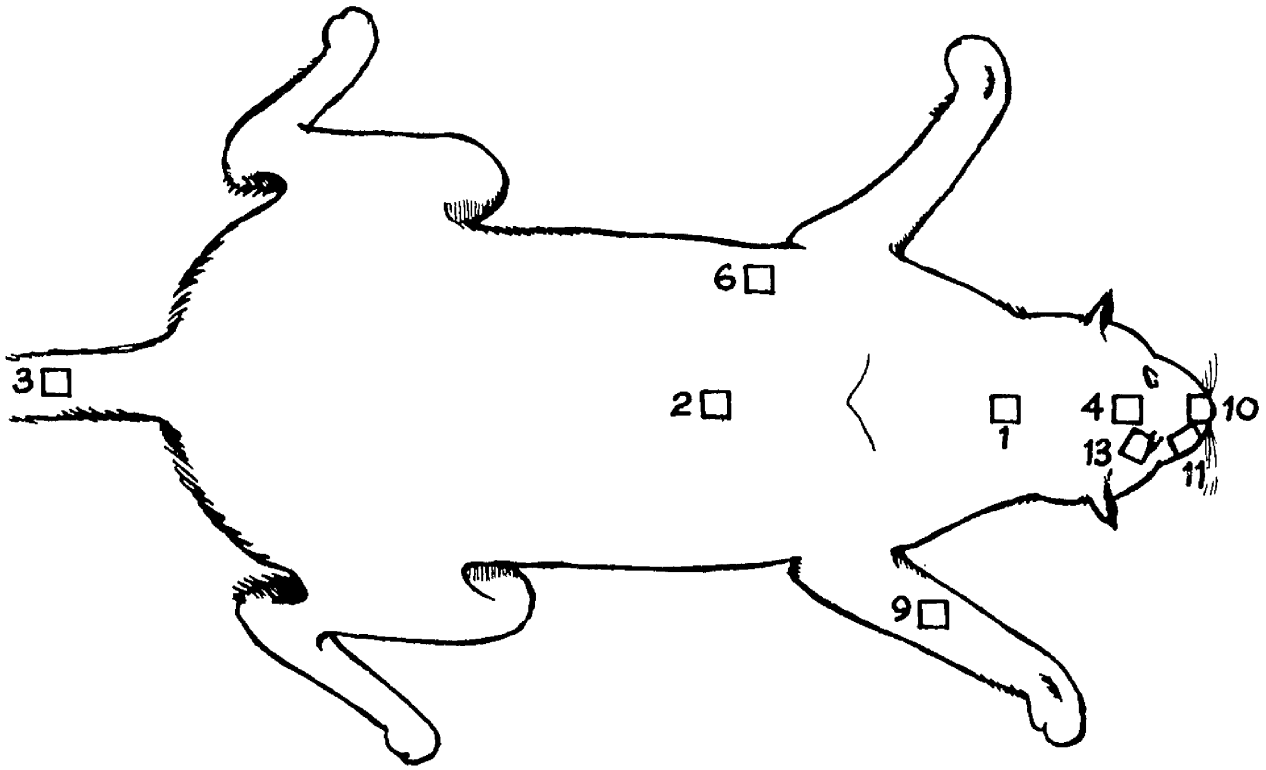
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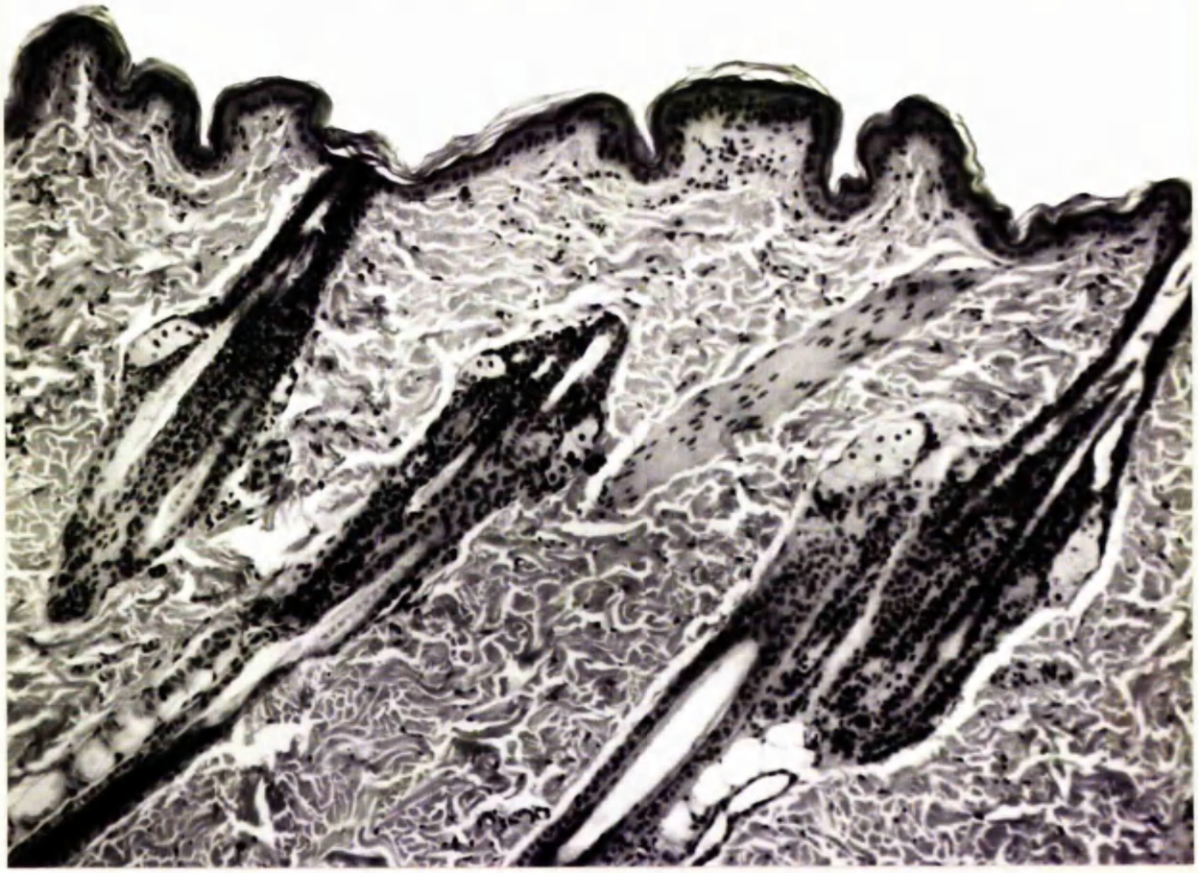
Fig. 2 Normal cat skin showing three compound hair follicles. Haemalum and eosin x180.

Fig. 3 Epidermal papilla showing thicker epidermis and well-vascularised dermis. Haemalum and eosin x450.



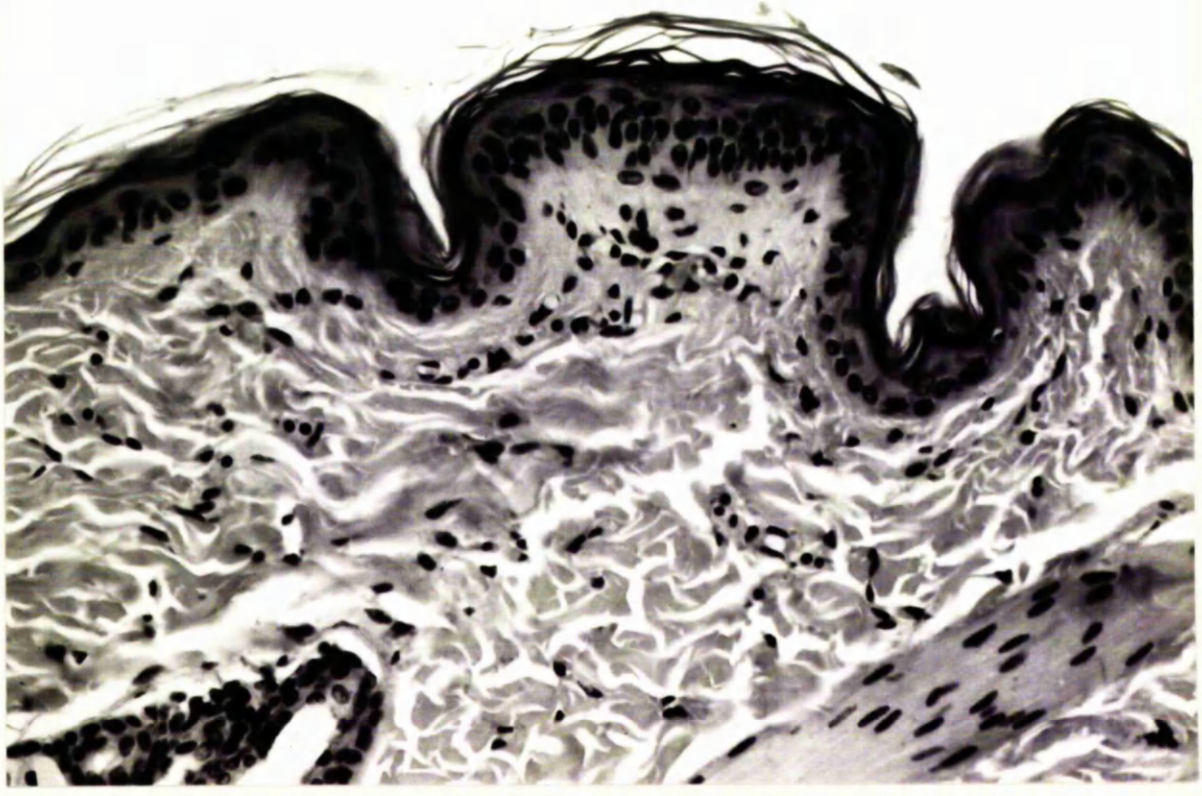
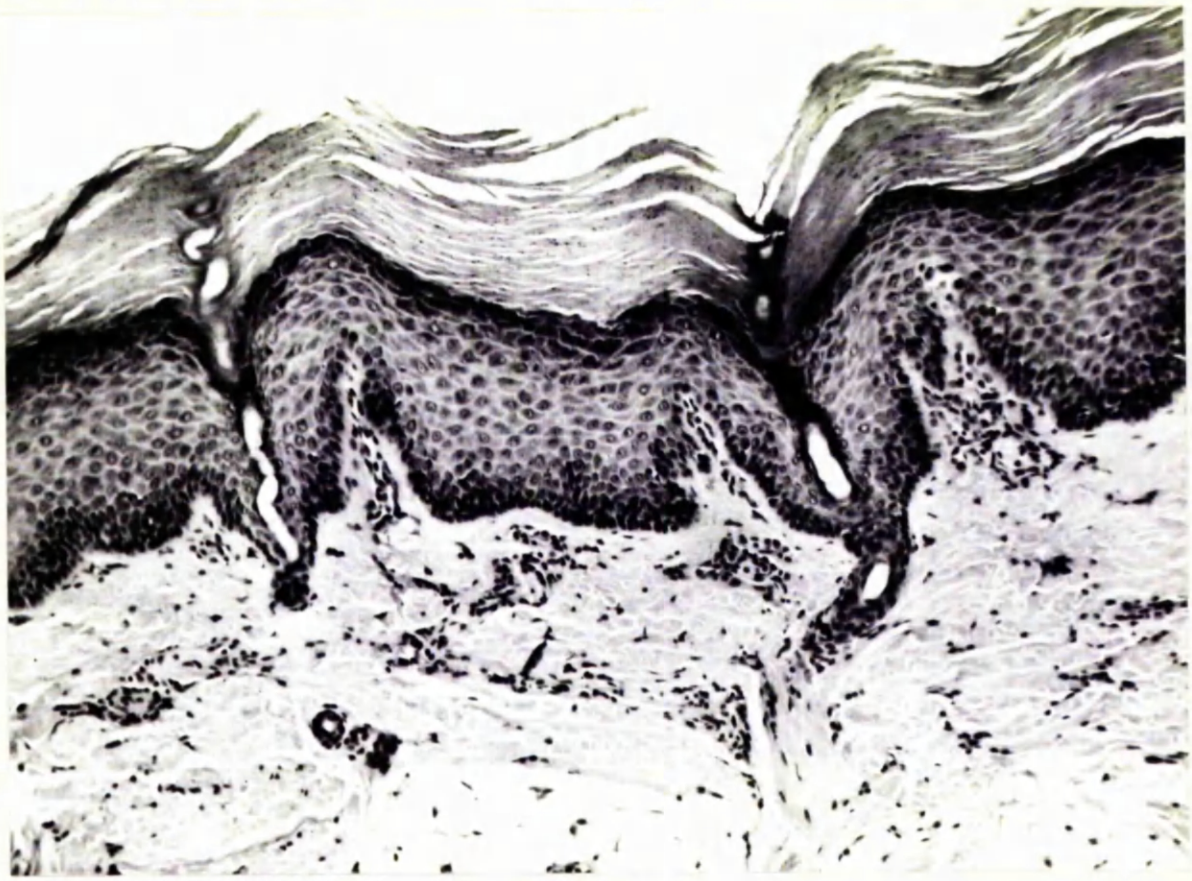


Fig. 4 Digital pad of the cat traversed by excretory ducts of eccrine glands. Haemalum and eosin, x150.

Fig. 5 Supracaudal organ with strongly positive acid phosphatase reaction in sebaceous glands and epidermis. Gomori, x150.



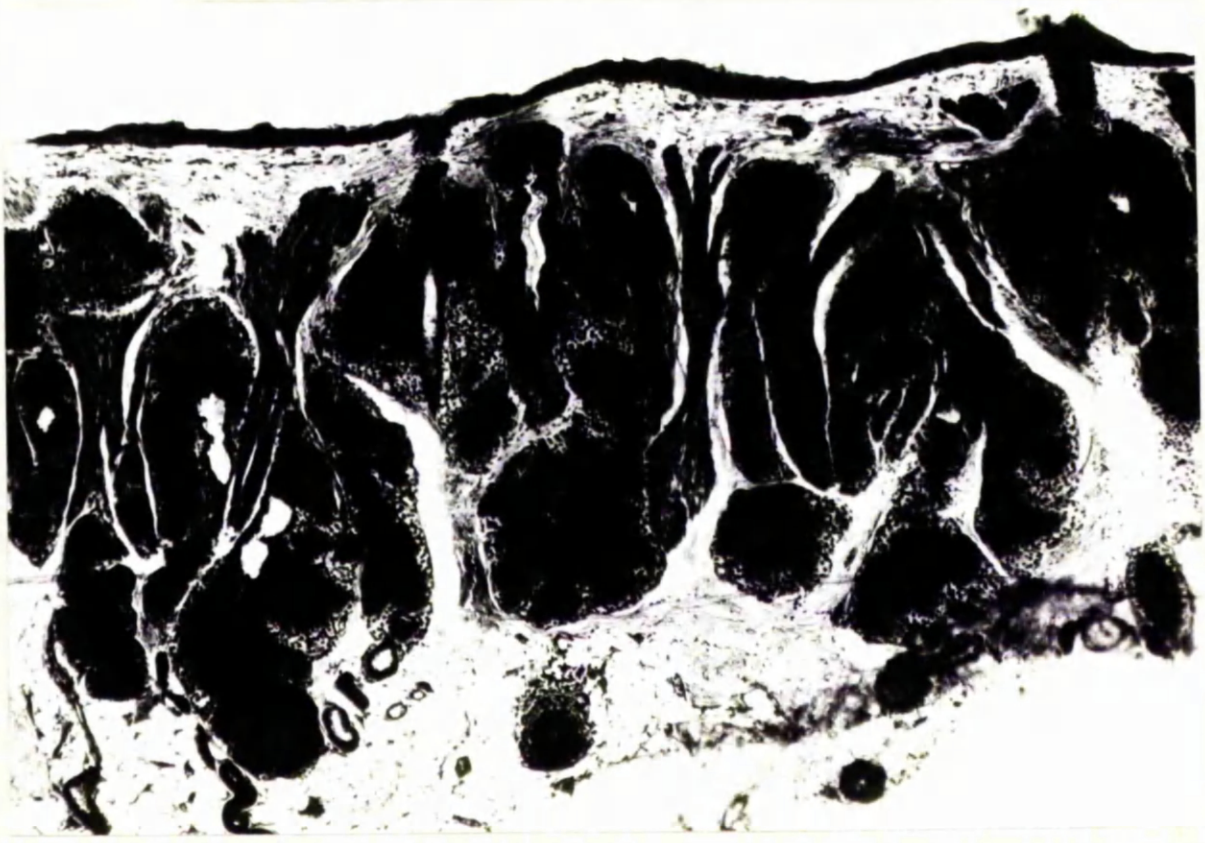
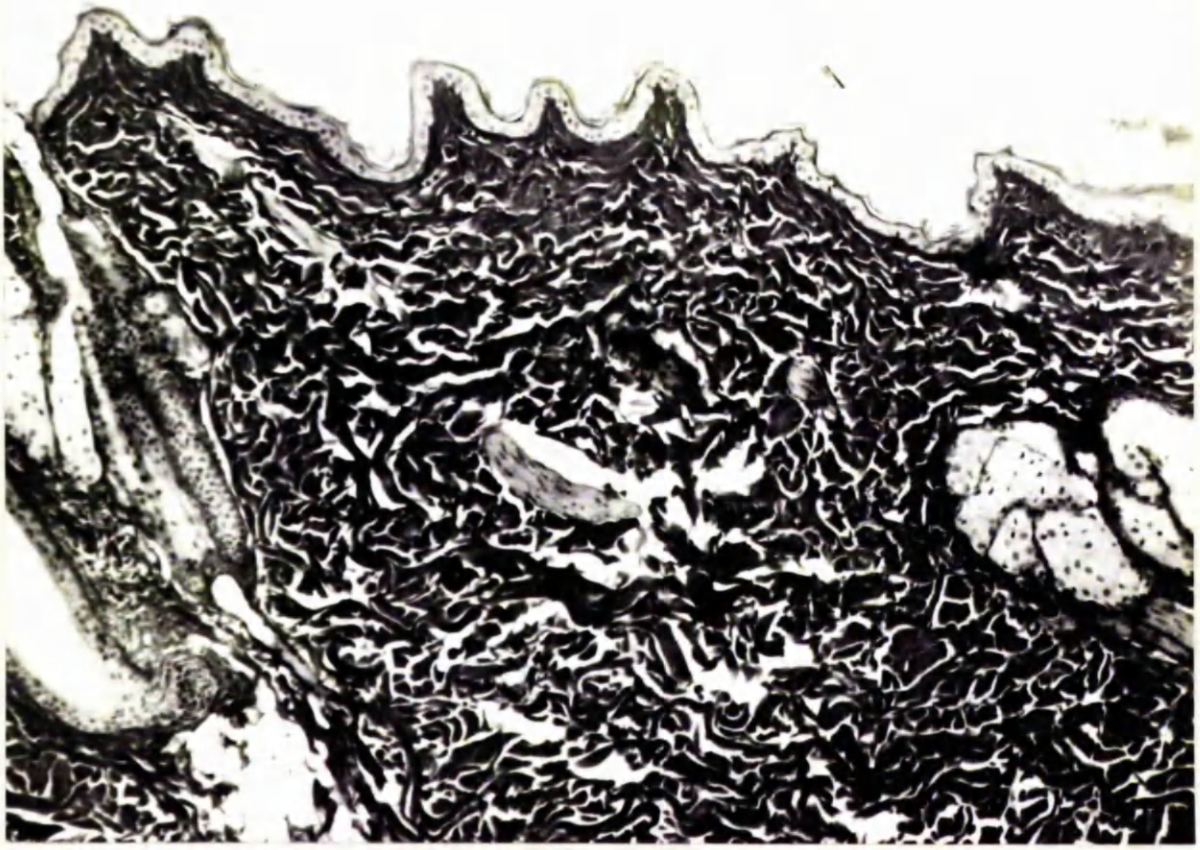


Fig. 6 Skin of cat showing condensation of collagen just below the epidermis. Van Gieson, x150.

Fig. 7 Dermo-epidermal junction in cat with meshwork of reticulin fibres. Gordon and Sweet, x150.



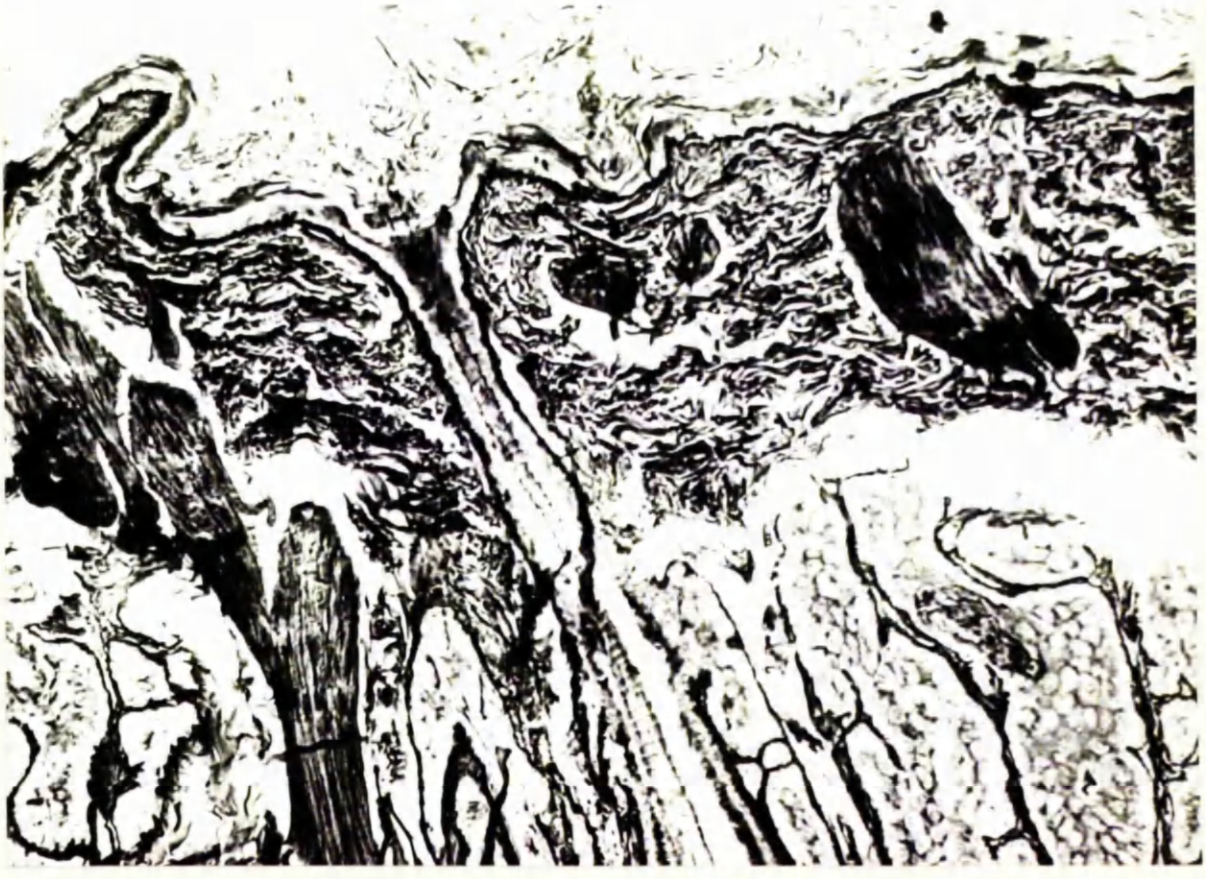
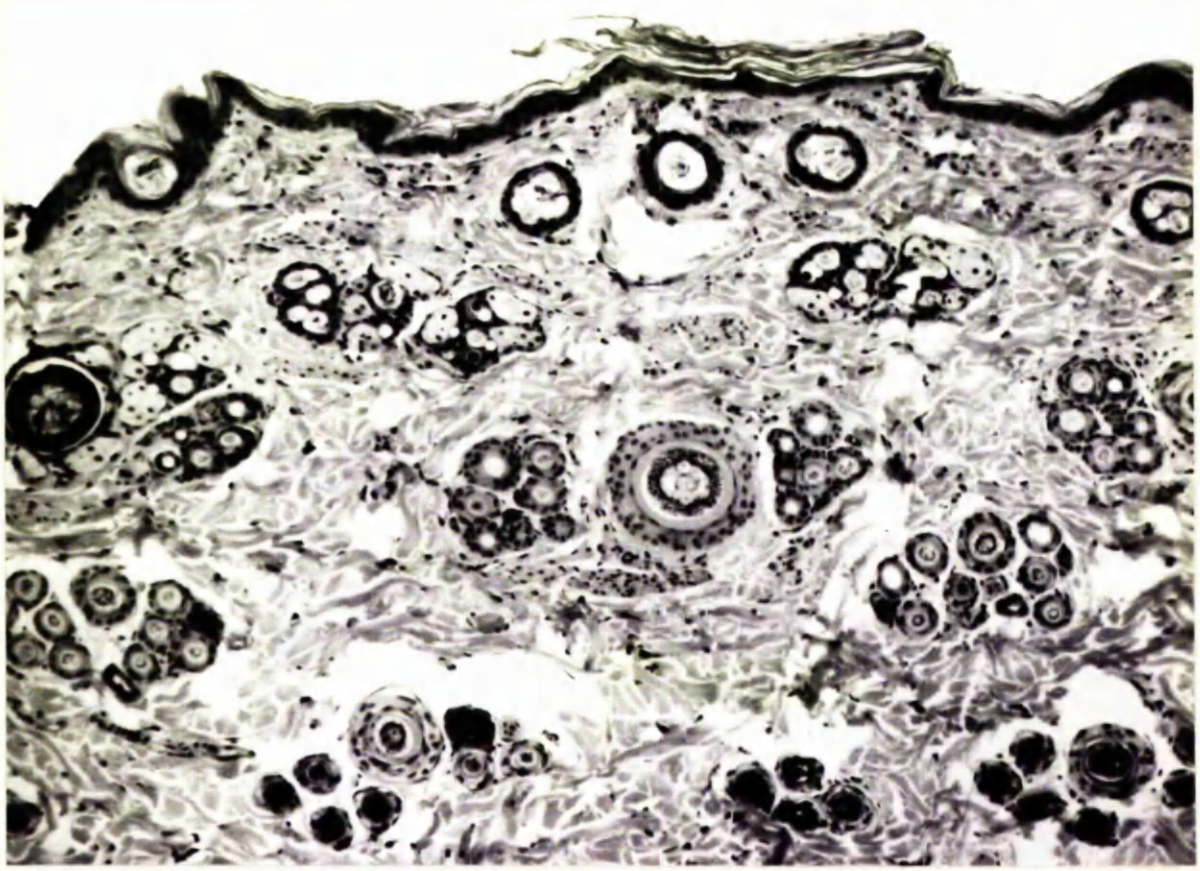


Fig. 8 Section of cut skin cut parallel with the surface to show guard hair with adjacent clusters of wool hair. Haemalum and eosin, x150.

Fig. 9 Guard hair erupting from a single follicular opening. Haemalum and eosin, x110.



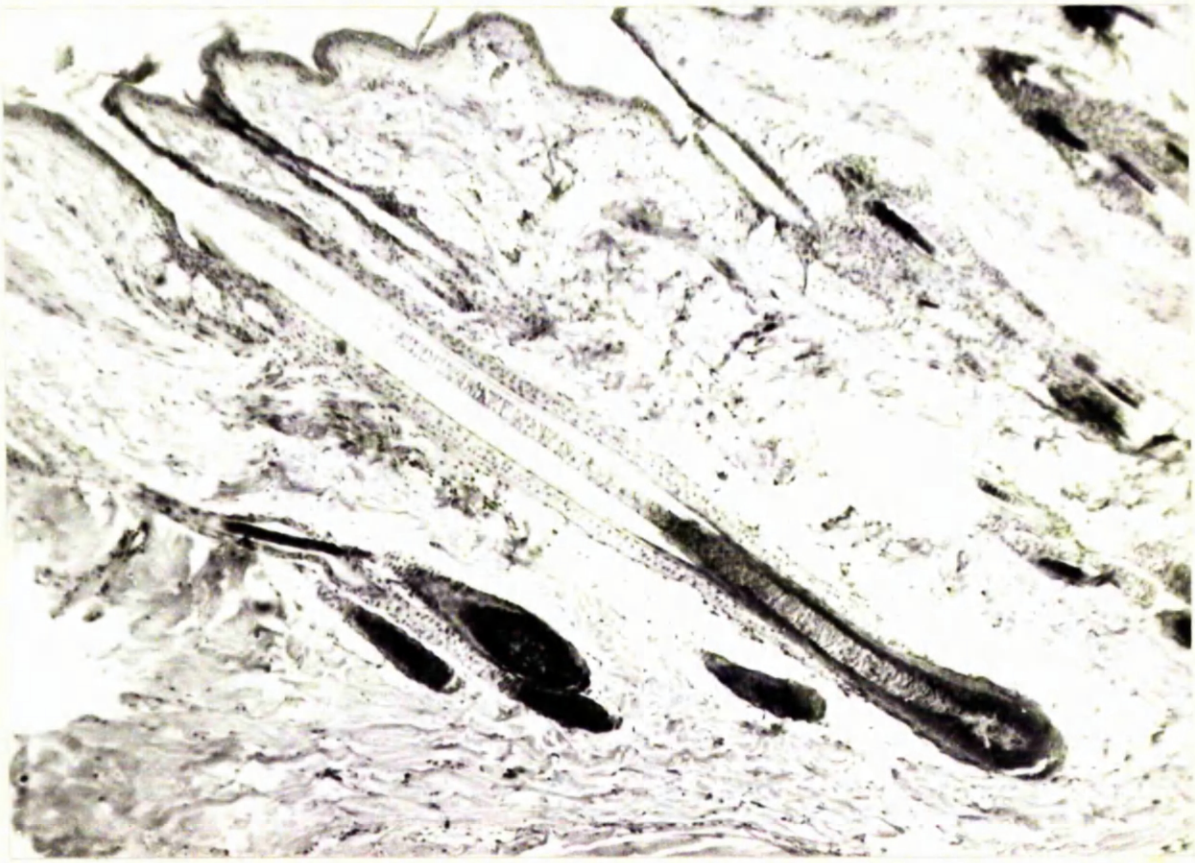
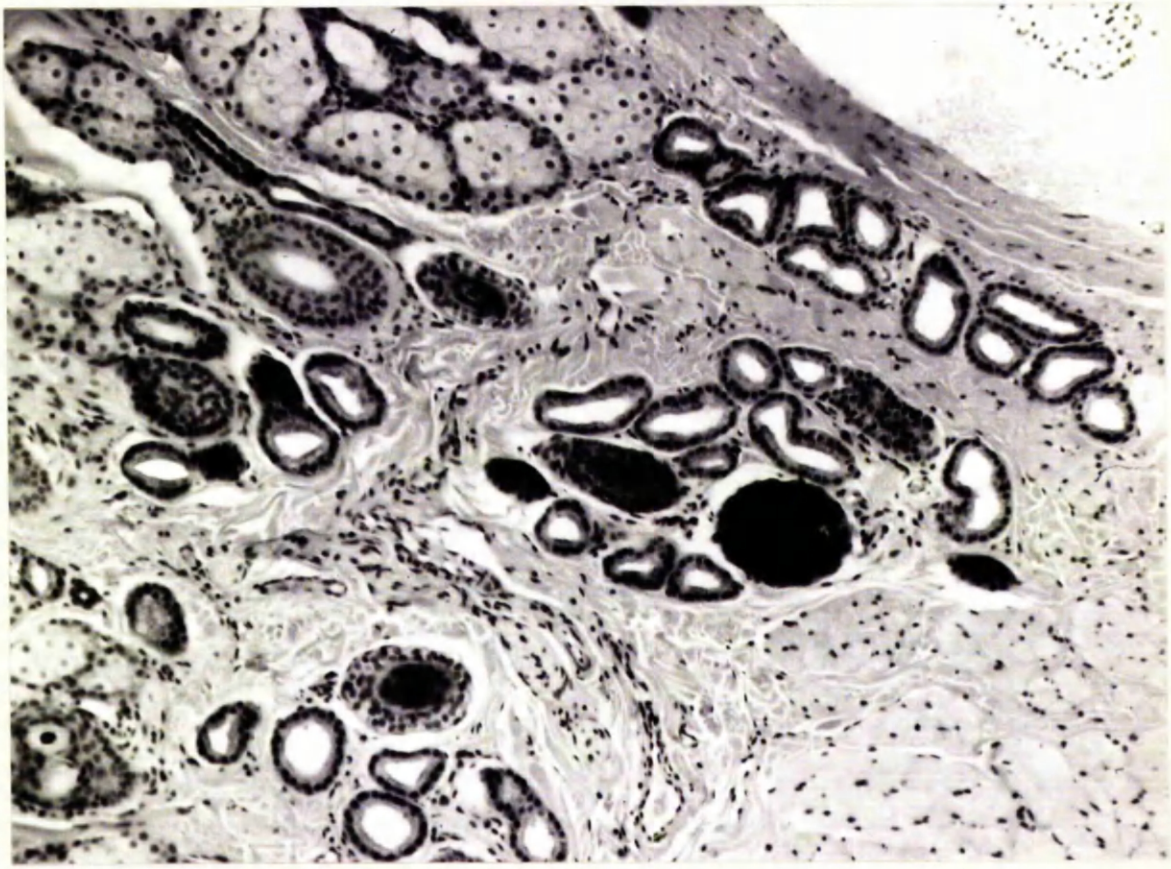


Fig. 10 Large apocrine glands of upper lip lined by columnar epithelium. Haemalum and eosin, x150.

Fig. 11 Secretory portion of eccrine glands of digital pad. Note prominent secretory vacuoles in lower right segment. Haemalum and eosin, x450.



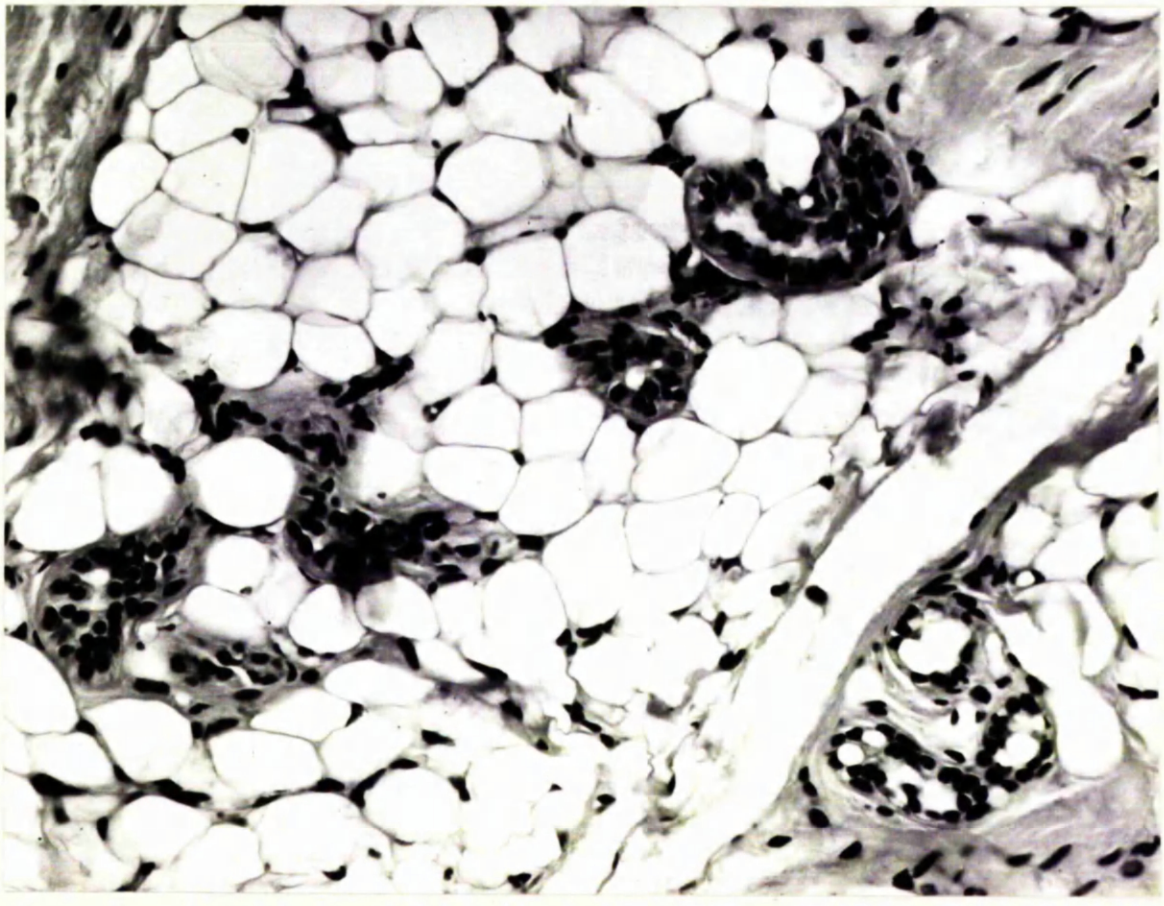
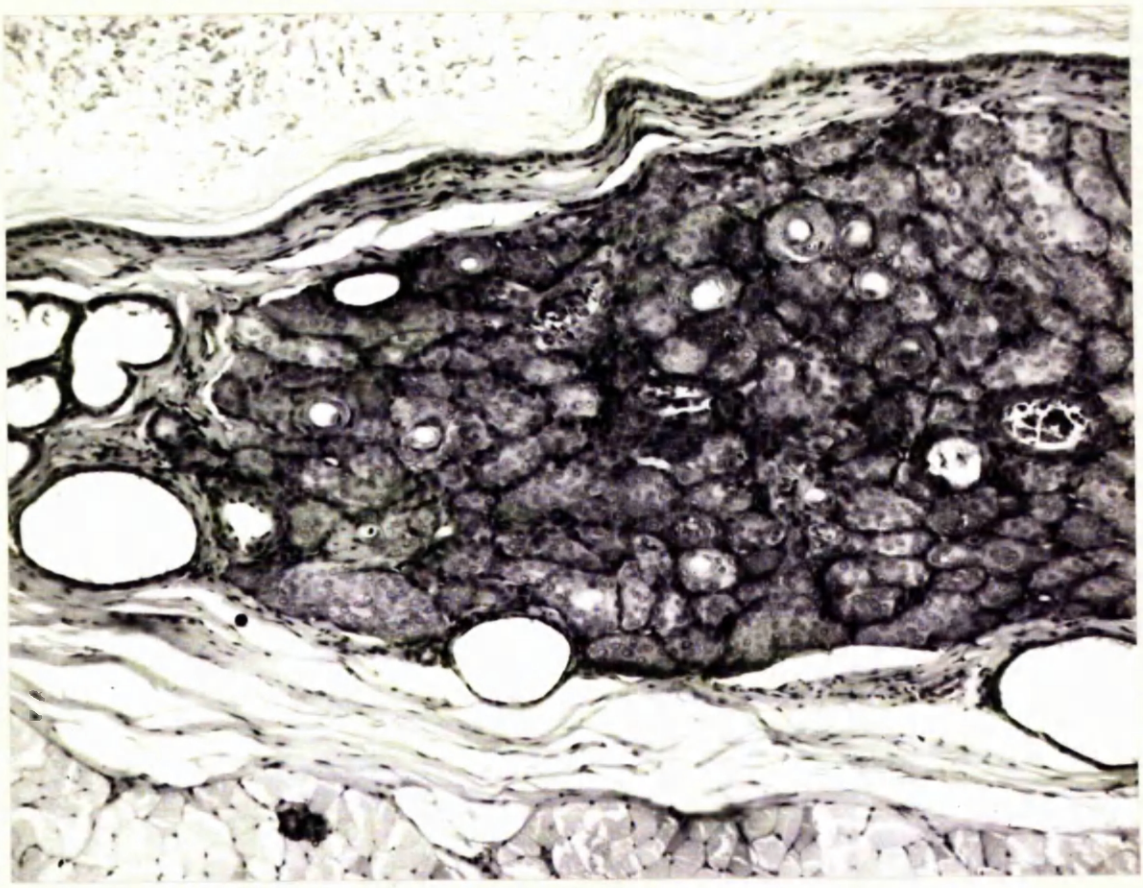


Fig. 12 Sebaceous gland of anal sac with coiled apocrine glands. Haemalum and eosin, x110.

Fig. 13 Intense birefringence under polarized light of sebaceous gland of supracaudal organ; indicative of cholesterol. Unstained section, x450.



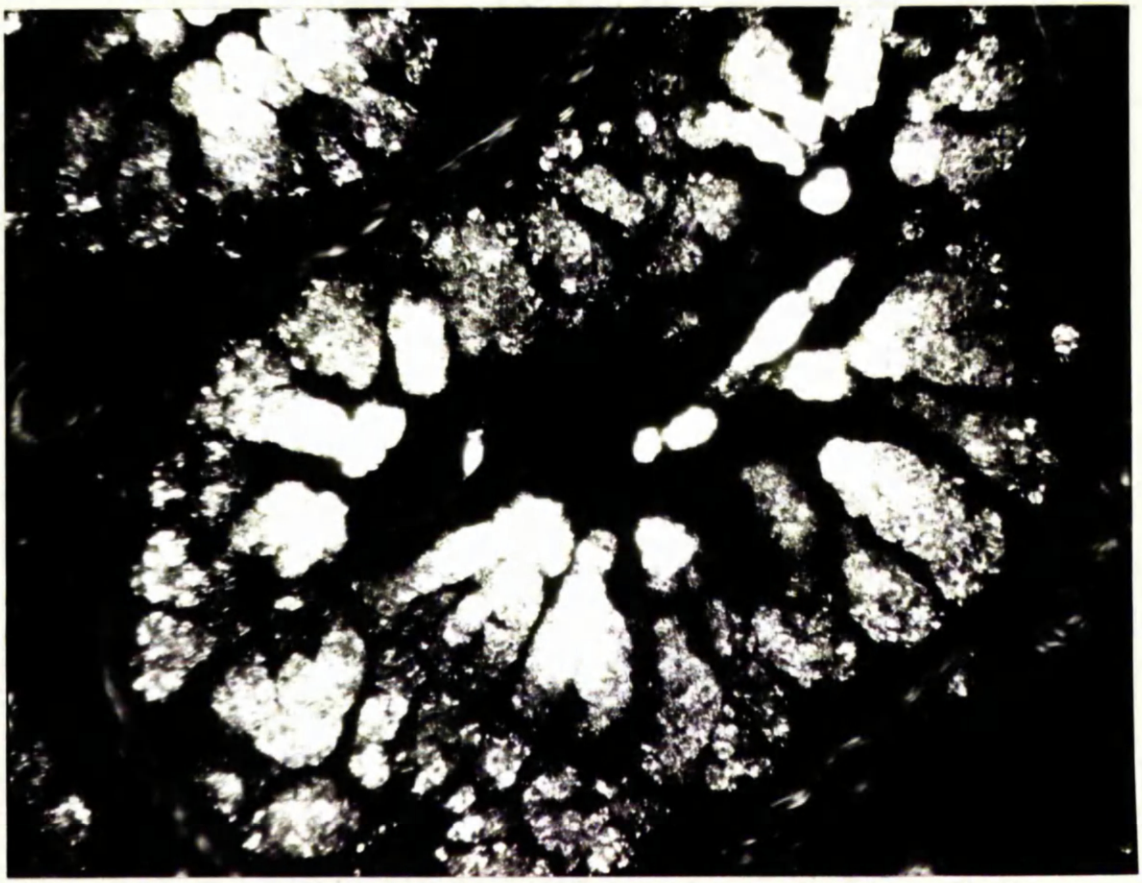
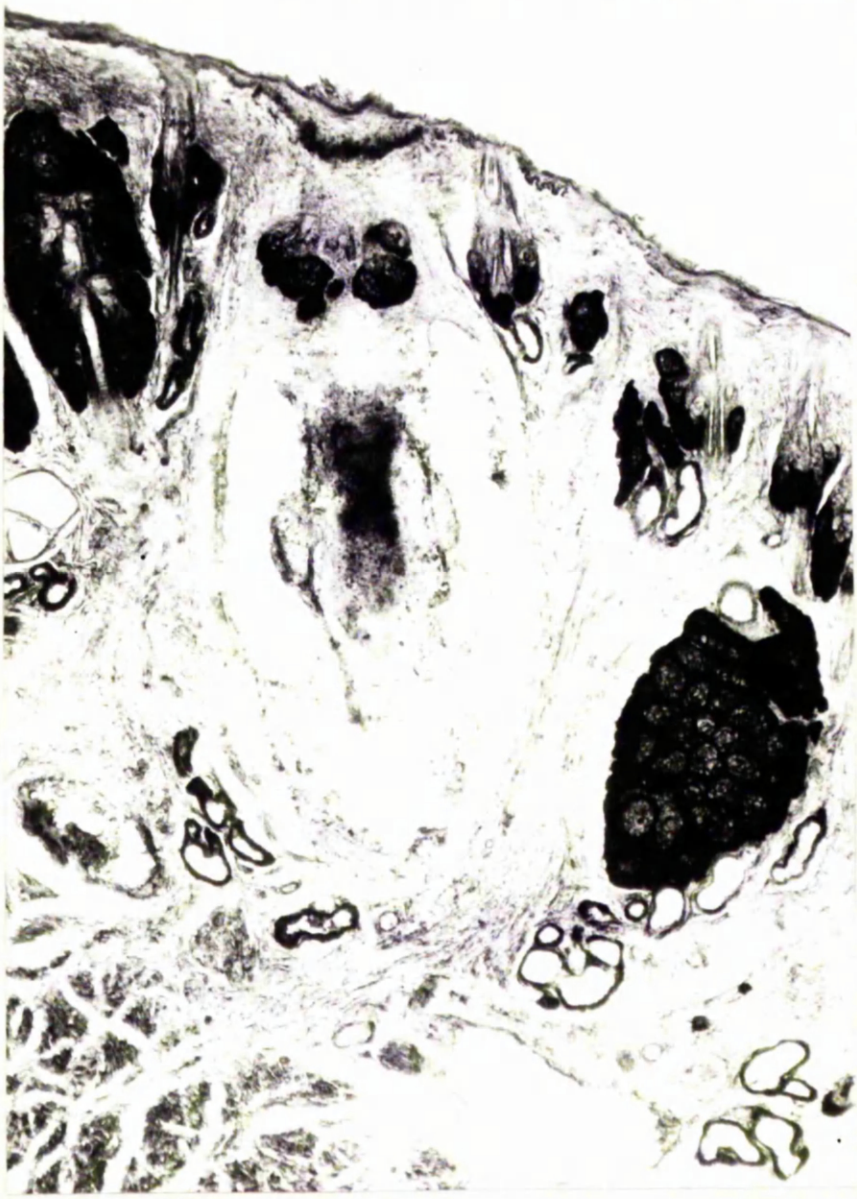


Fig. 14 Marked alkaline phosphatase activity in sebaceous gland of upper lip. Gomori, x80.



HISTAMINE AND MAST CELLS IN THE SKIN OF NORMAL CATS

Introduction

In many species of animals, mast cells have been found to occur frequently in the connective-tissues of the body, e.g. they are common in the skin of the cat, the canine liver, the bovine pleura as well as the guinea-pig lung and appear as large cells filled with granules which stain metachromatically with toluidine blue. The discovery that these cells contained the pharmacological agents, histamine and heparin as well as 5-hydroxtryptamine, and the fact that they increase in number in many morbid conditions has stimulated much research into the possible function of the mast cell in health and disease.

The distribution and the staining characteristics of the mast cells of normal feline skin as well as their association with histamine constitute the main subjects of study described in the ensuing section.

Review of the Literature

The mast cell was first described by Ehrlich in 1879 and was so called because of its high content of intracytoplasmic granules. Ehrlich considered it to be primarily a connective-tissue cell which increased in number in chronic inflammatory conditions and which exhibited the property of staining metachromatically with basic dyes. At the time, a specific function was not attributed to the mast cell

so that for many years that aspect aroused little interest and research was concentrated mainly on morphology, distribution and staining properties, which features have been reviewed by Michels (1938). A great impetus to research on mast cell physiology resulted from the discovery by Jorpes, Holmgren and Wilander (1937) that the cell contained heparin and thus might act as a source of anti-coagulant material. More recently, Riley and West (1953) demonstrated the presence of histamine in mast cells thereby producing further speculation concerning their possible role in both health and disease.

The Origin of the Mast Cell

Various theories have been advanced regarding the origin of mast cells but as yet there is not any single explanation of their presence in the tissues that is commonly accepted. Michels (1938) stated that in the adult body the supply of histogeneous mast cells is maintained by homoplastic and heteroplastic regeneration. The former is accomplished by mitotic division of pre-existing mast cells and the latter by an elaboration of mast granules in various types of connective-tissue cells. Fawcett (1955) suggested that the mast cell arose from undifferentiated cells in the walls of blood vessels and found that intraperitoneal injection of distilled water into the rat resulted in destruction of the mast cells of the mesentery. The latter structures were slowly replaced over a period of six weeks, initially, by means of differentiation of new mast cells from

fibroblastic precursors and, later, in consequence of cell division. At first, regeneration ensued slowly and only a few granule-containing cells were to be found in the adventitia of the small blood-vessels after a lapse of 8-12 days. These cells gradually enlarged in size over a period of weeks and moved away from the neighbourhood of the blood-vessels. During the later stages, mitotic and amitotic division of surviving mast cells contributed but slightly to repopulation of the mesentery which, in the main, was effected by new mast cells arising from undifferentiated units in the adventitia of small vessels.

Combs et al. (1961) claimed that, in the case of mice inoculated with tumour cells, mast cells originated from the reticular cells of the thymus and from medium and large lymphocytes as well as from epithelial cells. More recently, Ginsberg (1963) observed the development of mast cells in tissue-cultures of thymus cells that were grown on feeder layers of mouse embryo cells. At first, there was extensive degeneration of the small and the medium-sized lymphocytes which was followed by proliferation of undifferentiated cells scattered over the feeder layer. These undifferentiated cells, which Ginsberg termed 'mastoblasts', were similar morphologically to large lymphocytes. During the third and fourth weeks of culture, the mastoblasts developed intracytoplasmic metachromatic material which gave them a characteristic foamy appearance and were then termed young mast cells. From the 24th to the 30th day of culture, mitotic figures became

conspicuous but usually were observed only in circumscribed areas. From the 22nd to the 60th day the young mast cells gradually developed into mature mast cells. Of interest is the fact that Ginsberg found that thymus cultures derived from mice infected with Moloney leukaemia virus produced better mast cell preparations than did those obtained from control non-infected mice. The mechanism underlying that discovery remains unknown.

Morphology of the Mast Cell

Michels (1938) declared the mast cell to be, at times, round or oval and, at others, pyriform, fusiform or spindle shaped with a characteristically round or elliptical nucleus that is mainly of eccentric position. Binucleated mast cells are rather common. The great variation in their morphology may be due to factors such as genetic origin, amoeboid movement, physiological condition of the cell and mode of fixation. The most characteristic property of the mast cell is the accumulation of intracytoplasmic granules which stain metachromatically with basic dyes. According to Smith (1963), the granules are usually about 0.5 micron in diameter and may be sparse or closely packed within the cell. Where granules released from the mast cell have been phagocytosed by macrophages, differentiation of the latter from true mast cells may be a matter of difficulty.

Distribution of the Mast Cell within the Body

Histogenous mast cells occur in connective-tissue in all regions of the body in numbers that, as a rule, depend on the amount of the latter tissue (Michels, 1938). In the case of skin, Riley and West (1956) carried out a quantitative examination relative to the distribution of mast cells in the outer and inner layers of the dermis of the ear of pigs and cattle and in the abdominal integument of the mouse, rat, guinea pig, rabbit, cat, dog and man. Those authors found the mast cells to be most common in the dermis of the cat and the rat. In his review, entitled "The Tissue Mast Cell", Smith (1963) included a number of publications pertaining to counts of intradermal mast cells that have been carried out in various species of animals. Kelsall and Crabb (1959) reported that several anatomical areas of connective-tissue contain considerable numbers of mast cells, e.g. the mesentery of most normal animals. Again, mast cells are plentiful in the canine liver where they play an important part in anaphylactic reactions (Wilander, 1939). There, too, mast cells are to be found in the capsule of Glisson and not in the parenchyma. Other viscera rich in mast cells include the lungs, digestive tract, spleen and lymph-nodes (Michels, 1938). In morbid organs and tissues mast cells may be particularly numerous, for example, mast cell tumours of the dog, ox and cat; the lesions of the analogous human disorder, known as urticaria pigmentosa, are also rich in these cells (Head, 1958).

Accumulations of mast cells have been noted in many chronic inflammatory processes, e.g. chronic eczema, lichen planus and contact dermatitis (Asboe-Hansen, 1950).

Chemistry of the Mast Cell

Heparin

For long after Ehrlich's early work, interest in the mast cell rested mainly on morphology and distribution within the tissues. In 1937, however, it was discovered that heparin, the powerful anticoagulant recoverable from dog liver, stained metachromatically with toluidine blue and that a close correlation existed between the number of mast cells in a tissue and the amount of heparin obtainable therefrom (Jorpes et al., 1937). In view of the above findings, Jorpes and his colleagues considered that the mast cells were the main source of heparin in the tissues. At first, mast cells were thought to be wholly perivascular in location so that they could produce heparin and pour it into the blood stream. However, later study of the movement of perivascular mast cells showed that they can migrate away from the blood vessels and hence they probably produce their secretion for both blood and tissues (West, 1958). Further proof that mast cells contain heparin reposes in the fact that those cells selectively incorporate and retain substantial amounts of sulphur, which latter presumably becomes incorporated into sulphated mucopolysaccharide. By use of S^{35} injected as $Na_2 S^{35} O_4$

Asboe-Hansen (1953, 1954) and Asboe-Hansen and Levi (1959) demonstrated a high uptake of that material by mast cells which were present in large numbers around cutaneous papillomata of mice as well as in those in the vicinity of Rous sarcoma of the fowl. Direct proof that much of the sulphated mucopolysaccharide consists of heparin has been obtained from studies wherein heparin has been chemically identified after its isolation from extracts of rat peritoneal mast cells (Benditt, 1950; Schiller, 1963).

Further evidence that mast cells are rich in heparin is derived from experiments in which high levels of anticoagulant activity were found to characterize extracts of peritoneal mast cells of the rat (Archer, 1961), mouse mastocytoma (Furth et al., 1957), and dog mastocytoma (Oliver, et al., 1947).

Histamine

Riley and West (1953) reported that a close correlation between the mast cell population and the histamine content was found to exist in numerous normal tissues of the rat, dog, sheep, pig, cow and ox. They observed also that, when mast cells were few in tissues such as those of young animals and of foetuses, levels of histamine proved to be correspondingly low. Again, in the case of pathological organs and tissues, the mast cell was frequently increased in number and the content of histamine also was raised. Histamine liberators were found by those workers to bring about destruction of mast cells as

well as to effect the release of histamine from the mesentery of the rat *in vivo* and from the liver-capsule of the sheep *in vitro*. These findings have been confirmed by numerous other workers. As a result of the injection of distilled water into the peritoneal cavity of rats, Fawcett (1954) produced destruction of the local mast cells with release of histamine into the peritoneal fluid. Similar results attended analogous inoculation of compound '48/80' and it was further found that a second injection given several days after the first resulted in little, if any, liberation of histamine, indicating that the source of histamine was, indeed, the tissue mast cell.

Direct measurement of the histamine content of mast cells has been accomplished by a number of workers, including Benditt *et al.* (1955) who found that large amounts of the substance were released from isolated mast cells of the peritoneum that had been subjected to alternate freezing and thawing.

Techniques of cell fractionation have served to establish that histamine is contained within the cytoplasmic granules of mast cells encountered in the ox liver-capsule, the subcutaneous tissue of the rat and the substance of the Furth mouse mastocytoma.

In the case of a few tissues, close correlation between the number of mast cells and the amount of histamine has not been confirmed. Thus, Hota *et al.* (1956) recorded high levels of histamine to obtain in the gastric fundus and the duodenum of the rat when only

a few mast cells were to be found in those areas. Moreover, treatment with '48/80' did not reduce the histamine content of those tissues as it did in the instance of the skin, the tongue and the cardia of the stomach where a positive correlation existed between the number of mast cells and the histamine content.

Whether the mast cell synthesizes histamine or simply stores exogenous histamine in the cytoplasmic granules is a matter that has been investigated by Schayer (1956) who employed suspensions of cells obtained from the peritoneal cavity of rats. That author demonstrated that such preparations are capable of decarboxylating C-L-histidine and of binding the resultant C-histamine in stable form. Histidine decarboxylase was isolated from disrupted cells. Since none of the blood cells of the rat manifested those properties, it was inferred that the tissue mast cells were responsible for the decarboxylation and binding of histamine and that they contained histidine decarboxylase. Significant levels of histidine decarboxylase activity in mast cells have been demonstrated by Hagen et al. (1960) in the instance of the nonparticulate part of homogenates derived from Furth mouse mastocytoma and by Weissbach et al. (1961) in the case of the Dunn-Potter mouse tumour.

5-Hydroxytryptamine (5-HT; serotonin)

Benditt et al. (1955) demonstrated that the isolated peritoneal mast cells of the rat contained substantial amounts of 5-HT (serotonin)

which came to be released as a result of alternate freezing and thawing and was then chromatographically identifiable. In a later communication, Benditt et al. (1963) reported that 5-HT played an important part in local anaphylactoid reactions of the rat that were produced by injection of egg-white. The above experiments also revealed that partial inhibition of the reaction was obtainable by use of an antagonist to serotonin but that significant inhibition did not follow the administration of an antihistaminic substance alone. Benditt was of the opinion that 5-HT occurred in the cytoplasm of the mast cells only of the mouse and rat and that little, if any, evidence obtained for the presence of that substance in other species. In the case of the rat, Paasat and West (1957) found that over half the total amount of 5-HT in the body is located in the skin where, probably, the substance resides in two places, one of which is not the tissue mast cells. Dixon (1959) experienced a steady increase in the 5-HT content of whole rats to occur from birth until the 4th day post partum, after which the level remained static. Interestingly, administration of polymyxin B as a liberator substance has been noted to bring about disruption of mast cells with loss of histamine from the skin but with little change in the content of 5-HT (Paasat and West, 1957).

Histochemistry

(a) Mitochondrial enzymes

Histochemistry

(a) Mitochondrial enzymes

nase was detected by Glick and Potapragada

(1961) in suspensions of intact and disrupted mast cells isolated from the peritoneal cavity of rats. Hagen et al. (1959) found high levels of amine oxidase and fumarase to be associated with a particulate fraction which was lighter than that of the portion containing histamine, heparin and 5-HT.

(b) Proteolytic enzymes

Gomori (1953) observed that in tissue sections the mast cells hydrolyzed 3-hydroxy, 2-naphthoic acid anilide. Benditt and Arase (1959) showed marked similarities to exist between the properties of mast cell suspensions and chymotrypsin and have suggested that the latter substance may constitute the enzyme of the mast cell. Recently, Lagunoff and Benditt (1963) used isolated rat peritoneal mast cells to demonstrate the presence of a proteolytic enzyme which hydrolyzed casein, albumin and insulin. The enzyme occurred in active form in the characteristic large granules of the mast cell along with histamine and heparin and was readily extractable by means of 0.5 M KCNS. It was proposed that the enzyme should be known as mast cell chymase. The authors also found evidence of the existence in human and canine mast cells of a second enzyme which was homospecific with trypsin and suggested that, if it proved separable from chymase activity, it should be called mast cell tryptase.

(c) Other Enzymes

Coupland and Heath (1961) have presented histochemical evidence

that dopamine is a constituent of the mast cells in the liver capsule of the cow and the sheep and that dihydroxyphenylalanine decarboxylase is present in the non-particulate fraction recoverable from Furth mastocytoma. Smith (1963) has recorded a number of papers in which the mast cells are specified to contain sulphhydryl, oxidative and other enzymes.

Mast Cell Secretion

Depending on the nature of the stimulus, the mast cells appear to be able to liberate their contents by exercise of any one of three secretory processes. Thus, in response to treatment with distilled water or cortisone or to X-irradiation, changes characteristic of holocrine secretion are to be observed with disruption of the mast cell and consequent release of its cytoplasmic granules into the surrounding tissues (Smith, 1963). Hill (1957) has found evidence for apocrine secretion in that during the lipaemic phase following a meal of fat, the mesenteric mast cells of the rat liberate a part of their cytoplasmic granules but otherwise remain intact. As a consequence of intraperitoneal injection of toluidine blue or of protamine sulphate, Smith (1958) encountered a histamine discharge from the mesenteric mast cells of the rat that was not associated with any microscopical evidence of degranulation and so concluded that "secretion by the mast cell does not require cell disruption and death as much of the literature indicates but is merocrine in nature."

Selye (1965) considered that the available information on mast cell discharge did not favour a unitarian interpretation of degranulation and that the mechanism concerned was dependent on the degranulating agent, the species of animal and the area affected. In his opinion, the most popular concept of degranulation and histamine liberation is that of an "energy-requiring mechanism involving a lytic enzyme (probably phosphatase A or C) which is situated on the mast cell surface but normally remains inert because its active group is blocked by a special inhibitor. Only when the latter is removed by a histamine liberator does the enzyme become active; it then attacks the cell membrane and liberates the granules. However, trypsin and chymotrypsin-like enzymes may also be responsible for the activation of the mast cell-discharging mechanism."

Calciphylaxis and Calcergy

The newly-observed biological reactions, calcipylaxis and calcergy, have recently come to light as another possible function of mast cells. Apparently, discharged mast cell granules may furnish the organic matrix necessary for the binding of various metallic salts, particularly those of calcium (Selye, 1965). Calciphylaxis is defined by the above author as a phenomenon in which selective calcification of various organs is brought about by pre-treatment with a systemic calcifying agent, e.g. parathyroid hormone or vitamin D derivatives (the "sensitizer"), followed after an interval of time

(the "critical period") by an eliciting agent (the "challenger"). Calcergy differs from calciphylaxis in that it is produced without previous sensitization and in consequence of parenteral administration of so-called direct calcifiers or "calcergone", e.g. lead acetate, indium chloride and lanthanum chloride. Selye considers histamine liberators play an important part in calciphylaxis and calcergy through their mast cell discharging effect and that in rats, sensitized by means of dihydrotachysterol and subsequently given ferric chloride intravenously, topical calcification of the skin is producible at sites where histamine liberators are injected. Selye is of the opinion that discharged mast cell granules bind blood-borne iron which, after calciphylactic sensitization, come to attract calcium salts and that finally the mast cell granules disintegrate to release contained mineral which then becomes attached to adjacent connective-tissue fibres.

Materials and Methods

For histology and for histamine analysis two small blocks of skin (1 cm. square) were removed from adjacent parts of the dorsum of 20 normal rats immediately after they had been killed by an intra-peritoneal injection of nembutal. Samples for histamine analysis were stored for not more than 6 days at -20°C while those for histological examination were fixed in picro-formol and then processed

in the manner described in Part I for normal skin. Sections were cut perpendicular to the surface at a thickness of 6 microns and were stained by Mayer's haemalum and eosin, by toluidine blue (Gomori, 1952) at a pH = 4.0, by aldehyde-fuchsin (Gomori, 1950), by PAS and by Alcian blue with chlorantine fast red.

Counting technique

For this purpose, sections of skin were stained by toluidine blue and the number of mast cells in ten fields at a magnification of 400x was counted. The mean of ten such counts was taken as the mast cell value for each specimen. The fields of observation were chosen from the dermal papillary layer since the mast cells of feline skin are to be found mainly in that area (Riley, 1959). That procedure is not claimed to give an absolute mast cell value but has provided a reasonable degree of correlation with the amounts of histamine that have been extracted from a number of tissues (Riley, 1959). Only mast cells with granules and visible nuclei were counted.

Histamine analysis

Skin samples were trimmed to dimensions of 5 mms. by 4 mms., approximately, before storage at -20°C. On removal from the deep freeze, each sample was weighed and then cut on a refrigerated microtome or cryostat (Pearse Model by Slee Medical Equipment Limited) into sections, 15 microns thick, all of which were carefully collected into a small test tube placed at the foot of the section chute.

When cutting was almost complete, the tiny residuum of tissue was removed from the chuck and added to the rest in the test-tube. That procedure has been found to give a higher yield of histamine than is to be had from tissue ground up in sand. Two millilitres of $\frac{N}{3}$ HCl were then added to the tube and its content of sliced skin when the latter was placed in a water-bath at 100°C for one hour in order to extract the histamine. To prevent evaporation, a small piece of tin foil was placed on top of the tube and when cool, the material was centrifuged at 3,000 r.p.m. for 15 minutes. The supernatant was then poured off into a 25 ml. beaker to be neutralized by means of $\frac{N}{3}$ NaOH, with 0.1% neutral red as an indicator, when the volume of the final solution was measured. The extract was assayed on a preparation of isolated guinea-pig ileum obtained from the terminal 8-10 cms. of small intestine after the animal had been killed by a blow on the head. The portion of intestine was then placed into Tyrode's solution to have out from it a 2 cm. length, the contents of which were washed out by means of a Pasteur pipette. Without delay, the preparation was transferred to a tissue-bath, of 8 ml. capacity, containing oxygenated Tyrode's solution at 37°C, to which had been added atropine sulphate to prevent non-specific contraction. The isolated portion of ileum was connected by means of a thread to an all metal frontal writing point complete with lever, the movements of which were recorded on a khymograph. Two doses of the extract (u) and

and two of histamine standard (0.2 μg/ml.).(H) were used for a four-point assay and a tracing so obtained is shown in Figure 15. The amount of each solution was chosen to produce approximately equal contractions of the muscle. Administration was effected in "Latin square" design so as to minimize the effect of one dose on another and each amount of drug was allowed to act for 20 seconds, followed by a period of 60 seconds for recovery. The Tyrode solution in the bath was changed twice at the end of each period of contraction to wash out histamine and, at the close of each assay, the specificity of the test was checked by the addition of mepyramine maleate. After application of varnish, the height of each contraction was measured in millimetres and the mean of each group of four was plotted on graph paper, as shown in Fig. 16. The histamine content of the extract was then calculated in the following way:

Weight of skin = 0.0869 gm.
Volume of extract = 4.4 ml.

	A	B	C	D
	29 mm.	28 mm.	73 mm.	56 mm.
	31 mm.	29 mm.	70 mm.	55 mm.
	31 mm.	25 mm.	71 mm.	54 mm.
	25 mm.	22 mm.	62 mm.	32 mm.
Total	116 mm.	104 mm.	276 mm.	197 mm.
Mean	29 mm.	26 mm.	69 mm.	49.25 mm.

On the graph paper it was found that:

0.15 ml. Histamine Solution (H) ≡ 0.067 ml. unknown (U)

∴ 1 ml. H ≡ $\frac{0.15}{0.067}$ ml. U

∴ 4.4 ml. U contains $\frac{0.15 \times 4.4 \times 0.2}{0.067}$ μgms. histamine

i.e. 1.97 gms. histamine

∴ 1 gm. skin contains $\frac{1.97}{0.087}$ μgms. histamine

i.e. 22.6 μgms. histamine

Results

Histological examination of the skin showed an appreciable number of mast cells to be present in cat skin located mainly in the upper dermal layer from which they extended down the sides of hair-follicles, (Fig. 17). Only occasionally did cells of the type occur singly in the deeper parts of dermis and in the subcutis. The majority of the mast cells were polyhedral or round in shape and possessed a central nucleus which, in sections stained by toluidine blue, was largely obscured by the metachromatic granules of the cytoplasm. Frequently, the cells were to be found in groups of two or three and, to a large extent, were present around capillaries although some were also to be seen in areas of dermal connective tissue in which blood vessels were not visible. The results of differential staining of mast cell granules encountered in normal feline skin

Table 2

HISTAMINE AND MAST CELLS IN NORMAL FELINE SKIN

Cat Number	Mast Cells (average of 10 high power fields)	Histamine (μgms. per gram)
1	12.5	14.4
2	8.8	13.0
3	6.2	9.2
4	6.7	16.4
5	4.5	10.45
6	6.5	20.0
7	6.4	10.2
8	9.8	11.1
9	10.5	16.1
10	6.3	12.45
11	7.8	21.5
12	8.5	9.6
13	7.7	9.23
14	7.7	12.45
15	6.2	11.5
16	7.4	11.6
17	10.4	10.4
18	7.7	17.3
19	7.0	11.5
20	6.8	11.6
	Total 155.4	259.98
	Mean 7.7 $\pm$ 1.8*	13 $\pm$ 2.3*

* Standard deviation

are as follows:

Haemalum and Eosin	Negative
Periodic acid-Schiff	Positive
Toluidine blue (pH 4.0)	Metachromatic
Aldehyde-fuchsin	Deep reddish-purple
Acid phosphatase	Negative
Alkaline phosphatase	Positive
Alcian blue	Blue

In the case of sections of skin stained by toluidine blue, mast cell counts made over ten fields in the dermal papillary layer gave mean values that ranged from 4.5 to 12.5 mast cells per high power field with a mean for twenty animals of 7.7 $\pm$ 1.8 cells.

The histamine values recorded extended from 9.2 to 21.5 μgms. per gram of skin with a mean value for the group of 13.0 $\pm$ 2.3 μgms. These figures refer to the histamine base and are recorded in Table 2.

Discussion

Michels (1938) noted the presence of large numbers of mast cells in the various layers of the dermis, in the superficial and deep fascia, in and around hair-follicles and not infrequently in the epithelial layers. Throughout the present work, the presence of

considerable numbers of mast cells in the dermis has been confirmed but in the case of the cat they were confined mainly to the papillary layer of the dermis and only an occasional cell of the type was to be seen in the deep dermis. Contrary to Michels' findings, on no occasion were mast cells found in the epithelial strata although quite often they were present in close association with hair-follicles. Riley (1959) has stated that large numbers of mast cells occur in the skin of the rat as well as in that of the mouse and the cat. Those cells are to be found mainly in the papillary layer of the feline dermis and in the deep dermis of the rat. In the case of the mouse, cutaneous mast cells occur in two situations, one in the inner and the other in the outer layer of the dermis. Thus, there seem to be species differences in the distribution of mast cells in mammalian skin.

For long, the staining of mast cells in different species of animals has aroused considerable interest and most of the work so concerned has been applied to the discovery of the specific chemical substances that prevail within the cell.

In sections of feline skin stained with toluidine blue at a pH = 4.0 followed by decolourization in acid-alcohol and subsequent staining by the P.A.S. method, the mast cells which had exhibited metachromasia were constantly found to be P.A.S.-positive. This finding is at variance with the results of Lillie (1950) who found that, in sections of human appendix, rat omentum and salivary gland

and mouse stomach (in which the mast cells had been charted following staining by thionin after which they were decolourized with acid-alcohol and restained by the P.A.S. technique) "many or perhaps most of the mast cell granules" gave a negative P.A.S. reaction. Lillie interpreted his findings to indicate that mast cell granules do not possess a mucopolysaccharide as a major component. Compton (1952) noted that, in the hamster, mast cell granules do not stain constantly with P.A.S. and are more frequently unstained than stained. That author considered that his results were to be interpreted in the following ways: "(1) the hamster mast cell contains no heparin; (2) the hamster mast cell contains mainly the trisulfuric form of heparin; (3) irregularity of staining may represent different stages of esterification during the synthesis of heparin." In the case of the rat, Riley (1953) stated that immature mast cells (Type 1) were P.A.S.-positive and orthochromatic with toluidine blue, while the adult mast cell (Type 2) was P.A.S.-negative and metachromatic with toluidine blue and quoted Jorpes et al. (1948) who said that "heparin monosulphate reacts with P.A.S. while the di- and tri-sulphates do not." It was concluded by Riley that the Type 2 mast cells of the rat contain only the higher sulphates of heparin and that those cells are derived from Type 1 cells which originate in the adventitia of blood-vessels. Pearse (1960) states that a positive reaction with P.A.S. is manifest only by that proportion of mast cells which is

characterized by metachromasia and that many of those cells develop only a weak colour. He also refers to Jorpes et al. (1948) who showed fully sulphated heparin to be P.A.S.-negative. In the instance of canine mastocytoma, Head (1965) found that adult mast cells were both P.A.S.-negative and metachromatic with toluidine blue whereas the immature mast cell was P.A.S.-positive as well as orthochromatic.

The staining reaction exhibited by feline mast cells, characterized, as the latter are, by metachromasia with toluidine blue, show that they differ from the mast cells of the rat, hamster and dog in that the mature forms are P.A.S.-positive. Such dissimilarity may be due to difference in the degree of sulphation of the heparin of the feline mast cell or it may be attributable to some other chemical factor, or factors, as yet unknown. In their review, "Lymphocytes and Mast Cells", Kelsall and Crabb (1959) state it to have been found that "differences in reactions to the P.A.S. method occur (1) among mast granules within the same individual and tissue (2) in mast cells of different species and (3) in granules of a single mast cell." Those authors attributed the conflicting results obtained by the various workers in the field to a variety of factors that included variations of technique, apropos which they quoted Compton (1952) who found that mast cells were negative after 15 minutes, but commonly became positive when left for 2.5 hours in Schiff reagent. It was Compton who suggested that differences in

P.A.S. staining may be the result of "different functional states within the cell."

That feline mast cells contain acid mucopolysaccharide is manifest by their metachromasia at a low pH (= 4) and by their colouration by alcian blue with chlorantine fast red as a counter stain (Lison, 1954). The other staining results experienced are in accord with those obtained by Compton (1952).

The amount of histamine encountered in this series of normal cats (13.0 $\pm$ 2.3 μgm. per gm.) is in agreement with the values that have been recorded by other workers in the field. Thus in the case of the cat, Riley (1959) found levels of 20 μgm/gm. of histamine in the papillary layer of the dermis where the mast cells are numerous and of 6 μgms/gm. of histamine in the reticular layer where those cells are few in number. That information was incorporated in a combined mast cell-histamine profile of feline skin which served to demonstrate the close relationship that existed between the two components in cutaneous tissue. The lower histamine value of 13 μgms/gm. obtained during this work is probably ascribable to the mode of extraction of histamine from entire skin that was adopted throughout. In a comparison of the histamine content of the various tissues of the cat, Smith (1953) discovered that the abdominal skin of 10 cats yielded an average content of 20 μgm. of histamine per gram of skin, which figure is within the range of values obtained in the present work and by

Riley.

In spite of the volume of research which has attended the subject, the role of the mast cell in normal skin still requires elucidation. The part played by any cell needs to be related to the various chemical substances which it possesses and, clearly, the mast cell is a rich source of histamine, a substance of very high pharmacological potential. As has been already mentioned, mast cells may include other important substances such as heparin, 5-HT (in the instances of the mouse and rat) and various enzymes. It should be noted, however, that not all the histamine of the body occurs in mast cells and that the foetal tissue of various mammalian species (Rosengren, 1963) are endowed with a high histamine-forming capacity (HFC). Kahlson et al. (1960) found that histamine was formed in particularly large quantities in embryonic rat liver in which the histidine decarboxylase level was about a thousand times higher than that of maternal liver. In tissues undergoing rapid cellular multiplication, a high HFC has also been observed by Kahlson et al. (1963) who, in their review of the literature, cite references pertaining to the finding of a high HFC in embryonic tissue of rats and mice, in wound and granulation-tissue in the rat and in man as well as in rat hepatoma and in Walker rat mammary carcinoma. In adult sheep, the gastric mucosa contains appreciable amounts of histamine, not all of which is associated with mast cells, while in the duodenum of the rat, where very few mast cells exist, a

histamine value of 14.8 µgm/gm. has been recorded (Mota et al., 1956). In addition, the latter authors observed that a fall in both the mast cell population and the histamine concentration of the skin, tongue, oesophagus, cardia and fundus followed treatment of rats with compound 48/80 but were unable to detect any change in the histamine content of the duodenum, thereby providing further evidence that the histamine of the latter area is stored in structures other than the mast cells.

While there is fairly conclusive proof that mast-cell histamine is utilised as a vasodilator in tissue injury, convincing evidence has yet to be presented that the mast cell has a specific function in normal tissue. However, Scheyer (1964) was of the opinion that the vasodilatation of exercise was produced mostly by histamine formed in the tissues aided, possibly, by small amounts contributed from the tissue mast-cells. Riley (1962) proposed that mast-cell histamine played an important part in the metabolism of connective-tissue. His theory arose out of an earlier observation, recorded by Riley and West (1955), that a local zone of connective-tissue "activation" developed around an area of mast cell damage in which the connective-tissue cells became swollen, basophilic and amoeboid and eventually resembled cells in a tissue-culture. A similar reaction in mesenchymal tissue was producible experimentally in the rat and mouse by intraperitoneal injection of both histamine and heparin although intravenous inoculation of those substances failed to elicit any

reaction. Such findings led Riley (1962) to postulate a mast-cell cycle, in which damage to mast cells as a result of tissue injury led to the release of free histamine and of granules containing heparin. Diffusion of histamine so liberated was considered to stimulate phagocytic activity on the part of adjacent mesenchymal cells which then took up and metabolized the heparin-containing granules. The latter served to excite the activated connective tissue-cells to produce fresh mucopolysaccharide, which material contributes to the formation of extra-cellular ground substance. That, in turn, may be broken down and rebuilt into new mast cell granules where the histamine and heparin are stored for release. Burton (1963) arrived at a somewhat similar conclusion, namely, that "the function of mast cells is to remove or segregate some kind of polysaccharide and other related material from the environment, rather than to secrete." Since heparin is the main mucopolysaccharide present in the mast-cell, its primary function appears to be concerned with connective-tissue metabolism and its anticoagulant property may be of secondary importance in species other than the dog.

Fig. 15 Four-point assay of extract of cat skin.

A = 0.05 ml. extract (U) C = 0.1 ml. extract (U)
B = 0.08 ml. standard (H) D = 0.15 ml. standard (H)

Standard solution contained 0.2 g/ml. histamine base.

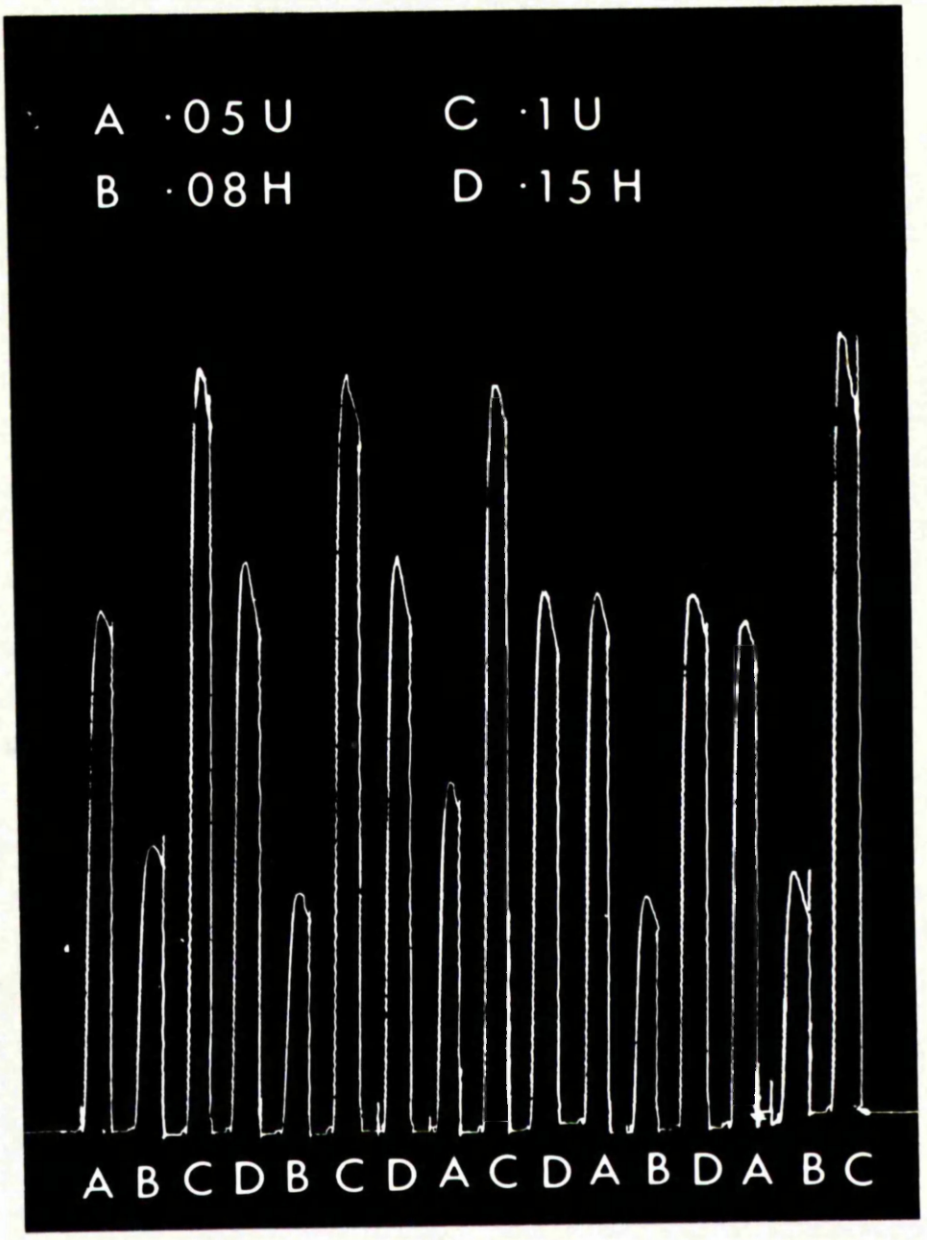

A ·05 U
B ·08 H
C ·1 U
D ·15 H
A B C D B C D A C D A B D A B C

Fig. 16 Result of four-point assay expressed graphically. Points A, B, C and D represent mean heights of contraction for each dose.

RESULT OF FOUR-POINT ASSAY EXPRESSED GRAPHICALLY. POINTS A, B, C, and D REPRESENT MEAN HEIGHTS OF CONTRACTION FOR EACH DOSE.

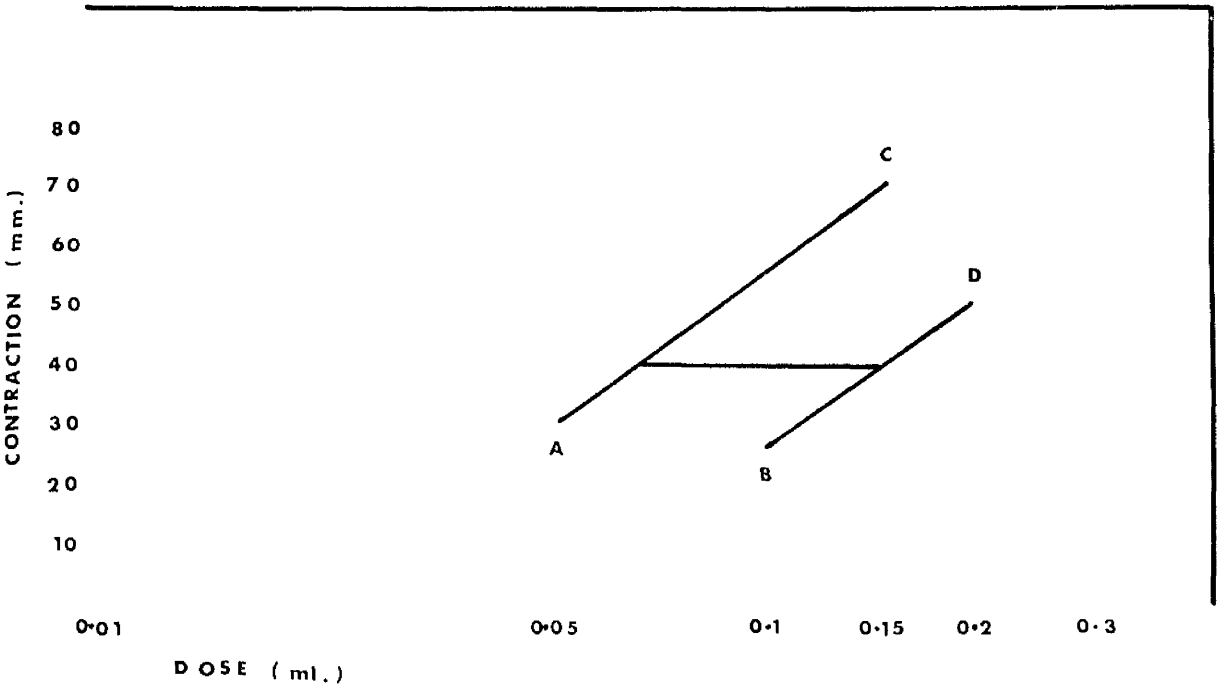
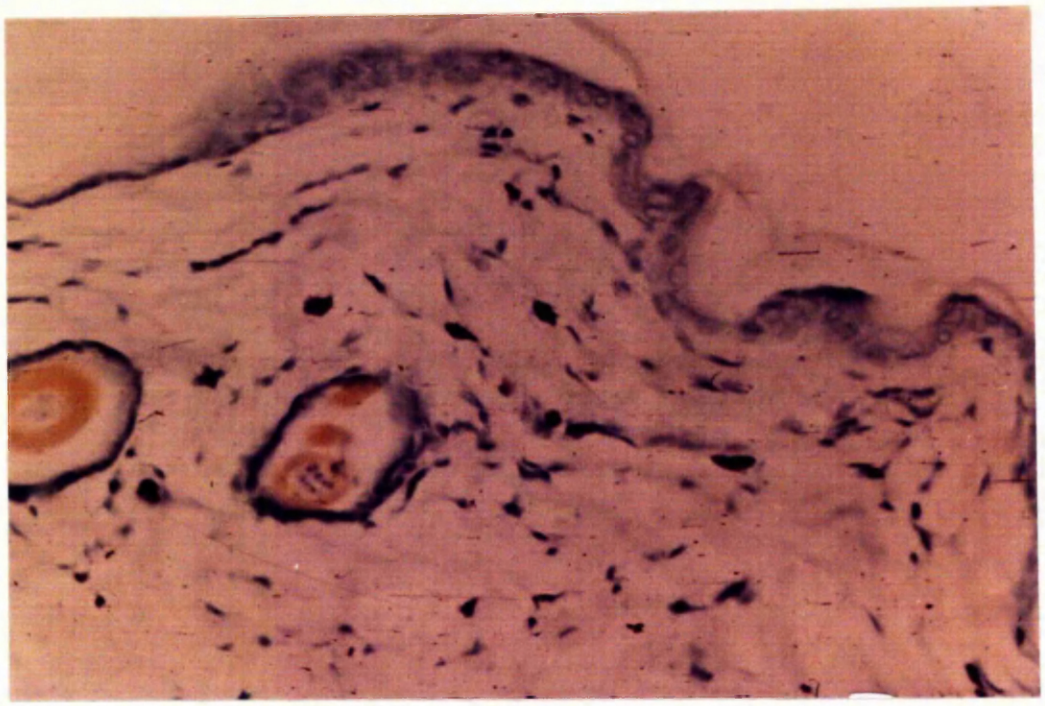
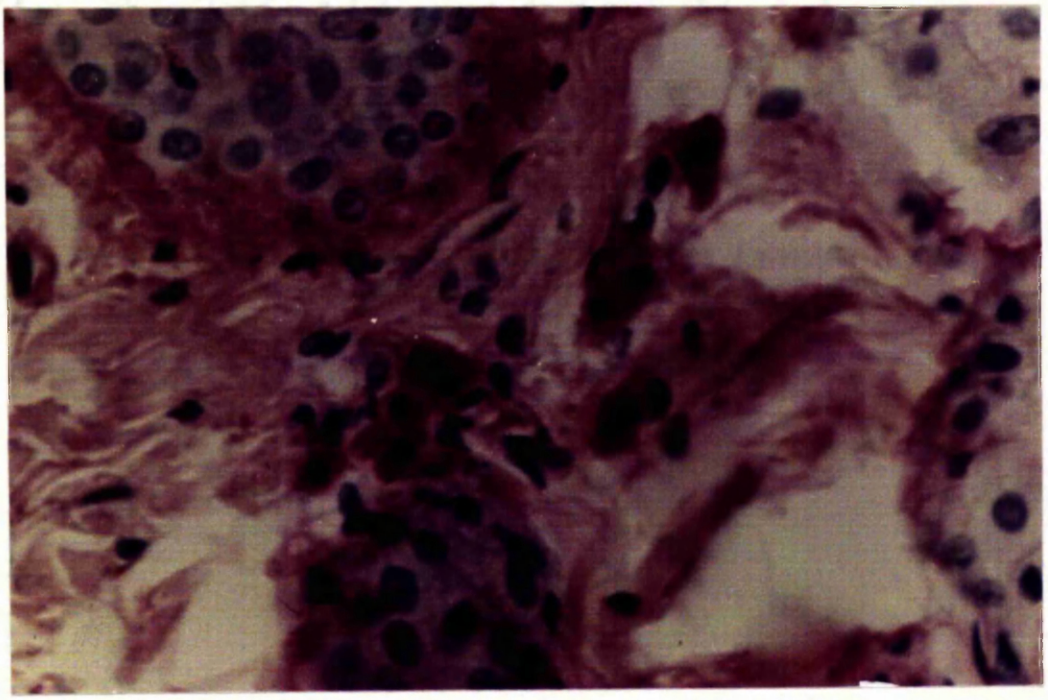


Fig. 17 Dorsal skin of cat. Note large metachromatic polyhedral mast cells in upper dermis. Toluidine blue, x110.

Fig. 18 Dorsal skin of cat. P.A.S. positive mast cells at base of hair-follicle. P.A.S., x400.





THE EFFECT OF CORTICOSTEROIDS ON THE SKIN OF THE NORMAL CAT

(1) BETAMETHASONE

Introduction

The adrenal cortex synthesizes a complex mixture of steroids, of which thirty-four have now been isolated although some of them are probably intermediate substances or degradation products produced during the elaboration of true hormones. These compounds have been classified by Lewis (1960) into:

(a) The mineralocorticoids which control salt and water balance in the body by an effect on the renal tubules and which cause retention of sodium, chloride and water; they increase the plasma level of sodium and reduce that of potassium. Important members of this group are desoxycorticosterone and aldosterone.

(b) The glucocorticoids which increase gluconeogenesis but have little effect on salt and water balance. They inhibit the formation of antibodies and reduce the response of the tissues to inflammation. This group includes cortisone, corticosterone, 17-hydroxycorticosterone and 11-dehydrocorticosterone.

(c) The sex hormones; oestrogens, androgens and progesterone.

Although the hormones of Groups (a) and (b) are mutually antagonistic, the mineralocorticoids have been shown to possess some glucocorticoid activity and vice versa.

Important among the actions of the corticosteroids, especially cortisone and hydrocortisone, is their anti-inflammatory and anti-allergic activity. The exact mechanism of that protective function remains unknown but White et al. (1961) have suggested that a number of factors may be involved, prominent among which are changes in the permeability of the membranes of both cells and mitochondria. Evidence that cortisone may possess the property of stabilising lysosomes in vivo is to be found in the fact that treatment of rabbits with cortisone prior to administration of vitamin A inhibits the lytic effect of the latter on cartilage (Thomas et al., 1963). Injection of prednisolone into one knee-joint resulted in selective protection of that articulation, indicating that cortisone acted directly on cartilage. The injurious effect of vitamin A on cartilage is believed to be the result of the release of cathepsin from the lysosomes of intact cells. Further evidence of the protective effect of cortisone on lysosome structure has been provided by Weissman (1964) who showed that pretreatment of lysosomes with hydrocortisone in vitro and in vivo considerably retarded the release of enzymes following ultra-violet irradiation.

In the skin of normal animals treatment with cortisone or its synthetic analogues elicits a number of interesting changes. Thus, Ghadially and Green (1957) have shown a marked decrease in the epidermal mitotic rate to occur in the mouse. Other authors have dem-

onstrated a decrease in epidermal thickness and atrophy of the sebaceous glands and hair-follicles. Asboe-Hansen (1952) has reported the finding, in the case of cortisone-treated skin, not only of a reduction in the identifiable mast cells but also of morphological changes which included diminution in size, degranulation and vacuolation. According to Brody et al. (1953), regression and almost complete disappearance of mast cell tumours occurs in the dog as a sequel to cortisone therapy. More recently, Zachariae (1964) found that a fall in the histamine content of human skin followed administration of betamethasone by the oral route for 14 days, which event was considered to support the hypothesis that tissue reserves of histamine are, in part, regulated by the adrenal cortex.

One of the main problems in the use of steroids as therapeutic agents in man has been their tendency to produce deleterious changes, the more important of which have been listed by Robson and Stacy (1962) as follows:

(1) Action due essentially to excessive mineralocorticoid activity, i.e. sodium retention, potassium loss, oedema and hypertension.

(2) Other excessive metabolic effects, i.e. osteoporosis and nitrogen depletion, development and accentuation of diabetes.

(3) Adverse effects on tissue repair and healing, notably peptic ulceration and its complication, increased liability to infection.

(4) Complications due to inhibition of the anterior

pituitary, and notably of corticotrophin secretion; those occur on cessation of treatment.

In an attempt to overcome those difficulties a number of synthetic steroids have been prepared although in some cases these have been found to produce new toxic effects. Among those substances are prednisolone and prednisone, derivates of hydrocortisone and cortisone, respectively, which are four or five times as active as cortisone in respect of glucocorticoid activity and anti-inflammatory action but do not exert any increased mineralocorticoid action so that the effects on sodium retention and potassium depletion are minimized. Other synthetic steroids include triamcinolone, fluorohydrocortisone, dexamethasone and betamethasone. The rationale involved in the elaboration of the new synthetic steroids has been the finding that minor alterations in molecular structure may at once greatly enhance their anti-inflammatory effect and reduce their mineralocorticoid activity. Betamethasone, (16-methyl-9-fluoro-1, 2-dehydrocortisol), potently anti-inflammatory in action and considerably more active than cortisone although of reduced mineralocorticoid effect, was used in the present experiment on cat skin in order to study the histological changes which follow corticosteroid therapy and to allow subsequent comparison with the alterations produced with cortisone acetate. They will also be compared with the results produced in other species of animal by a number of workers.

Materials and Methods

Twelve adult male cats were used in this experiment. Six served as a control group while the remainder were given 0.5 mg. betamethasone by subcutaneous injection each day until death. The animals were killed, by intravenous administration of nembutal, at intervals of 10, 21, 36, 47, 59 and 65 days, one treated and one control cat being sacrificed on each occasion. Each experimental animal was weighed before the start of the experiment and again immediately before death. For histological examination and for measurement of the epidermal thickness samples of skin, ½-inch square, were taken from the dorsum, the mid-abdomen and the lateral thoracid wall. An additional small sample of dorsal skin was stored at -20°C until required for histamine analysis. The skin was fixed in picro-formol solution for 24 hours and then processed as has been described in Section I for normal skin. Paraffin sections were cut at 5 microns and stained by haemalum and eosin, periodic acid-Schiff, (Lillie, 1950), aldehyde-fuchsin, (Gomori, 1950), toluidine blue (Gomori, 1952) and van Gieson. Counts were made of the mast cells in the upper part of the dermis of sections of dorsal skin stained by toluidine blue at pH = 4.0. The total number of mast cells in ten high power fields (x400) was ascertained and the mean of that figure was taken as the mast-cell value for the piece of skin under consideration.

A calibrated Wild, Model M20-KGS, microscope was used to measure the epidermis of skin sections from each of the three areas mentioned above at the places chosen at random and the mean of each ten measurements was regarded as the epidermal thickness of that particular piece of skin. The average of the three means was taken as the epidermal "index of thickness" for each cat. Analysis of variance (Snedecor, 1959) was performed on the mean values obtained in each of the three areas to determine whether, or not, any significant difference in the results obtained. The student 't' test (Hill, 1961) was applied to the figures obtained from the two groups to ascertain whether, or not, there was any significant difference in values. The histamine content of the skin was determined on the atropinized guinea pig-ileum preparation as already described.

Results

(a) Histology

In the cats under treatment, the epidermis showed a marked decrease in thickness and the constituent cells had assumed an elongated appearance with only occasional nuclei to be seen here and there. The changes were most conspicuous in the stratum germinativum and the stratum spinosum (Fig. 20) while the stratum granulosum, although still present, was discontinuous. Appreciable alteration of the stratum corneum was not observed. The sebaceous glands appeared smaller in

size and there was evidence of increased cellular breakdown, pyknotic nuclei being more common than they are in normal glands. In all parts of the skin, the cytoplasm of the cells of the sebaceous glands was vacuolated and weakly stained and, in the case of the supracaudal organ of the tail, large, clear spaces present in the central part of each gland indicated excessive breakdown of cells.

The hair-follicles were atrophied and most of the associated bulbs were in the catagen phase in which the outer root-sheaths were distorted. A feature of the hair-bulbs was their almost complete lack of pigmentation.

In the group of treated cats the index of epidermal thickness (Table 3) was 0.9 $\pm$ 1.15 microns while that of the control group was 13.1 $\pm$ 1.41 microns, a difference which was found to be significant by use of the 't' test, ($p = < 0.05$).

The changes in the dermis consisted mainly of fragmentation of the elastic fibres together with a condensed appearance of the collagen. Most of the mast cells were of normal structure but a few were vacuolated. In all the cats, adipose tissue was very much reduced in amount and little, if any, fat was visible between the dermis and the cutaneous muscle.

(b) Histamine and mast cells

The results of this part of the investigation are given in Table 4. The mast cell value of the treated group was 6.5 $\pm$ 1.4 with a

Table 3

CHANGES IN EPIDERMAL THICKNESS FOLLOWING BETAMETHASONE THERAPY

Day of Death	Cat No.	Treated	Cat No.	Control
10	1	10.6 microns	7	13.1 microns
21	2	9.03 "	8	13.4 "
36	3	0.6 "	9	14.9 "
47	4	7.6 "	10	14.5 "
59	5	9.8 "	11	11.6 "
65	6	8.16 "	12	11.16 "
	Total	53.79 "		78.66 "
	Mean	8.9 $\pm$ 1.5*		13.1 $\pm$ 1.4*

* Standard deviation

Table 4

HISTAMINE AND MAST CELLS IN SKIN OF

NORMAL AND BETAMETHASONE TREATED CATS

Treated				Control		
Day of Death	No.	Mast Cells (10 fields)	Histamine (μgm./gm.)	No.	Mast Cells (10 fields)	Histamine (μgm./gm.)
10	1	not counted	12.5	7	6.5	9.2
21	2	6.5	14.1	8	3.5	11.2
36	3	8.5	20.0	9	6.8	10.2
47	4	4.5	8.13	10	9.8	10.3
59	5	6.8	14.0	11	10.5	15.6
65	6	6.3	16.4	12	6.5	7.27
	Total	32.6	85.13		43.6	63.77
	Mean	6.5 $\pm$ 1.4*	14.5 $\pm$ 3.9*		7.2 $\pm$ 2.5*	10.8 $\pm$ 2.8*

* Standard deviation

histamine content of 14.5 ± 3.9 μ gms. per gram of skin while that of the control cats was 7.2 ± 2.5 mast cells per high power field and 10.0 ± 2.7 μ gms. of histamine per gram of skin. Both pairs of figures are well within the normal range of values obtained from 20 normal cats, namely, 13 ± 2.3 μ gms. and 7.7 ± 1.8 mast cells per high power field.

Discussion

Under the conditions of this experiment parenteral administration of betamethasone was found to produce marked changes in the epithelial components of the feline skin. The greatest alteration occurred in the epidermis in which the cells became elongated and flattened with, in some areas, absence of the nuclei. Reduction in the number of cell layers resulted in marked thinning of the epidermis (Fig. 20). Such results are in agreement with those obtained in the rat by Baker and Whitaker (1948) who experienced a decrease in the total thickness of the skin, attributable to diminution in size of the panniculus adiposus and thinning of the epidermis, following parenteral administration of adrenocorticotrophin. The authors considered the above effects to have been mediated by the adrenal glands and, in support of that view, cited the findings of Evans et al. (1943), namely, that inhibition of growth did not occur in adrenalectomized rats in consequence of treatment with adrenocorticotrophin. Castor

and Baker (1950) found a thinning of the epidermis in the rat after prolonged percutaneous application of adrenocortical hormones. In male rats, atrophy of the epidermal cells was conspicuous but in female rats results were inconclusive as far as epidermal cell size was concerned indicating, perhaps, a sex difference in the response to corticosteroid therapy. In the present study, in addition to the epidermal changes there was a noticeable degree of atrophy of the pilo-sebaceous unit in the cat. Such a change is in accord with the finding of Morill and Herman (1961) that, in mice, daily subcutaneous injections of cortisone inhibited the growth of hair in plucked areas of the dorsum, which latter procedure would ordinarily have induced hair growth. When treatment was continued for thirty-three consecutive weeks, it was found that cortisone inhibited the development of new hair but did not come to affect the natural growth already in progress until three or more days after the injections began. Moreover, during prolonged steroid administration the inhibitory effect came to an end and, after a number of months, the plucked areas became completely covered with hair although continued administration of cortisone affected the character of the new coat, which latter manifested bizarre patterns due to patchy differences in the length and density of the regenerating hair. That finding is of particular interest inasmuch as a similar response may occur in the cat. Thus, the thinnest epidermis was present in cat No. 4 which was sacrificed

after 47 days of treatment and there was an increase in epidermal thickness in subsequent animals although the values never reached the level of 13.1 ± 1.4 microns that distinguished the control cats. However, such information cannot be considered conclusive because of the few animals that were involved in the experiment.

Still largely unknown is the mechanism by which cortisone brings about the aforesaid epidermal changes but one factor may be depression of the mitotic rate, as has been described in the case of the rat by Ghadially and Green (1957), resulting in reduced cellular proliferation and defective replacement of cells which are normally labile in character. The phenomenon of depressed mitotic activity in the cells may be a sequel of increased catabolism of epidermal proteins arising from continued corticosteroid therapy.

It is of interest that in this experiment betamethasone failed to produce any appreciable effect on the histamine content and the mast cell counts of the skin. The results obtained of 6.5 ± 1.4 mast cells and 14.5 ± 3.95 μgms. histamine per gram of skin in the treated animals are comparable to the values of 7.2 ± 2.5 mast cells per high power field and 10.8 ± 2.79 μgms. histamine per gram of skin in the control group, allowing for the degree of variation inherent in the mast cell counting technique employed and in the method of biological assay used for histamine estimation. Histologically, in the case of the cats under treatment, most of the mast cells were of normal appearance and

only a few exhibited vacuolation of the cytoplasm. In the instance of cat No. 4, which exhibited the thinnest epidermis and which was killed after treatment for 47 days, the mast cell and histamine values were the lowest of all. Investigation of a larger group of animals is essential in order to test the hypothesis that in the cat, just as in the rat, prolonged corticosteroid therapy is likely to render an animal refractory to the depressant effect on mitosis and, if so, to determine whether, or not, that influence is extended to the histamine-mast cell component of the skin.

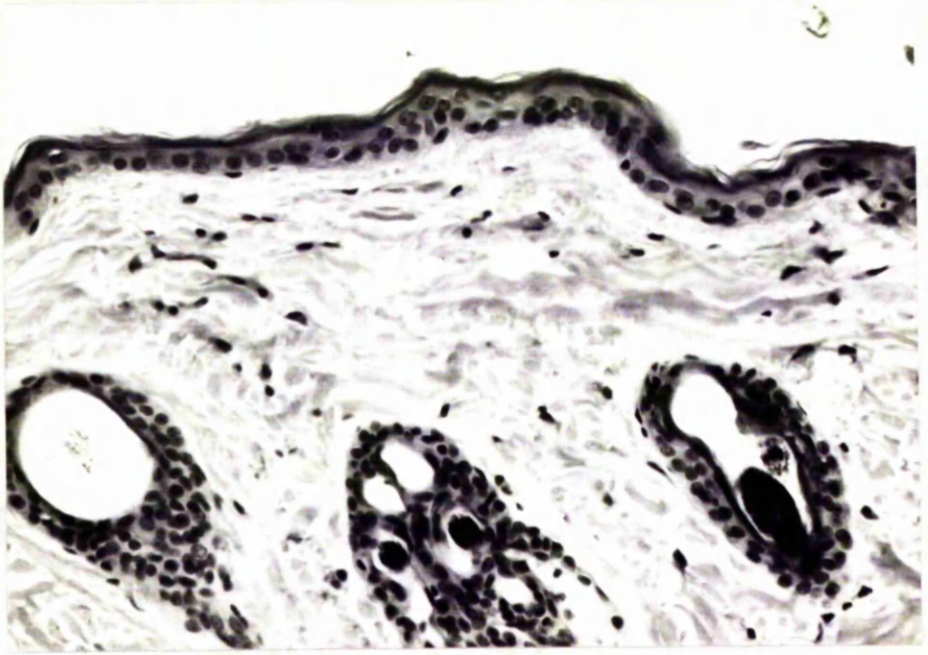
Another factor which may explain the failure of betamethasone to produce a profound fall in the histamine and mast cell content of feline skin is the dose of the drug employed. Thus, each cat of average weight 2,719 gms. received only 0.5 mgms. of betamethasone daily in the above experiment in contrast with the much larger amount of 2 mgm. per 10 grams mouse weight of cortisone acetate used in the experiments of Ghadially and Green (1957). Even allowing for the greater potency of betamethasone each cat received only a small amount of corticosteroid compared with that given to mice in the experiments quoted above. It may well be, therefore, that in some species of animals very large amounts of cortisone require to be administered before degranulation of mast cells will occur. In that connection, it is not without interest that Bloom (1952), in the treatment of canine mastocytoma, gave 100 mg. of cortisone daily for 9 days to

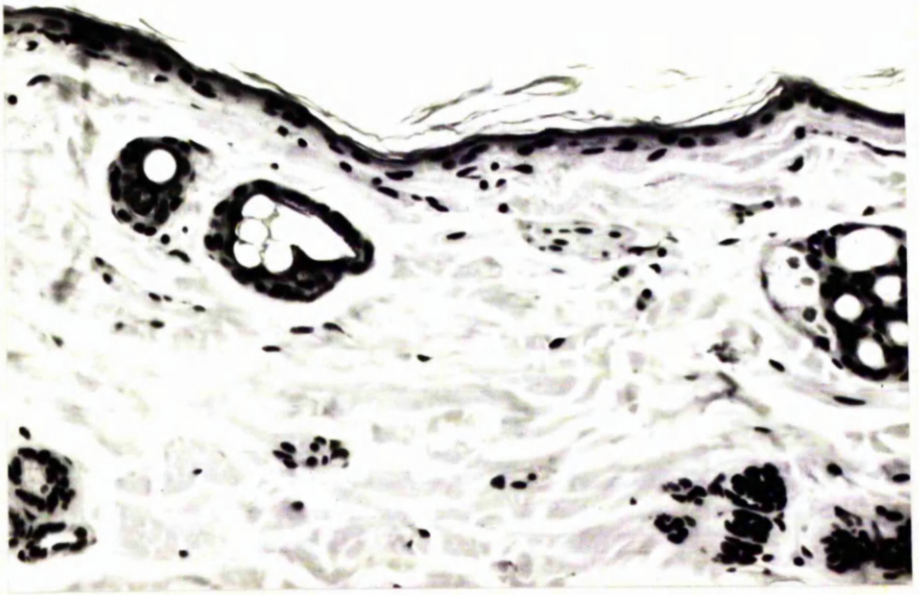
effect a rapid regression in size of the lesions so that by the ninth day they had completely disappeared. Eight weeks later, however, the dog had developed a new nodule which the author presumed to be a mast-cell tumour. Brody et al. (1953) reported a diminution in the size of mast-cell tumours in three dogs to have attended the use of amounts of cortisone similar to those employed by Bloom but in each case a decrease in dosage or cessation of therapy was followed by a marked increase in tumour size which eventually necessitated euthanasia.

Thus, feline skin responds to betamethasone by the onset of atrophy of the epidermis, the hair-follicles and the sebaceous glands in a manner similar to that manifested in the rat and mouse. The comparatively small dose of betamethasone used in this experiment, however, failed to produce any marked change in either the mast-cell population or the content of histamine.

Fig. 19 Normal cat skin from dorsum. The epidermis is 3–4 cells in thickness. Haemalum and eosin, x110.

Fig. 20 Dorsal cat skin after betamethasone treatment. Note thin epidermis and atrophy of pilo-sebaceous units. Haemalum and eosin, x110.





(2) A COMPARISON OF THE EFFECTS OF CORTISONE ACETATE AND BETAMETHASONE ON THE SKIN OF THE CAT

Introduction

In the preceding section, it was shown that betamethasone administered at the rate of 0.5 mg. daily over a period of time varying from 14 to 65 days failed to produce a significant fall in the histamine content and the mast-cell population of feline skin. That negative result may have been due to chemical differences between cortisone acetate, which has been shown to produce degranulation of mast cells in the dog (Bloom, 1952), and betamethasone or to a species difference on the part of mast cells or to the comparatively small dose of betamethasone that was used. In order to test whether or not there is a difference between the action of cortisone acetate and of betamethasone on feline mast cells as well as to assess the effect of a higher steroid dosage on the histamine and mast cell components of the skin, an experiment was conducted on eight adult male cats, divided into two groups of four, one of which received cortisone acetate and the other betamethasone by daily subcutaneous injection for 15 days.

Materials and Methods

Two groups of four male cats were used for this experiment. Each animal was maintained in its own cage and fed on a diet of tinned meat,

milk and water. The first group was given cortisone acetate by subcutaneous injection at a rate of 25 mg. daily for 15 days, while the second group received 3 mg. of betamethasone daily by the same route. The smaller amount of betamethasone was used because that drug is considered to be much more potent than is cortisone acetate.

Prior to treatment, a sample of dorsal skin was taken by biopsy and fixed in picro-formol. At the close of therapy, twin biopsies were removed from the same area of the body; from one of these the histamine was extracted and estimated on the oxygenated guinea pig-ileum preparation and the other was fixed in picro-formol solution for processing in the manner described in Section I. Sections were cut at a thickness of 5 microns perpendicular to the surface and were stained by haemalum and eosin, by periodic acid-Schiff as well as by toluidine blue at pH = 4.0.

Both before and after treatment, mast-cell counts were made on the toluidine blue stained sections in the manner described in Section I, 2. The histamine value (13 ± 2.3 μgms/gm of skin) obtained from the twenty normal cats as detailed in Section I, 2 was used as a control for the results obtained from the treated animals.

Results

(a) Group 1 - Cortisone acetate

On histological examination, the skin showed thinning of the

Table 5

GROUP 1 - CORTISONE ACETATE
(25 mgms. daily for 15 days)

Cat No.	Mast Cells (Mean of 10 fields: x400)		Histamine (μgm./gm.)
	Before Treatment	After Treatment	After Treatment
1	6.8	6.8	9.3
2	8.7	7.9	8.5
3	9.8	10.5	13.6
4	7.6	5.7	13.0
Total	32.9	30.9	44.4
Mean	8.2 $\pm$ 1.3*	7.7 $\pm$ 2.0*	11.6 $\pm$ 2.5*

* Standard deviation

epidermis similar to that seen in the cats treated with a low dose of betamethasone. A few mast cells exhibited vacuolation of the cytoplasm but most of them were normal in appearance and exhibited a well granulated metachromatic cytoplasm in sections stained by toluidine blue (Fig. 21). The histamine content of the skin ranged from 8.5 - 13.6 μg/g. of skin with a mean value of 11.6 $\pm$ 2.5 μg/g., while the mast cell counts ranged from 6.8 to 9.8 per high power (x400) field with a mean of 8.2 $\pm$ 1.3 (Table 5). Those figures were not significantly different from the normal histamine values of 13 $\pm$ 2.3 μg/g. skin and 7.7 $\pm$ 1.8 mast cells that were obtained in respect of 20 normal cats.

(b) Group 2 - Betamethasone

A similar but rather more extreme atrophy of the epidermis was noted in this group. Although differential counts of vacuolated and normal mast cells were not carried out, more of those cells appeared to exhibit vacuolation and degranulation of the cytoplasm, as is illustrated in Fig. 22, yet many mast cells were of normal appearance. The histamine content of the skin in this series showed a drop to a group mean of 6.1 $\pm$ 2.04 μg/g. of skin with a range of 4.8 to 8.4 μg/g. of skin while the mast cell value for the group had fallen from 8.3 $\pm$ 1 to 6.4 $\pm$ 1.3 cells per high power field (Table 6). Use of the 't' test revealed the fall in the histamine content to be significant ($p = < .01$) but there was not any significant difference

Table 6

Group 2 - Betamethasone
(3 mg. daily for 15 days)

Cat No.	Mast Cells (Mean of 10 fields: x400)		Histamine (μ gm./gm.)
	Before Treatment	After Treatment	After Treatment
5	8.3	6.8	7.3
6	7.2	4.5	4.8
7	9.6	5.4	4.0
8	8.1	7.1	8.4
Total	33.2	23.8	24.5
Mean	8.3 ± 1*	6.4 ± 1.3*	6.1 ± 2.0*

* Standard deviation

in the mast cell values before, or after, treatment.

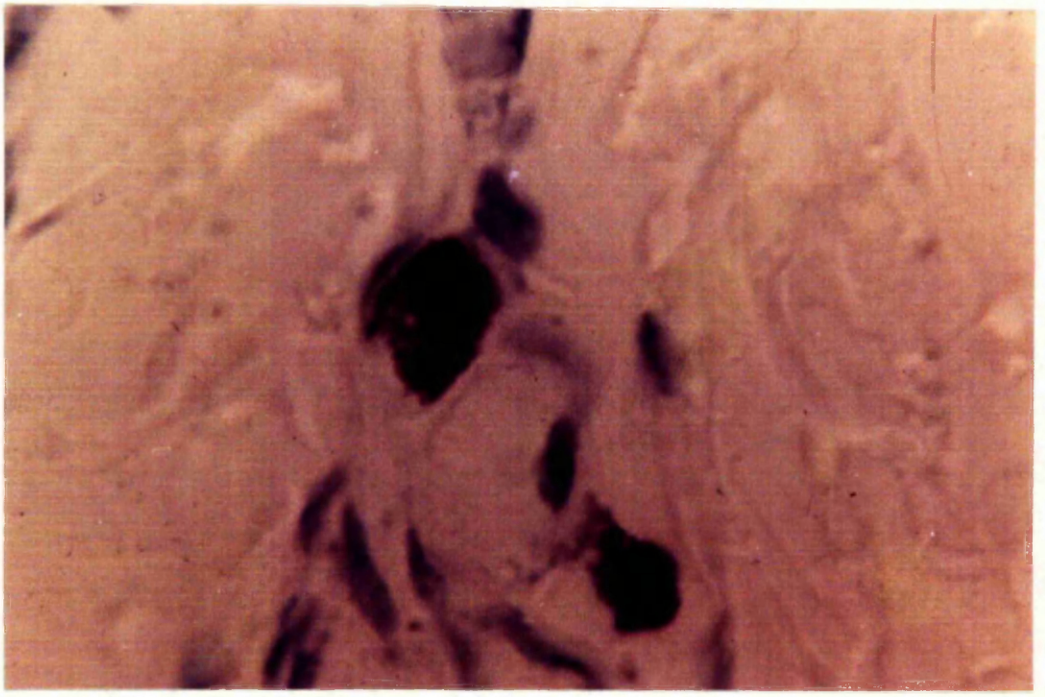
Discussion

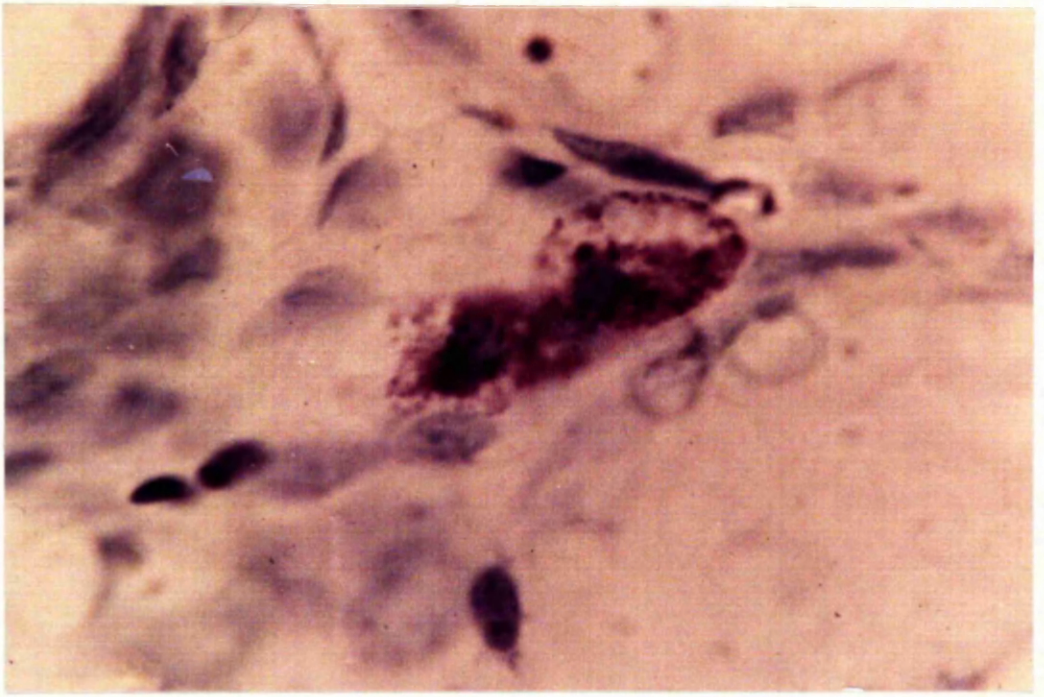
The foregoing results indicate that betamethasone given subcutaneously to the cat at a rate of 3 mgms. daily exerts a more profound effect on the mast-cell population and the histamine content of the skin than does an amount of 25 mgms. of cortisone acetate similarly administered. Since, in both groups of animals, the epidermis was affected more than the mast cells, it would appear that the latter are more resistant to the influence of corticosteroids than are the epidermal cells. Such an effect may be due to the fact that the cells of the epidermis have a higher rate of turnover than the mast cells. However, the degree of mast cell damage is probably not reflected in the mast cell values obtained since it was difficult to decide whether cells showing mild degranulation had been significantly affected by the steroid therapy. Severely affected cells were certainly to be seen (Fig. 22) and, moreover, manifested that vacuolation of the cytoplasm which had been noted in the mast cells of man, rabbits, mice and guinea pigs by Asboe-Hansen (1952). That author, however, did not carry out histamine estimations as part of his investigation. As a result of oral administration of 2 mg. betamethasone daily for 14 days, Zacharias (1964) caused a reduction in the average histamine content of apparently normal human skin and suggested that the lowered

histamine content may partly explain some of the therapeutic effects of glucocorticoids on human skin. From the present experiments it would appear that, in the cat, betamethasone affects the histamine content of skin more potently than does cortisone acetate. Comparison with the results of the previous experiment shows that the degree of response to betamethasone varies with the amount administered.

Fig. 21 Normal mast cells in feline skin. Toluidine blue, x1000.

Fig. 22 Mast cells in feline skin following administration of betamethasone. Note degranulation and vacuolation of the cytoplasm. Toluidine blue, x1000.





ECZEMA IN THE CAT

Introduction and Review of Literature

In this section the changes encountered in the skin of cats affected by eczema have been studied and compared with the lesions that have been found to occur in both naturally occurring and experimentally produced allergic reactions of the skin in man, the horse, the dog and the guinea-pig. Alterations of the histamine content and the mast cell population of the skin have been evaluated as well as the therapeutic effect of betamethasone on feline eczema.

According to Lever (1961), in human dermatology the term dermatitis and eczema are used synonymously and refer to that inflammation of the skin which is the result of an allergic response to a variety of agents, including chemicals, proteins, bacteria, fungi and ectoparasites. The exciting allergen may act on the skin from either the outside or the inside of the animal.

Various forms of eczema, or dermatitis, have come to be recognized and are simply classifiable as acute, subacute or chronic in type. Clinically, acute dermatitis is distinguishable by primary lesions which consist of macules, papules and vesicles. Coalescence of macules and papules tends to produce diffuse areas of erythema. Secondary changes comprise scaling, crusting, lichenification and fissuring. Lever noted that areas of dermatitis are not sharply

demarcated from the surrounding skin with which, in fact, they tend to merge in a gradual manner. In most cases, a moderate to severe pruritus is present.

Percival, Montgomery and Dodds (1962) consider that, from a clinical standpoint, eczema may be separated into several well-defined groups, each of which exemplifies a single process, the eczema reaction, of which the clinical features, prognosis, incidence and response to treatment are similar irrespective of the nomenclature adopted by different authors. Further, the eczematous reaction always presents the same basic histological pattern regardless of the clinical group from which the tissue has been obtained. Thus, the changes are located mainly in the epidermis and the vascular and cellular reactions of the dermis seem to play a minor role. The epidermal disorder is essentially one of wide separation of groups of cells of the rete mucosum by oedematous fluid, which process is known as spongiosis. In some cases, cells are completely detached from their neighbours.

These changes appear to result from the action of an allergen on an epidermis which has an inherent or acquired allergy towards that substance. The allergic response due to a single contact with the allergen persists for an appreciable time and, if such contact is repeated, the duration of both the reaction and the clinical eczema may be correspondingly prolonged. Cappell (1964) considers the initial lesion of eczema to be an intercellular oedema which results

in separation of the prickle cells followed by lymphocytic infiltration together with degeneration and liquefaction of the cells and ultimate formation of intra-epidermal vesicles. Coalescence of the thin vesicular walls gives rise to larger vesicles or bullae, within which fibrin along with degenerated epithelial cells, polymorphonuclear leukocytes and lymphocytes are to be found while parakeratosis develops as a result of interference with the nutrition of the stratum corneum. In the dermis the main changes comprise oedema, vascular dilatation and congestion accompanied by perivascular aggregration of lymphocytes, eosinophils and polymorphonuclear leukocytes.

In subacute eczema, the vesicles are less evident while acanthosis and parakeratosis becomes more marked. On the surface of the lesion there is an exudate composed of a mixture of fibrin, degenerating leucocytes and bacteria. Rupture of a superficial vesicle may expose a dermal papilla covered by fibrin and debris, constituting the so-called "eczema pit". The chronic phase of eczema is characterized by marked acanthosis with elongation of the rete ridges and by hyperkeratosis and parakeratosis. Intercellular oedema may still be visible but vesicles are absent.

In the dog, eczema occurs very commonly and is associated with as wide a variety of exciting factors as in man. Thus, Roy (1954) has postulated that the sweat glands may play a role in dogs affected by hyperhidrosis inasmuch as excessive secretion of alkaline sweat

(in normal dogs the skin has a pH of 5.5 to 7.2 but in hyperhidrosis there may be a pH of 8.2 to 8.9) may irritate the epidermal surface and so cause eczematous lesions. Jennings (1953) has briefly described instances of acute moist eczema in the dog in which the lesions were circumscribed, hot, painful and oedematous and were accompanied by exudation of serum. Vesicles were not seen in those cases. The aetiology of canine eczema remains obscure although a relationship with infestation by fleas has been postulated by Holmes (1933) and by Pugh (1947). Moreover, Jennings (1953) found 24 cases of canine eczema out of a total of 53 to be associated with similar infestation while other 15 animals were parasitized by lice. Schwartzman and Orkin (1962) described an acute moist eczema of the dog that was usually complicated by secondary bacterial infection and declared it to be initiated by hypersensitivity to flea-bites, resulting in pruritus that led to self-inflicted lesions which became infected by bacteria. Such a condition was designated "pyo-traumatic dermatitis". The same authors also employed the expression, "chronic flea-bite dermatitis" or "summer eczema" in reference to a disorder which they ascribed to flea-infestation and flea-bite hypersensitivity and consequent self-inflicted trauma. Gross examination of that condition revealed areas of skin on the lower part of the back which were erythematous, irregularly pigmented and had a thickened scaly appearance. At histological examination, sections of skin showed hyper-

keratosis and parakeratosis with occasional abscesses below the stratum corneum. In the upper part of the dermis there was a cellular infiltrate composed mainly of lymphocytes, macrophages and plasma cells. In the mid-corium, macrophages, polymorphonuclear cells, plasma cells and mast cells were evident together with a concomitant hyperaemia.

Jennings (1953) considered eczema to be a common disease of cats with a clinical picture closely resembling that of canine eczema. He mentioned the frequent association of the disorder with ingestion of fish but pointed out that many cats were not affected by a similar diet and that, in a series of 12 affected animals, 6 did not receive any fish while other 6 were so fed only on occasion. Again, animals with the disease were cured while they were maintained on a diet composed entirely of fish. The same author, out of 76 cases of feline eczema, was able to demonstrate flea-infestation in 61 cats, an incidence of 80.2 per cent., and in the instance of one animal, which had never had the disease, induced eczema as a result of the release of 5 fleas into its coat. Within four weeks the animal had developed severe eczema from which it recovered two weeks later following treatment with an insecticide. In this connection, it is pertinent to mention the experiments of Hudson et al. (1960) in the guinea-pig, in which animal local cutaneous hypersensitivity developed in response to flea-bites. Furthermore, guinea-pigs sensitized by bites

of the flea, Ctenocephalus felis, developed within 5-7 days a hypersensitivity which was manifested by delayed skin reactions characterized by induration and erythema at the locus of bite. Later Benjamini et al. (1960) used extracts of fleas to show that the response was a systemic one in which reactions of both immediate and delayed type were elicited.

Another factor contributory to the causation of feline eczema may be endocrine imbalance. Thus, in the British Veterinary Association Handbook, "Aspects of Skin Disease of the Dog and Cat", (1961), testosterone deficiency as a cause of miliary eczema in the cat is discussed and good results are reported to have followed treatment of such animals by means of testosterone implants.

The close correlation between the mast cell population and the histamine content of the skin in the normal animal as well as in pathological conditions has been demonstrated in many species during recent years. Thus, in the case of a mast cell tumour, that was removed from a ten year-old female Cocker Spaniel, Riley (1959) obtained a histamine value of 1,290 μg/g. tissue, an extraordinarily high value compared with that of normal dog skin which is usually only 5-10 μg/g. The same tumour was found to have the high heparin value of 110 i.u./g., a figure which is more than twice the heparin content of normal ox-liver capsule which yields up to 50 i.u./g. Although haemorrhage was not noted at surgical removal of the above

tumour, despite the presence of such large amounts of the anticoagulant heparin, Bloom et al. (1958) reported a prolonged clotting time to have characterized a series of cases of canine mast cell tumours. In the instance of the cat, Riley (1959) mentioned three cases of mastocytoma which were associated with high histamine values although figures for heparin relating to two of the animals were very much lower than those for the dog. Increased numbers of mast cells have been noted in a number of chronic inflammatory conditions of human skin, e.g. Asboe-Hansen (1950) noted a higher population of those cells in neurodermatitis, lichen planus, urticaria as well as in acute and chronic eczema.

Because of the above findings and in view of the fact that in chronic feline eczema large numbers of mast cells are present in the dermis, it was decided to investigate the histamine content and the mast cell population of the skin of cats suffering from the condition.

Materials and Methods

Clinical

50 cats affected with eczema were examined at a small animal clinic in Glasgow. For each animal, there was completed a clinical record containing details of sex, age, duration of illness, distribution and description of lesions, presence or absence of external parasites together with an outline diagram of the body surface

(Fig. 23), on which the distribution of the lesions was sketched. In addition, any other illnesses were noted.

Pathological techniques

Two biopsy specimens, one for histological examination and the other for histamine analysis, were taken from the affected areas of skin of 20 cats. The biopsy instrument consisted of a powered electric drill into which was fitted a hollowed-out steel bit that rotated at 2,000 r.p.m. and was similar to that described by Evans et al. (1957). To prevent penetration below the subcutis, a perspex guard was fitted to the drilling instrument. The specimen for histological examination was fixed in picro-formol solution for 24 hours and processed as has been described in Section I for normal skin while that destined for histamine analysis was stored at -20°C until required. Sections were cut at right angles to the epidermal surface at a thickness of 5 microns and were stained by haemalum and eosin, toluidine blue, periodic acid-Schiff, aldehyde-fuchsin for mast cells and picro-Mallory for collagen, muscle and fibrin. In the upper part of the dermis in sections stained by toluidine blue mast cell counts were made in ten fields at a magnification of x400 and the mean of the ten counts was taken as the mast cell value of the biopsy. Only those cells which showed metachromasia with toluidine blue and which contained nuclei were counted. Histamine analysis was performed on the atropinised guinea pig-ileum preparation

that has been described in Section I.

Results

Clinical

In the majority of cases, the clinical features consisted of alopecia together with raised erythematous papules which spread over the dorsum and, in some cases, extended to the head, neck and abdomen (Fig. 24). The skin was commonly thickened and scaly in appearance, although four of the cats had extensive areas of ulceration (Fig 25) caused by licking or scratching in an effort to relieve itching. Exudation was present in only 7 out of the 50 animals. The disease varied in duration from a week to over one year and was limited to adult cats, in only four of which were fleas detected. A sex-incidence was not established inasmuch as 26 of the cats were females and 24 were males.

Histopathology

Histological examination of samples of skin from the series showed two forms of the condition to occur, viz. acute and chronic.

In the acute case which may be either primary and result from an initial attack of the disease or secondary when there has been a recurrence of the condition, the most characteristic change was acute dermatitis with a fibrinous exudate on the surface of the skin that contained large numbers of neutrophils as well as macrophages and

red blood cells. Occasionally, the inflammatory material was seen to infiltrate, and so to split, the stratum corneum. In the acute phase of the disease, areas of the epidermis became eroded with resulting ulceration (Fig. 26). Acanthosis and parakeratosis also were present but were not of the degree distinctive of the chronic disease. Many epidermal cells were seen to undergo hydropic degeneration with subsequent development of intracytoplasmic vacuoles and loss of staining affinity. Intercellular oedema resulted in separation of the epidermal cells but vesiculation occurred in only one case in which it was of very limited extent.

In the dermis, especially in the papillary layer, there was a cellular infiltrate which was composed of macrophages, polymorphonuclear neutrophils together with a few lymphocytes and mast cells but eosinophils were only rarely to be seen. Capillary hyperaemia was a prominent feature as was also oedema of the dermis which was most conspicuous at the dermo-epidermal junction. The mast cells were mainly perivascular in location and in the acute stage showed evidence of degranulation in sections stained by toluidine blue. The degranulation was most distinct in the upper part of the dermis and in many cases metachromatic granules were recognized which had escaped from the mast-cell cytoplasm into the interstitial spaces. In the deeper parts of the dermis and also in the subcutaneous fatty tissue there was a perivascular reaction which was mainly lympho-

cytic (Fig. 28) in character. Many sweat glands were dilated as a result of compression and blockage of the follicular openings. A few cases of the acute type were complicated by secondary bacterial infection which was attended by very severe epidermal necrosis and widespread infiltration of the dermis by polymorphonuclear neutrophil leucocytes.

In the chronic stage of feline eczema, the main epidermal changes noted were acanthosis and parakeratosis (Fig. 27) but ulceration was absent. The cells of the stratum germinativum often showed hydropic degeneration while intercellular oedema was still apparent. In the deeper part of the dermis there was a marked increase in the population of mast cells, most of which were large and well-granulated in appearance and exhibited metachromasia in sections stained by toluidine blue (Fig. 29). Another prominent feature was subepidermal fibrosis denoting the onset of healing. The mast cells of this area showed marked loss of staining affinity and in many of the sections studied were almost completely degranulated.

Histamine and mast cells

On average, mast cell counts in the twenty cats examined were 23.79 cells per high power field with a range of from 10.9 to 63.6 cells. The histamine values obtained from analysis of samples of skin were markedly increased to a mean of 56.3 μgms. of histamine per gram of skin with a range of values of from 12.2 to 288 μgms.

Table 7

HISTAMINE AND MAST CELLS IN ECZEMATOUS FELINE SKIN

Cat No.	Histamine (μgms. per gm. of skin)	Mast Cells (average of 10 high-power fields)
1	68.0	41.7
2	12.2	16.1
3	50.0	11.3
4	33.8	12.1
5	53.5	14.4
6	45.9	30.6
7	36.4	14.0
8	228.0	63.6
9	61.2	20.4
10	67.5	15.3
11	89.8	42.9
12	29.0	22.3
13	32.0	19.3
14	66.6	26.7
15	24.16	16.7
16	43.2	10.9
17	67.0	26.6
18	40.1	24.6
19	38.0	33.1
20	41.5	13.3
	Total 1127.8	479.9
	Mean 56.4	23.8

(Table 7). The average histamine value in normal cat skin was 13 μgms. per gram of skin.

Discussion

The close association of feline eczema with flea-infestation reported by Jennings (1953) has not been clearly established as a result of this work since only 8.0 per cent. of the cats were so afflicted. Since, however, only a mild burden of fleas may result in the production of the disease, it may well be that in some cases such ectoparasitism was not detected. The lesions grossly resemble those of canine moist eczema as well as those described by Riek (1953) in the case of horses parasitized by sand-flies (Culicoides robertsi). Histologically, they are quite similar to the changes distinctive of acute human dermatitis but differ in so far as vesiculation is not a prominent feature. Possibly, the latter alteration occurs at a very early stage of feline eczema to be quickly followed by cellular infiltration and exudation. As all the cases examined were brought by their owners to a small animal clinic, it is probable that most of the cats had had the disease for at least a few days before they were examined so that the above change had been superseded. On the other hand Jennings (1953) emphasized that vesiculation is uncommon in canine eczema and Riek (1953) does not mention that change in association with equine allergic dermatitis

whereas in human eczema the production of vesicles is typical of the condition. In the early stages of the feline disease, the cellular infiltrate is similar to that which is found to occur in man and the dog and is composed mainly of macrophages and lymphocytes with only a few eosinophils but differs from eczema in the horse in which the latter cells are prominent throughout.

Chronic eczema of the cat was marked by epidermal acanthosis and parakeratosis, both of which may be attributable to the increased blood supply available as a part of the inflammatory response. Parakeratosis is a reflection of hyperplasia of the stratum germinativum to the extent that the new cells have not sufficient time to undergo the normal process of keratinization (Van Scott, 1964). The greatly increased mast cell population noted particularly in the upper dermis is of interest in relation to mast cell function. The decrease in granularity of those cells in the area may indicate a connection between the mast cells and the new connective-tissue produced in sub-epidermal fibrosis. In that case, the alterations may be associated with the mast cell-fibroblast interaction described by Riley (1962) in which the fibroblasts of a region of tissue injury are sensitized by the histamine released from damaged mast cells to take up the heparin-rich granules of those cells and are thereby stimulated to produce mucopolysaccharide of their own which is utilized for further formation of collagen.

The high histamine value found in the skin in feline eczema associated with the increased number of mast cells is in accord with the findings of Asboe-Hansen (1950) that mast cells are increased in many skin diseases of man. Likewise, in canine mastocytomata there exists a close correlation between the marked accumulation of mast cells and the histamine content (Riley, 1959). Although the function of that histamine is unknown, evidence that it plays a part in healing is provided by Boyd and Smith (1959) whose work with histamine-depleted rats revealed that repair of an aseptic linear wound was retarded compared with that encountered in normal animals.

Fig. 23 Distribution of lesions in a typical case of eczema indicated by hatched areas.

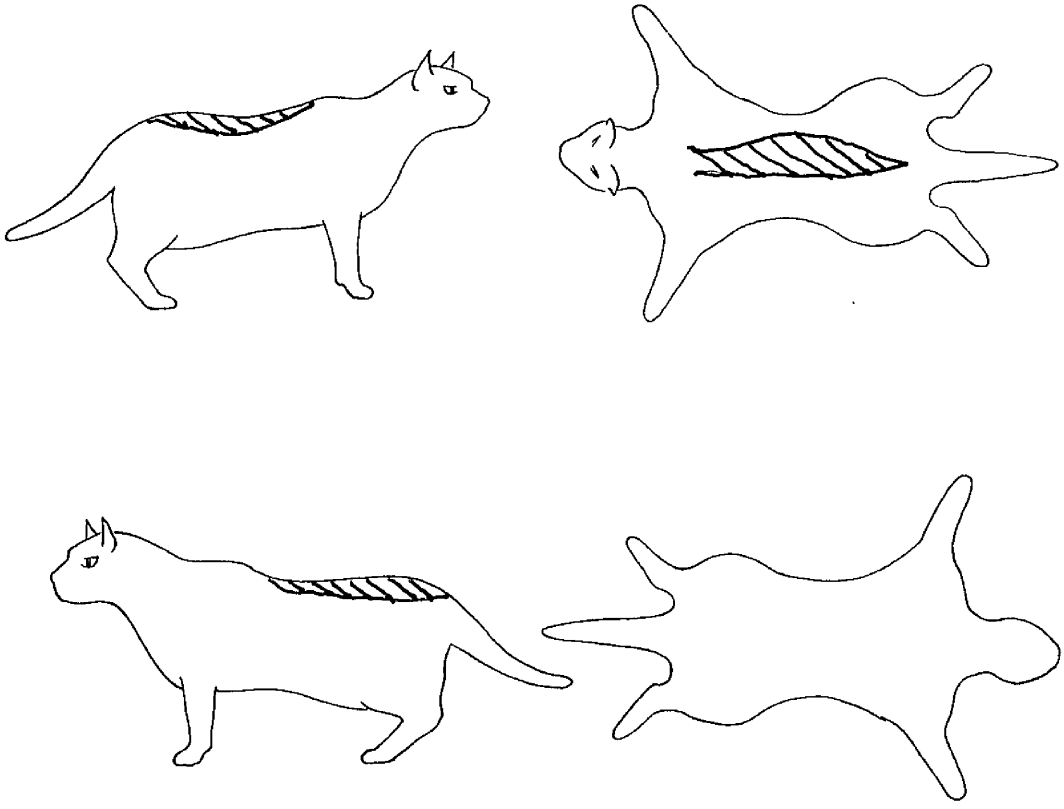


Fig. 24 Miliary eczema. The lesions extend along the dorsum and have a dried fibrinous exudate on the surface. There is also diffuse alopecia.

Fig. 25 Acute miliary eczema. Note large area of ulceration surrounded by multiple papular lesions and also alopecia.

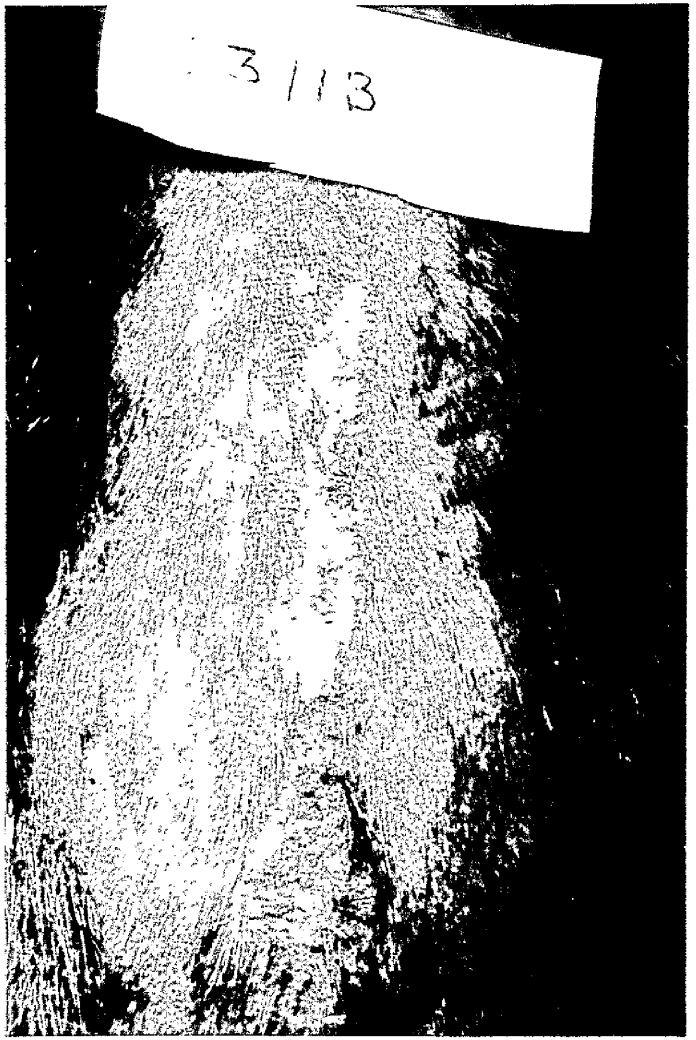

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Fig. 26 Section of skin in acute eczema of the cat showing a fibrinous exudate on the surface together with thickening and ulceration of the epidermis and cellular infiltration of the dermis. Haemalum and eosin, x110.

Fig. 27 Section of skin in chronic eczema of the cat exhibiting acanthosis and parakeratosis and cellular infiltration of dermis. Haemalum and eosin, x110.

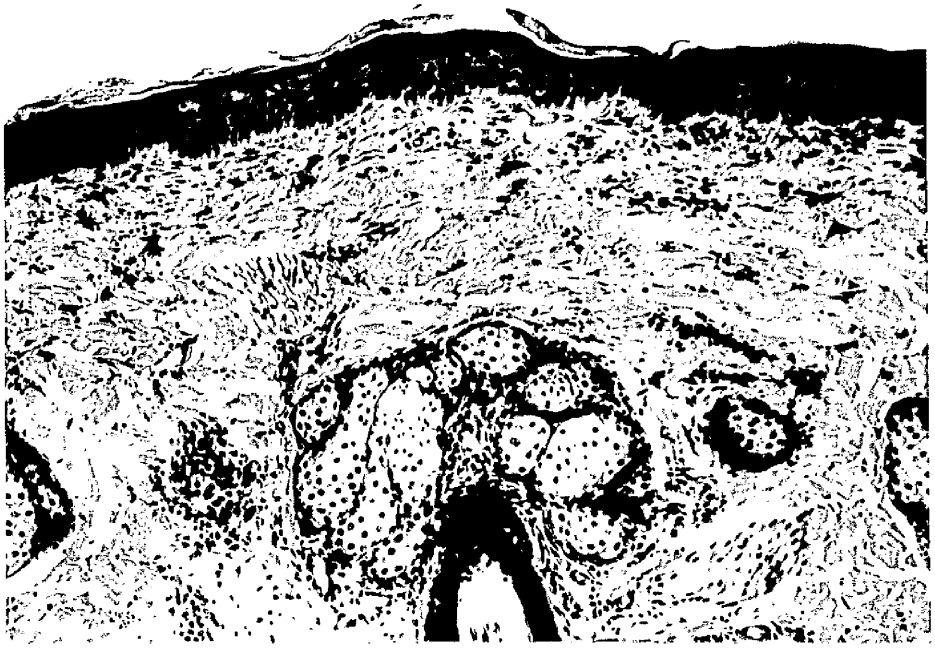
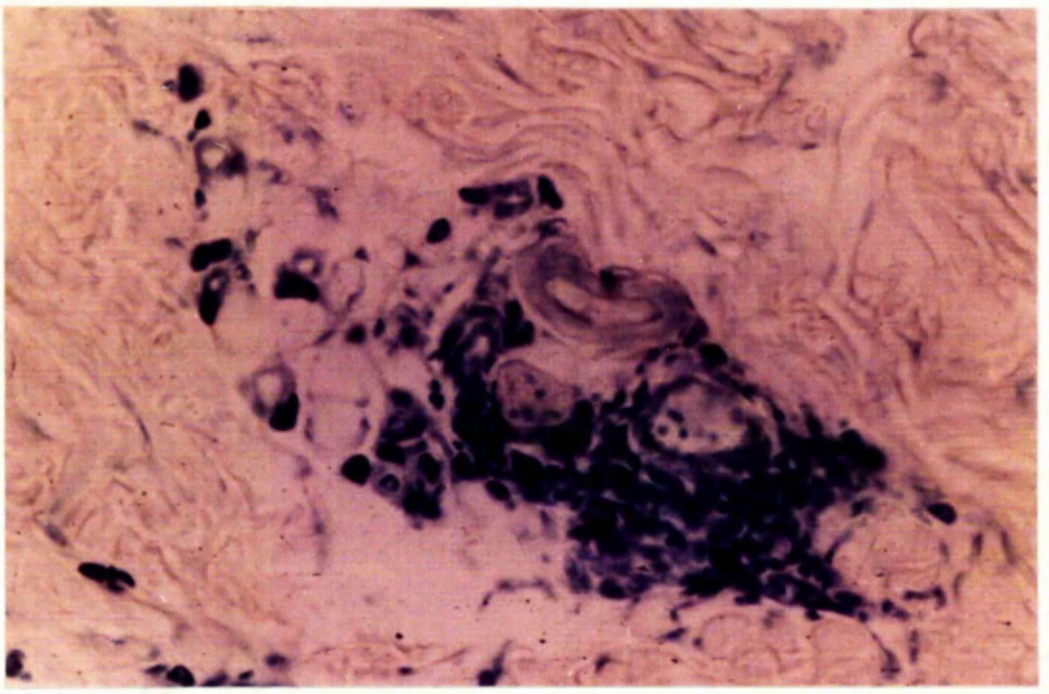
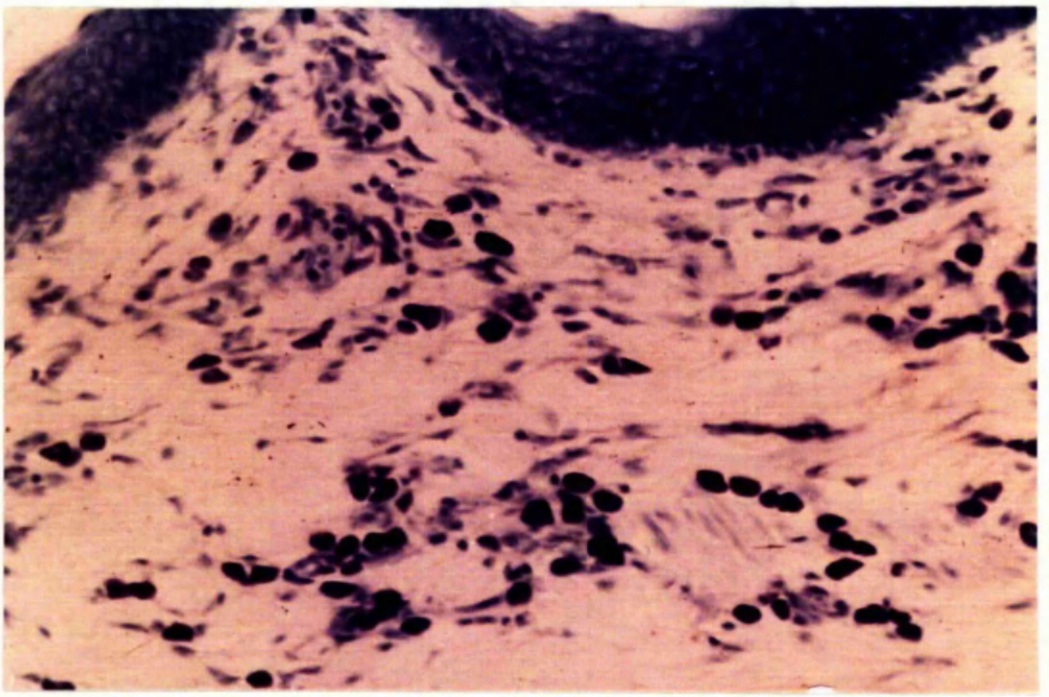


Fig. 28 Perivascular reaction in deep dermis in acute eczema. Note large mast cells around capillaries. Toluidine blue, x180.

Fig. 29 Section of skin from a case of chronic miliary eczema showing acanthosis and an increased dermal mast cell population. Toluidine blue, x180.





THE EFFECT OF BETAMETHASONE ON ECZEMA IN THE CAT

Introduction

In recent years cortisone and its derivatives have been found to be of value in the treatment of a number of chronic inflammatory conditions, including those of the skin. Thus, Halpin (1955) recorded a remarkable improvement with remission of pruritus in seven cats suffering from miliary eczema to have followed parenteral treatment with A.C.T.H. in doses which varied from 10-60 units. Russell et al. (1955) used 2.5 per cent. hydrocortisone acetate with favourable effect in the treatment of lichen simplex, discoid eczema, otitis externa and ano-genital pruritus. Church (1955) treated 105 cases of eczema and dermatitis in man with 1 per cent. or 2.5 per cent. hydrocortisone ointment and obtained good results in 76 per cent. of instances. The author considered such therapy to be most effective in acute contact dermatitis, nummular eczema, atopic eczema and perianal dermatitis but warned that infection, unless previously controlled, was likely to be spread by the ointment. Schwartzman and Orkin (1962) recommended the use of cortisone acetate for the cure of a number of skin conditions in the dog including cutaneous pollinosis and urticaria while, as a result of a clinical trial on canine eczema, Crichton et al. (1965) showed that fluocinolone acetonide was more effectual than was either hydrocortisone or the

emollient base in which both chemicals were suspended. In the light of the above findings and also of the reports by Bloom (1952) and by Brody et al. (1953) on the influence of cortisone on the mast cell, it was decided to study the effect of the synthetic steroid, betamethasone, on the skin of cats suffering from eczema in an effort to assess the value of the drug as a therapeutic agent as well as a mediator of mast cell degranulation and of histamine depletion of the skin.

Materials and Methods

To permit of proper study under uniform conditions, 10 cats with lesions of miliary eczema were brought into the veterinary hospital where they were kept for a period of 16-18 days. On arrival, each animal was subjected to thorough clinical examination and the distribution of the skin lesions was marked on the form of the record illustrated in Fig. 23. The animals were maintained on a diet of tinned meat and water. Treatment consisted of subcutaneous injection every third day of one milligramme of betamethasone. From all of the animals two skin biopsies were taken by means of a powered biopsy drill, one of the specimens to be fixed in picro-formol solution for purposes of histopathology and the other was stored at -20°C until required for histamine analysis. After fixation the samples for histological examination were processed in the manner

already described for normal skin and were then embedded in paraffin-wax. Thereafter, sections were cut at 5 microns and stained by haemalum and eosin, toluidine blue, periodic acid-Schiff and, on occasion, by alcian blue. Histamine analysis was carried out in the manner previously described in Section I using the four-point assay technique on the isolated guinea pig-ileum preparation. At the end of fourteen days, the cats were again examined clinically and two further biopsy specimens were taken from the affected areas for histological examination and histamine analysis.

Results

Clinical

In this group of cats the lesions occurred mainly on the dorsum where they stretched from the tail-head over the sacrum to the thoraco-lumbar junction. In three cases, lesions were also noted on the head and neck and in one of those instances a patch of wet eczema with a raw, red and weeping surface, 2 cms. x 1.5 cms. in size, occurred on the ventral surface of the neck. A similar area was observed on the abdomen of another case. All cases showed diffuse alopecia of the affected areas with thickening of the skin and papular eruptions about 1 mm. in diameter. A brief description of the lesions before and after treatment in each cat is given in Table 8, which reveals that clinical improvement occurred in 9 of the 10

Table 8

THE THERAPEUTIC EFFECT OF BETAMETHASONE ON ECZEMA IN THE CAT

Cat No.	Before Treatment	After Treatment
1	Multiple small crusty lesions on head and neck with alopecia.	Lesions healed and hair growing.
2	Multiple papular lesions on back with erythema and alopecia.	Condition much improved with only a few residual lesions.
3	Dry crusty lesions on dorsal surface and patch of wet eczema on ventral surface of neck.	Patch of wet eczema healed but dry lesions still present.
4.	Dry scaly lesions with alopecia of lumbar area.	Lesion completely healed and hair growing.
5	Dry scaly lesion with alopecia of lumbo-sacral area.	Lesion healed and hair growing.
6	Dry scaly lesion of lumbo-sacral area and patch of wet eczema on abdomen.	Marked improvement on back. Abdomen completely healed.
7	Dry crusty lesion on back with papules on either side.	No improvement.
8	Dry crusted lesions on back, neck and ears.	Marked improvement with regression in size of lesions.
9	Large lesions on shoulder and neck with multiple papules.	Marked improvement with regression in size of lesions.
10	Multiple papular lesions on back with erythema and alopecia.	Marked improvement with only a few papules left.

cats and, in most instances, was very marked. In only one animal did the lesions persist and, in fact, the condition actually worsened. Figures 30 and 31 illustrate a typical case before and after treatment.

Histology

Before treatment

The main changes exhibited by the cats prior to treatment were acanthosis, congestion and oedema of the dermis and a cellular reaction which was mainly of mononuclear type and consisted of lymphocytes, macrophages and mast cells. In only one animal was there to be found more than the occasional eosinophil. Four cats had a fibrinous exudate on the surface of the skin in which masses of neutrophils were present. Sub-epidermal fibrosis was present in five animals and it is of interest that many of the abundant mast cells of the area exhibited loss of granules and were identifiable solely by a faint rim of metachromatic material in their cytoplasm. In the deep dermis and subcutis, there was noted perivascular aggregations of lymphocytes, among which latter a few mast cells were recognizable. The sweat glands were dilated, probably, as a result of occlusion of their orifices while the sebaceous glands were frequently enlarged and hyperplastic in appearance.

After treatment

There was an appreciable thinning of the epidermis in 5 animals,

all of which were classified as chronic cases at initial clinical examination. In one of the above cats there was an increase in epidermal pigmentation and most of the melanocytes were situated in the basal layers of the epidermis. In four of the animals, the fibrinous exudate present on the epidermal surface prior to treatment had disappeared at the end of the 14 days. After therapy, the layer of fibrous connective-tissue below the epidermis appeared granular and embryonic but there was little obvious change in the mast cell concentration. In the deep corium, oedema and congestion were still present as was, too, a perivascular mononuclear reaction.

Histamine and Mast Cells

In all ten animals under treatment the initial levels of histamine were increased by approximately four times the value obtained for normal cat skin (13 micrograms per gram) to a group average of 59.5 micrograms of histamine per gram of skin. Values ranged from 24.16 micrograms of histamine in cat No. 72 to 173 micrograms in cat No. 81. After treatment, there was a slight fall in the group average value for histamine that was attributable to the decline in the case of cat No. 81 from a pre-treatment level of 173.4 μgms. per gram of skin to a value after betamethasone therapy of 85.7 μ gms. histamine per gram of skin. Moreover, the significance attachable to the reduction in histamine is rendered more doubtful by the fact that there was a coincident increase in the group average for mast cells of from 23.67 to 27.63 per high power field (Table 9).

Table 9

HISTAMINE AND MAST CELLS IN ECZEMATOUS CATS BEFORE AND AFTER BETAMETHASONE THERAPY

Cat No.	Pre-treatment		Post-treatment	
	Mast Cells	Histamine (μgm./gm.)	Mast Cells (Average of 10 h.p. fields)	Histamine (μgm./gm.)
71	26.7	66.6	33.8	80.0
72	16.7	24.16	15.7	25.0
73	10.9	43.2	30.5	49.3
74	26.6	59.7	23.6	67.0
75	24.6	40.1	32.7	28.6
76	33.1	38.0	40.5	25.4
79	24.7	72.3	19.1	54.2
80	16.5	41.5	22.0	61.4
81	43.1	173.4	39.6	85.7
82	14.0	36.4	18.8	44.9
Total	236.7	595.3	276.3	521.5
Mean	23.67	59.5 $\pm$ 40*	27.63	52.1 $\pm$ 19*

* Standard deviation

Discussion

Of the ten cats under experiment nine showed a very satisfactory clinical response to administration of the drug, a finding which is in accord with results obtained in the therapy of various forms of eczema in other species of animals. On the other hand, perusal of the records of 20 other cats which had been similarly treated at a small animal clinic revealed that 14 cases had been returned for care following recurrence of the disease. It would seem, therefore, that in the case of the experimental cats only the clinical manifestations of the disease had been controlled by betamethasone therapy and that the exciting cause or causes of the condition had not been eliminated. That experience emphasizes the need for further research into the aetiology of cutaneous diseases in domestic animals, especially those not assignable to any evident pathogen. In that connection, the observations of Walton (1965) on skin sensitivity in the dog to a variety of materials are of interest. Walton found that furnishing fabrics and bedding, chrome salts, wood preservatives, rubber matting, acriflavine, cetrimide, iodine and topically applied streptomycin were all capable of eliciting skin reactions in the dog. Again, Schwartzman and Orkin (1962) described a cutaneous pollinosis of the dog of which they suspected ragweed to be the prime cause and cited a recent report by Patterson (1959) of a case of canine ragweed dermatitis that was characterized by lacrimation, conjunctivitis and

severe pruritus attended by an erythematous scaly eruption over the back and forelegs. In respect of the cat, there is little real evidence of the existence of any specific skin sensitizing agents apart from a recent report by Parrish (1965) in which cutaneous sensitivity to cow's milk was recorded.

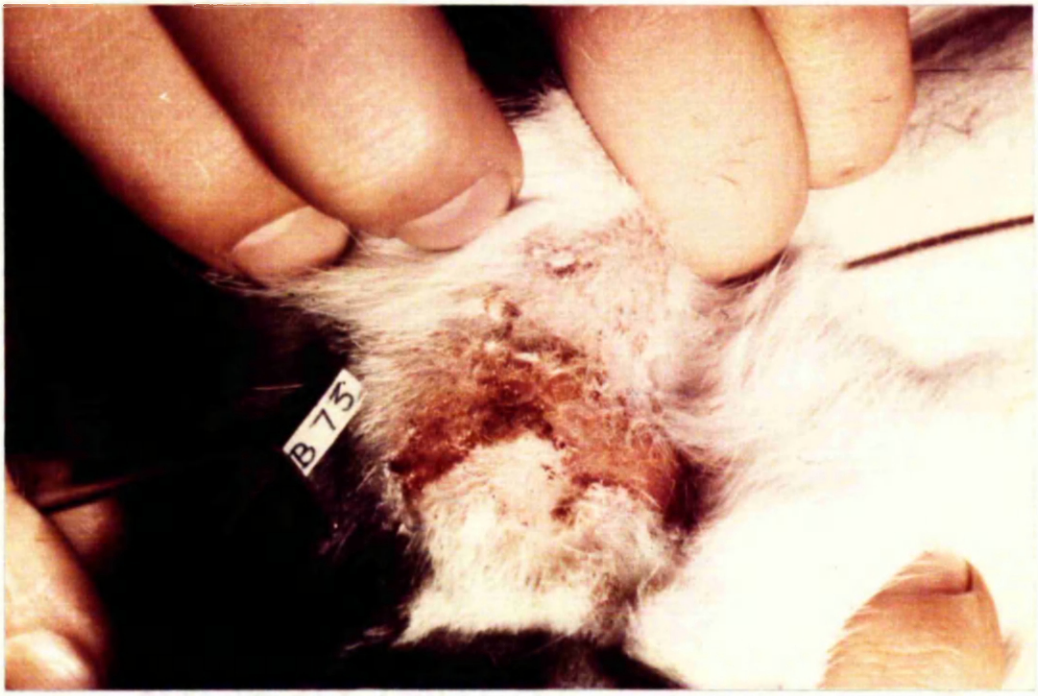
The fact that significant change in the mast cell values of the skin was not experienced either before or after treatment with betamethasone is of peculiar interest in view of the findings of Asboe-Hansen (1952) that treatment with cortisone in man, rabbits, mice and guinea pigs resulted in a decrease in the population of cutaneous mast cells. The latter were either perfectly normal in appearance both before and after treatment or exhibited degranulation and vacuolation of the cytoplasm. Bloom (1952) reported that cortisone given parenterally in a dose of 100 mg. daily for nine days effected complete clinical regression of multiple mastocytomata. Histologically, the mast cells of the tumour showed vacuolation of the cytoplasm, conglomeration and altered staining reaction of the metachromatic basophilic granules, disappearance of granules and cellular destruction. More recently Zachariae (1964) revealed a small but statistically significant decrease in the histamine content of the skin of people suffering from various dermatological diseases. The samples of skin were taken from areas of the back which appeared normal on gross examination. In the present series

of observations the fall in histamine content from 59 ± 40 μgms. to 52.15 ± 19 μgms. per gram of skin was found to be significant when analysed by the 't' test ($P = < 0.01$). The failure to evoke a reduction in the total number of mast cells may be due to errors of technique or to species differences or to other factors as yet unknown. Since in only three instances did the total histamine level decline after treatment and there was not any appreciable alteration in a fourth case, the need for further elucidation of the problem is beyond doubt.

Although conclusions cannot be drawn from this experiment regarding the mode of action of betamethasone, in the case of a cat affected with eczema, fall in tissue histamine is unlikely to be an important factor in the regression of lesions since the mean level of that amine persisted at rather more than four times that of normal feline skin.

Fig. 30 Acute miliary eczema before treatment with betamethasone.

Fig. 31 Lesion which has nearly healed after betamethasone therapy.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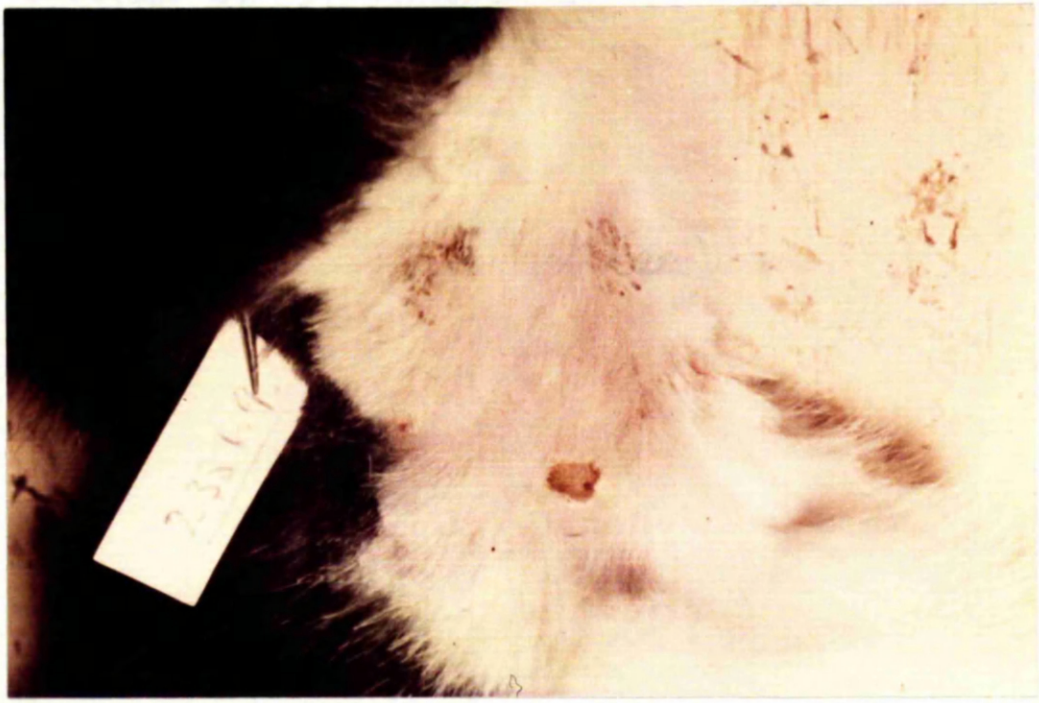


THE SEROLOGICAL RESPONSE OF THE CAT TO SOME FOREIGN PROTEINS

Introduction

Despite the extensive use which has been made of the cat in experimental physiology and pharmacology, remarkably few observations on the immunological responses of that animal have so far been published. Although Akcasu (1963) and Wilson and Miles (1964) suggest that the cat is unresponsive to foreign proteins, there are recorded instances of such responses, e.g. Gotschlich and Stetson (1960) prepared cat antiserum to human C-reactive protein and showed that it was capable of eliciting passive cutaneous anaphylaxis (PCA) in the guinea-pig. Parrish (1965) has reported the production of PCA in a cat by the use of homologous antisera to milk proteins, while further evidence of the response of the cat to various antigens has been provided by Miller-Ben Shaul (1965).

The ensuing section records some observations that have been made on the serological responses of cats to heterologous serum and to some defined foreign proteins.

Materials and Methods

Animals

Hybrid cats of both sexes, all over one year of age, were used

in this study. They were maintained in individual cages and fed on a standard diet of tinned meat, water and milk.

Antigens

As well as bovine normal serum (BNS) and rabbit normal serum (RNS) two commercially purified fractions of bovine plasma were employed for immunization of the animals and for serological procedures. The proprietary products were crystallized bovine serum albumin (BSA) and bovine gamma globulins (BGG) fraction II, obtained from Armour Pharmaceutical Company, Ltd.

As shown by electrophoresis, BSA is 98% albumin and on immunoelectrophoresis gives a single line with rabbit anti-bovine normal serum (R-a-BNS) (Fig. 32a). Assessed by electrophoresis, BGG is 95% pure and by immunoelectrophoresis with R-a-BNS three globulin components are revealed (Fig. 32c).

Antisera

Rabbit antisera to BNS and to cat normal serum (CNS) were obtained from rabbits subjected to standard immunization with the appropriate heterologous serum. Briefly, the procedure involved two intramuscular injections of serum emulsified in Freund's complete adjuvant (Difco) on days 1 and 15 respectively followed by an intraperitoneal and an intravenous injection of diluted serum (1 : 3) on days 29 and 30. Antisera were obtained by periodic bleeding conducted from day 36 onwards during the height of the

antibody response.

Cats were immunized in various ways. With BSA and BGG they received either (1) a single intravenous injection of soluble antigen or (2) single or multiple injections of antigen in Freund's adjuvant. With BNS and RNS the course of immunization was as described above for rabbits. Blood samples were taken at intervals during, and after, the courses of immunization.

All antisera were stored without preservative at -10°C. When required for serological procedures they were thawed at room temperature and centrifuged for thirty minutes at 1500 g.

Ion Exchange Chromatography of BGG

Separation of the three components of BGG was achieved by column chromatography on DEAE Sephadex A50 obtained from Pharmacia (Great Britain), Ltd. As BGG proved to be but sparingly soluble in 0.02M phosphate buffer of pH = 6.6, the column was equilibrated with 0.05M NaCl in 0.02M phosphate buffer before application of BGG in the same solvent. The protein fractions were eluted by a NaCl gradient (0.05 - 0.25M) in 0.02M phosphate buffer (Fig. 33). The 'β globulin' fraction so isolated was dialysed against 0.075M NH_4HCO_3 and freeze-dried. For serological use it was reconstituted in 0.15M NaCl.

Ultracentrifugation

Analytical ultracentrifugation of β-globulin isolated from

BGG was carried out on a Spinco Model E ultracentrifuge at 59,780 r.p.m. at 20°C with a protein concentration of 0.5 mg. per ml. and 0.15M NaCl as diluent. During the 90-minute period of centrifugation exposures were made at intervals of 16 minutes.

Trace-labelling of Proteins with I^{131}

BSA and the isolated β-globulin component of BGG were trace-labelled with I^{131} by the iodine monochloride method of Macfarlane (1958). During post-labelling dialysis against 0.15M NaCl some of the labelled β-globulin precipitated and was discarded. Labelling efficiency was determined by measuring the distribution of activity between precipitate and supernatant after total protein precipitation with 20% trichloracetic acid of a pre-dialysis aliquot of the labelled protein. Specific activities of labelled proteins were determined by measuring the activity of a post-dialysis aliquot of known protein concentration (optical density at 278mμ). The efficiency of labelling of BSA was 60% and the dialysed preparation had a specific activity of 719 counts/sec./mg. protein. For the β-globulin preparation a labelling efficiency of 53% and a specific activity of 195 counts/sec./mg. of protein were obtained. All radioactivity determinations were carried out in a well-type scintillation counter (Isotope Development Co., Ltd.) using an Ekco Scaler, Type N530D.

Serological Methods

As all serological methods used in this work have been fully

specified elsewhere, they will here be described only briefly.

(a) Immunodiffusion: this was carried out in 1% agar gel in the conventional manner (Crowle, 1961).

(b) Immunoelectrophoresis: all immunoelectrophoretic analyses were performed with immunophor equipment (LKB-Produkter, Sweden), using veronal acetate buffer of pH = 8.6 and of ionic strength = 0.1. Generally, antigens were electrophoresed for one hour at a field strength of 9 volts/cm. Full details of the technique and appropriate apparatus have been given by Hirschfield (1960).

(c) Quantitative precipitin reaction: Quantitative precipitin analysis conformed with approved techniques (Kabat and Mayer, 1961). To facilitate such estimation, the antigen was trace-labelled with I^{131} but iodination was so slight as not to interfere with serological specificity or reactivity (Talmage and Maurer, 1953). To a series of tubes containing a constant volume (0.2 ml.) of antiserum, increasing amounts of antigen were added and the mixtures then incubated at 37°C for 30 minutes before they were refrigerated overnight. The resulting precipitates were deposited by centrifugation for thirty minutes at 5°C at 1500g., washed once in 0.5 ml. ice-cold saline and finally dissolved in 0.1N NaOH. Antigen and antiserum controls were similarly treated. Total protein of the precipitates was estimated by the method of Lowry as described by Kabat and Mayer (1961). The radioactivity of the supernatants was used as a measure

of unprecipitated antigen.

(d) PBO test: this test assesses the antigen-precipitating capacity of an antiserum by determining the dilution required to precipitate 80% of a standard amount of trace-labelled antigen. Arbitrary as it is, the figure of 80% represents a point of slight antigen excess, which corresponds to the position of maximal precipitation on a typical rabbit quantitative precipitin curve. The test was used in the present work to determine the BSA-precipitating capacity of sera obtained from 4 cats immunized with BNS. The sera were tested at five different dilutions (1:2, 1:5, 1:10, 1:25, and 1:50) against a standard I^{131}-BSA preparation containing 4 μg. BSA-N/ml., 0.5 ml. aliquots of serum dilution and antigen being used throughout. Full details of the procedure adopted have been given by Campbell et al., (1963).

(e) Antigen-binding capacity (ABC) test: the binding of antigen by antiserum, as compared with actual precipitation of antigen, is measurable by means of the test devised by Farr (1958) and is applicable to systems in which serum albumin constitutes the antigen. It is based on the differential precipitation of albumins and globulins by ammonium sulphate. Thus, firmly formed, yet soluble, complexes of antigen and antibody are precipitable by half saturation with the salt while unbound albumin is unaffected and remains in the supernatant fluid. The use of trace-labelled

antigen facilitates measurement of its distribution between precipitate and supernatant. The serum dilution which binds 33% of a standard amount of antigen serves as an arbitrary end-point from which may be calculated the amount of antigen that would be bound by 1.0 ml. of undiluted serum. The resultant, or 'ABC-33', value is used to express the antigen-binding capacity of the serum. The four cat sera subjected to the PBO test were also submitted to an ABC test involving similar dilutions of serum and the same antigen. Details of procedures have been described by Campbell et al. (1963).

Results

Precipitin Response

The number of cats that produced precipitating antibody to BSA, BCG and heterologous serum is shown in Tables 10-12. A positive precipitin response to BSA (Table 10) was found to obtain in only 3 out of 10 cats immunized with that antigen in three different ways. The best response resulted from a single intravenous injection of 10 mg. BSA per kilogramme of body weight (group C), in which instance precipitating antibody was demonstrable on days 10 and 11 after injection. Antibody was not detectable in two animals that had been given a moderate course of treatment with BSA in Freund's adjuvant (group A) and only one of 4 cats subjected to similar but more intensive management (group B) yielded a positive precipitin response.

Even in cases where a response was obtained the reaction was very weak (Fig. 32b).

Similarly, the response to BGG (Table 11) was poor since only 4 out of 15 cats produced precipitins. Although a response was not obtained in four cats in receipt of a single intravenous injection of 20 mg./Kg. of BGG (group F), precipitating antibody was detected in 2 out of 4 cats inoculated with 120 mg./Kg. by the intravenous route (group G). Of the 5 cats in group D given a single injection of BGG in Freund's adjuvant only one produced antibody. Likewise, antibody was demonstrated in only one of two cats (group E) subjected to an intensive course of antigen with adjuvant. This was detectable at 22 days after the first injection, was still present three weeks later and persisted for at least 11 months without further stimulation. Surprisingly, in each case the antibody proved to be homologous with a minor component of BGG that possessed the electrophoretic characteristics of a β-globulin (Fig. 32f).

Ten cats were injected with heterologous serum (Table 12). Repeated subcutaneous injection of 1 ml. of BNS (Cat H) or administration of 1 ml. of BNS in Freund's adjuvant by various routes (group I) failed to elicit precipitating antibody. All four cats given multiple injections of 2 ml. BNS in Freund's adjuvant (group J) followed by an intraperitoneal and an intravenous injection came to yield precipitating antibodies to α - and β-globulins of BNS. Two

Table 10 - IMMUNISATION PROCEDURES FOR AND SEROLOGICAL RESPONSES OF CATS INJECTED WITH BSA

Group	No. of Cats	Immunisation Procedure				Precipitin Response	
		Day of Experiment	Ag. dose mg./kg.	Adjuvant	Route*	Day of Experiment	No. of positive cases
A	2	1 19 36	1 2 10	+ + +	d.p. d.p. i.m.	10 19 23 36 43	0/2 0/2 0/2 0/2 0/2
B	4	1	15	+	d.p. i.m. i.d. s.c.	22	1/4
		6	15	+	d.p. i.m. i.d. s.c.	29	1/4
		10	15	+	d.p. i.m. i.d. s.c.	36	1/4
		14	15	+	d.p. i.m. i.d. s.c.	43	1/4
C	4	1	10	-	i.v.	5 10 11	0/4 1/4 2/4

Key * d.p. = digital pad; i.m. = intramuscular; i.d. = intradermal; s.c. = subcutaneous; i.v. = intravenous

Table 11 - IMMUNISATION PROCEDURES FOR AND SEROLOGICAL RESPONSES OF CATS INJECTED WITH EGG

Group	No. of Cats	Immunisation Procedures				Precipitin Response	
		Day of Experiment	Ag. dose mg./kg.	Adjuvant	Route*	Day of Experiment	No. of positive cases
D	5	1	15	+	i.m. s.c. d.p.	8	0/3
						10	0/2
						12	0/3
						15	1/2
E	2	1	15	+	d.p. i.m. i.d. s.c.	22	1/2
		6	15	+	" "	29	1/2
		10	15	+	" "	36	1/2
		14	15	+	" "	43	1/2
F	4	1	20	-	i.v.	6	0/4
						8	0/4
						10	0/4
G	4	1	120	-	i.v.	5	0/4
						7	0/4
						9	2/4
						12	1/4

Key * i.m. = intramuscular; s.c. = subcutaneous; d.p. = digital pad; i.v. = intravenous

Table 12 - IMMUNISATION PROCEDURES FOR AND SEROLOGICAL RESPONSES OF CATS INJECTED WITH HETEROLOGOUS SERUM

Group	No. of Cats	Immunisation Procedures				Precipitin Response	
		Day of Experiment	Ag. dose (ml.)	Adjuvant	Route*	Day of Experiment	No. of positive cases
H (one cat)	1	1	1 ml. BNS	-	s.c.	22	1
		3	"	-	"	29	1
		6	"	-	"	36	1
		10	"	-	"	43	1
I	3	1	1 ml. BNS	+	i.m. s.c. d.p.	8	1/3
						15	3/3
J	4	1	2 ml. BNS	+	i.m. s.c.		
		10	"	+	"	27	3/4
		27	"	+	"	41	4/4
		41	1 ml. BNS	-	i.p.		
		42	0.3 ml. BNS	-	i.v.	50	4/4
K	2	1	2 ml. RNS	+	i.m. s.c.	19	1/2
		19	"	+	"	33	2/2
		33	1 ml. RNS	-	i.p.	40	2/2
		34	0.3 ml. RNS	-	i.v.		

Key * s.c. = subcutaneous; i.m. = intramuscular; d.p. = digital pad; i.p. = intraperitoneal i.v. = intravenous

cats similarly treated with BNS (group K) likewise produced precipitating antibodies to a limited number of the proteins of rabbit serum. The range of antibodies produced by cats to BNS and RNS proved to be more limited than was the response of rabbits to BNS and CNS to identical procedures of immunization (Fig. 32d, h, c, g.). Manifestly, the response of cats to RNS was better than that to BNS but in both cases there was a lack of antibodies capable of precipitating either serum albumin or gamma globulin.

Elimination of Antigen

The mode of removal of I^{131}-BSA from the circulating blood of four cats in receipt of antigen for the first time and from that of four cats previously immunized with BSA in complete Freund's adjuvant is shown in Figs. 34 and 35 respectively. In each of the four normal cats the pattern of elimination was diphasic and characterized by a rapid fall in concentration that occurred during the first 24 hours due to the equilibration of the antigen between intra- and extravascular fluids and was followed by a slower catabolic disappearance of antigen, the apparent biological half-life ($t_{\frac{1}{2}}$) of which was 84-98 hours. Although antibody was detectable in two of the cats on the tenth day after injection, there was not any accelerated clearance of antigen.

An accelerated immune clearance of antigen was seen in 3 of 4 previously immunized cats, the time required for complete removal of

antigen from the circulation being 3 days in B43 and 8 days in B41 and B42. In the instance of B44 the pattern of elimination was similar to that encountered in normal cats but the $t_{\frac{1}{2}}$ was only 70 hours. Only in cat B43 was circulating antibody detectable within the 12-day period of observation.

In Figs. 36 and 37 are shown the antigen elimination patterns of I^{131}-BGG in three normal and three previously immunized cats. Although all of the normal animals came to produce precipitating antibody by the tenth day after injection, only in B51 was there clear evidence of accelerated immune clearance which occurred on the 5th day after injection of antigen. The $t_{\frac{1}{2}}$ values in those three animals were 36, 49 and 50 hours. In the case of two (B45, B47) of the previously immunized animals, antigen was rapidly removed from the circulation so that by the 6th day it was not detectable while in the third animal (B46) there was a diphasic clearance in which the $t_{\frac{1}{2}}$ of the second phase was 44 hours. Two of the cats (B45, B47) produced precipitating antibody but the duration of that response was limited to 2-3 days.

The Globulin Nature of Cat Antibody

To determine whether the ABC test might serve to detect the antigen-binding capacity of cat serum, cat antibody had first to be identified as a globulin capable of precipitation by half-saturation with $(NH_4)_2\ SO_4$. The globulin character was readily demonstrable by

reversing the roles of the reactants in immunoelectrophoresis of a cat-a-BGG serum. Cat antiserum was electrophoresed from a central well and the trough thereafter charged with BGG. The resulting arc of precipitate was found to be located in the electrophoretic position typical of gamma globulin (Fig. 38a). When cat-a-BGG was half-saturated with $(NH_4)_2\ SO_4$ and immunodiffusion tests carried out on supernatant and redissolved precipitate, antibody activity proved to be restricted to the precipitate and a reaction of complete identity was found to occur between the lines formed by antibody in whole serum and antibody in the globulin precipitate (Fig. 38b).

PBO and ABC Tests

In none of the four cat sera used in the PBO test was precipitation observed but all exhibited some binding of I^{131}-BSA although to varying degree (Fig. 39), the ABC - 33 values ranging from 30.4 to less than 2.6. Binding of antigen by a control normal serum was quite negligible.

Isolation of the Precipitating Antigen from BGG

Chromatography of Armour's BGG yielded three distinct peaks (Fig. 33). By means of immunoelectrophoresis, the last peak was identified as the precipitating antigen (Fig. 40) which, because of its electrophoretic mobility, was designated a β-globulin. Approximately 6 mg. of the β-globulin was obtained from 100 mg. BGG. As a result of analytical ultracentrifugation, the isolated β-globulin

gave only a single peak, the calculated sedimentation coefficient of 6.3S suggesting a molecular weight of 150,000.

Quantitative Precipitin Reaction between Cat Antiserum and Bovine β-globulin

The results of the quantitative precipitin reaction between the β-globulin isolated from Armour's BGG and pooled cat antisera are given in Fig. 41. Although specific measures were not taken to eliminate complement, all sera had been stored for some months before use and control values for non-specific precipitation were always found to be negligible. The amount of total protein precipitated gradually rose to a maximum of 900 μg. as the quantity of added Ag was increased from 3 to 156 μg. but thereafter declined. Two anomalies were noted in the curve describing the percentage of antigen precipitated at each point. One was the failure to separate more than 65% of the antigen, even, under conditions of antibody excess and the other was the very small amount of antigen precipitated when less than 30 μg. of antigen were used in the test. The antibody/antigen ratio fell steeply from a value of 20 in the region of presumed antibody excess to level out through the area of maximal precipitation and presumed antigen excess where values ranged between 0 and 4. Although limited amounts of antigen precluded complete precipitin analysis of individual cat sera, it was possible to study the reaction in the pre-antigen excess zone of a single cat serum. The results were in general agreement with those

obtained with the pooled antiserum.

Discussion

The paucity of data on the serological response of the cat to foreign proteins suggests that it may be difficult to produce such a response using conventional immunization procedures and to some extent this is borne out by occasional failures reported (e.g. Akcasu, 1963). However, the results show that cats can be induced to form precipitating antibody to purified protein antigens such as BSA and BGG and to heterologous sera such as BNS and RNS. The responses to BSA and BGG were poor both in terms of the number of animals in which precipitating antibody was produced and in the intensity of the serological reactions. Thus, of 10 cats immunized in various ways with BSA in only 3 was precipitating antibody detected. Likewise, precipitins were found in the sera of only 4 of 15 cats treated in a similar fashion with BGG.

Although better responses were obtained from cats immunized with BNS and RNS the antibodies produced were directed only to α- and β-globulins and not to albumin or γ-globulins (Figs. 32d, h). Such a hiatus in response may represent an immunological paralysis as these latter proteins are present at high concentration in normal serum and the amounts used for immunization may therefore have been excessive. The formation of antibody to only the minor β-globulin

component of BGG (Fig. 32f) may be another example of the same phenomenon. In this context, it is interesting to note that tolerance to antigens of BSA and BGG type can readily be induced in cats younger than 3-4 weeks by parenteral or oral administration of the appropriate antigen (Miller-Ben Shaul, 1965). Conceivably in kittens having access to bovine milk at a very early age, intestinal absorption of milk proteins known to correspond immunologically to BSA and BGG (Hanson and Johanssen, 1959) could modify subsequent immune responses to these and serologically related antigens. On the other hand not all cats were immunologically inert to BSA, precipitin responses being sometimes obtained (Fig. 32b) when the antigen was given intravenously, a route that has also been successfully employed by Miller-Ben Shaul (1965) for production of precipitating antibodies to protein antigens. On the basis of Mitchison's (1964) description of a BSA-paralysis in mice occurring at both high and low zones of antigen dosage it is possible that the immunogenic efficacy of the intravenous route depends upon the relatively rapid elimination of antigen given in this way (Fig. 34) compared to the prolonged release of antigen from a Freund's adjuvant depot.

In any event there is a wide variation in the ability of different species to produce precipitating antibody; the fowl and the rabbit are good precipitin producers whereas it is extremely difficult to elicit precipitating antibody in the rat, and the cat would appear

to fall between these two extremes.

Akcasu (1963) has suggested that in his experiments the inability of materials such as egg albumin or cow's milk protein to elicit immune responses in the cat was due to the rapidity with which those proteins were cleared from the circulation, which process is completed in less than 24 hours. The antigen clearance studies reported here using I^{131}-BSA and I^{131}-BGG have shown both proteins to behave as typical heterologous plasma proteins with apparent biological half-lives of approximately 3.8 days (BSA) and 2.8 days (BGG). While no figures are available for the half-lives of homologous albumin and gamma globulin in the cat they are probably comparable to those of the rabbit which are 5.7 and 4.6 days respectively for albumin and gamma globulin (Weigle, 1957). The half-lives of BSA and BGG in the rabbit have been determined by the same worker as 4.3 and 2.2 days respectively.

All three cats given an intravenous injection of I^{131}-BGG for the first time produced precipitating antibody (Fig. 36), in each case directed to the minor β-globulin component of BGG. However, one cat (B51) exhibited a phase of accelerated immune clearance of antigen, suggesting that this animal may have produced antibodies capable of combining with all the components of BGG. The antibodies directed against the other two components of BGG may have been produced in amounts too small to detect by the serological methods used.

or may have been of the non-precipitating variety. The failure of cats 52 and 53 to exhibit immune clearance even when their sera contained an antibody for β-globulin, indicates that in these animals no antibody was produced to the components of BGG other than the β-globulin.

In none of the cats given a primary injection of I^{131}-BSA was an accelerated phase of antigen clearance observed (Fig. 34). Despite this, antibody was detected in the serum of two animals 10-11 days after injection. The co-existence of circulating antigen and antibody is difficult to explain although the weakness of the associated serological reaction suggests that the antibody may have been of poor avidity and perhaps readily dissociable from its antigen in vivo. That accelerated immune clearance of antigen can occur with BSA was shown by the antigen elimination patterns of three of the cats previously sensitized with this protein. Thus, appropriate immunization does result in the formation of antibody capable of combining with and removing BSA from the circulation. Further evidence of the combining capacity of cat antibody to BSA can be found in the results of the ABC test (Fig. 39) although the ABC values obtained were extremely low, being at least ten times less than those found in rabbits given a similar course of treatment (Farr, 1958).

Most quantitative precipitin studies have been carried out with rabbit antiserum (Kabat and Mayer, 1961). The typical quantitative

precipitin curve rises with increasing antigen concentration until it reaches a maximum value and thereafter declines. By analysis of the supernatants for uncombined antigen and antibody the curve may be divided into three zones, (a) the section related to antibody excess where the curve is rising and all antigen is precipitated but free antibody can be detected in the supernatant, (b) the zone of equivalence in which both antigen and antibody are completely precipitated and (c) the zone of antigen excess which is characterized by a progressive inhibition of precipitation and increasing quantities of free antigen in the supernatant. Maximal precipitation generally occurs in slight antigen excess. In general, the characteristics of the cat precipitin system resemble those described above but complete zonal analysis of the curve was not possible as precipitation of antigen was never found to be complete. However, the curve describing the total amount of protein precipitated rose to a maximum value of 900 μg. protein when 156 μg. of antigen were used and then began to decrease presumably as a result of inhibition by excess antigen. The antibody-antigen ratio curve followed a pattern similar to well characterized systems, and fell steeply in the early part of the curve levelling out in the region of maximal precipitation and antigen excess with values varying between 8 and 4. The ratios approximated to those expected in a system involving an antigen of molecular weight around 150,000 g.g. with rabbit antiserum the value

cited for human gamma globulin (M.W. 160,000) as antigen in the region of slight antigen excess is 3.5 (Kabat and Mayer, 1961).

The cat precipitin system differed from the typical precipitin reaction in two ways. At no point was more than 65% of the antigen precipitated and in the region of extreme antibody excess the precipitation of antigen was even less than 6%. Failure to achieve complete precipitation of antigen hints very strongly at the presence throughout the curve of soluble antibody-antigen complexes, whose precipitation may require adjustment of electrolyte concentration or of pH, a situation known to occur in other species such as the fowl (Aitken and Mulligan, 1962) and in certain systems in the rabbit (Kleczkowski, 1965). The less than 6% precipitation occurring in the early part of the curve suggests that there is some degree of inhibition of precipitation in extreme antibody excess. In this respect the cat anti-bovine β-globulin system resembles the type of reaction seen in horse anti-toxin systems (Kabat and Mayer, 1961), occasionally in rabbit anti-protein systems (Feinberg, 1958) and regularly in dog anti-albumin systems (Patterson, Chang, Pruzansky and Portney, 1963; Patterson, Chang, Pruzansky, 1964).

Detection of soluble complexes of cat antibody and bovine β-globulin in the supernatant could be achieved by treating the supernatants with a rabbit anti-cat globulin serum, previously absorbed with bovine β-globulin, which would precipitate labelled

antigen firmly bound to cat antibody. In the present work limited amounts of antigen prevented the use of this procedure but the characteristics of the quantitative test strongly suggest the existence of soluble antibody-antigen complexes.

As far as is known this is the first description of the quantitative precipitin reaction involving cat antiserum but the results should not be regarded as typical for this species since the reaction was carried out on pooled antisera and not on serum from individual cats. Further, no specific step other than ageing of serum was taken to remove complement from the system, and finally the isolated antigen proved to be somewhat unstable as shown by spontaneous precipitation during post-labelling dialysis. While the reason for this instability is not clear, it is worth noting that isolated H-chains of rabbit antibody are only sparingly soluble in neutral salt solutions (Reholt, Radzimski and Freeman, 1965).

Although the experiments described in this paper have indicated that the cat gives only a feeble precipitin response it will be necessary to investigate the antigenicity of a much wider range of proteins of both animal and plant origin, to characterise the serological response to them, and to study the properties and behaviour of cat antibody before the immunological capacity of this species can be fully appreciated. It does seem that the immune response of the cat to heterologous serum differs in a quantitative

way from that of the common laboratory animals such as the rabbit. From a comparative point of view further knowledge of the immunological behaviour of the cat to defined antigens may help to elucidate the general mechanism of antibody synthesis.

Fig. 32

Immunoelectrophoresis of heterologous serum proteins using rabbit and cat antisera

a. Rabbit-a-BNS versus BSA

b. Cat-a-BSA " BSA

c. Rabbit-a-BNS " BNS

d. Cat-a-BNS " BNS

e. Rabbit-a-BNS " BGG

f. Cat-a-BGG " BGG

g. Rabbit-a-CNS " CNS

h. Cat-a-RNS " RNS

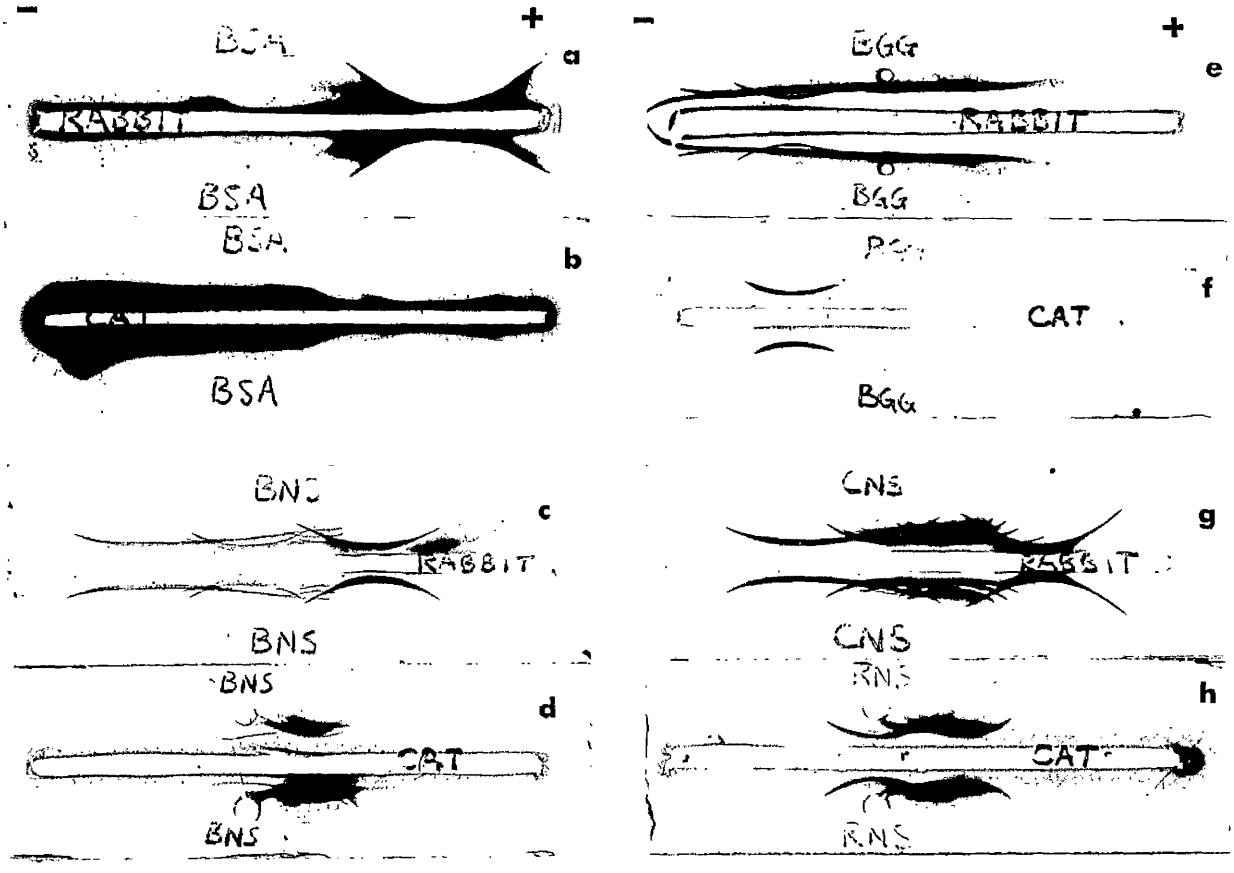

−
+
BSA
a
RABBIT
BSA
BSA
b
CAT
BSA
BNS
c
RABBIT
BNS
BNS
d
CAT
BNS
−
+
EGG
e
RABBIT
BGG
BGG
f
CAT
BGG
CNS
g
RABBIT
CNS
RNS
h
CAT
RNS

Fig. 33 Chromatographic fractionation of Armour's BCG on DEAE Sephadex A50.

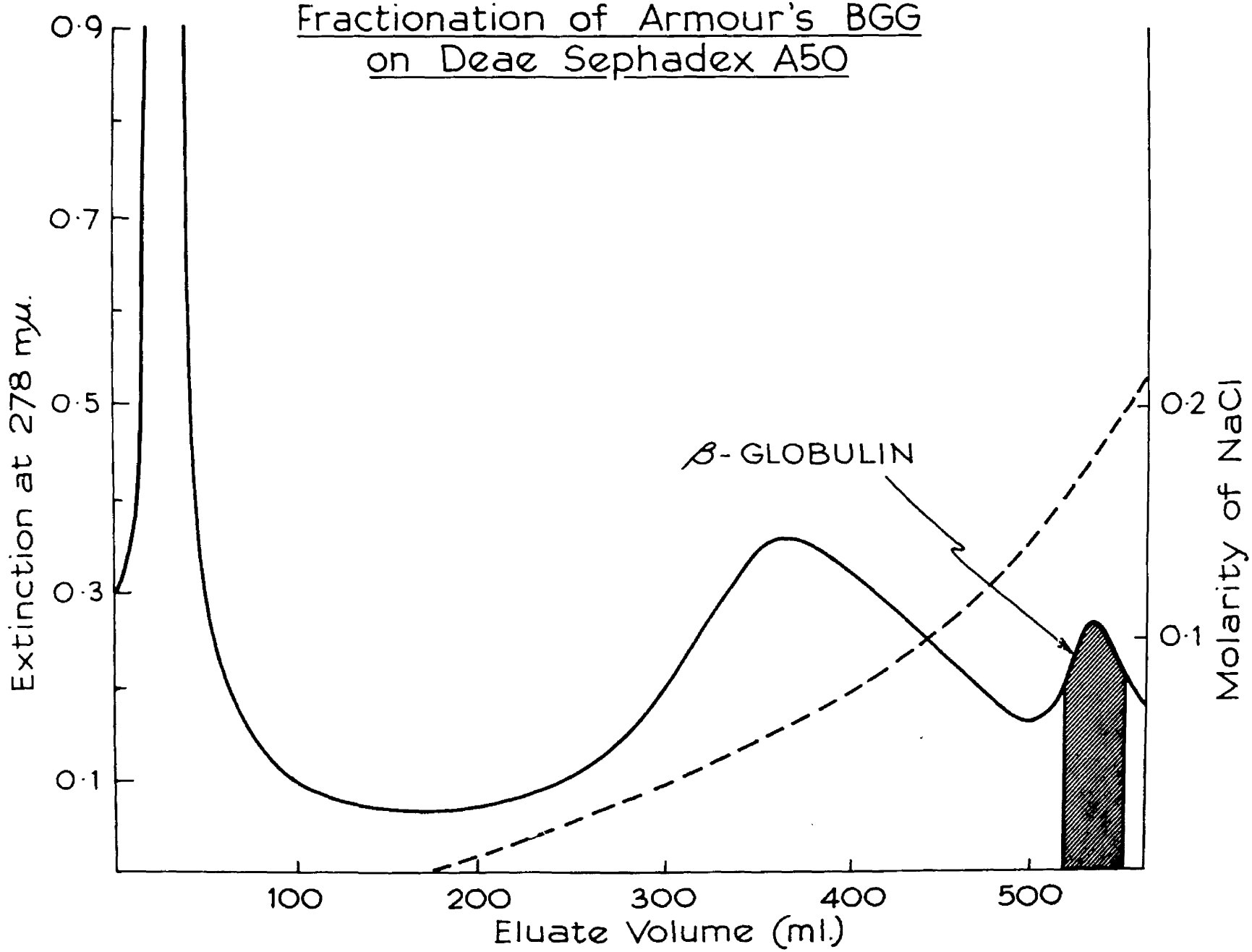
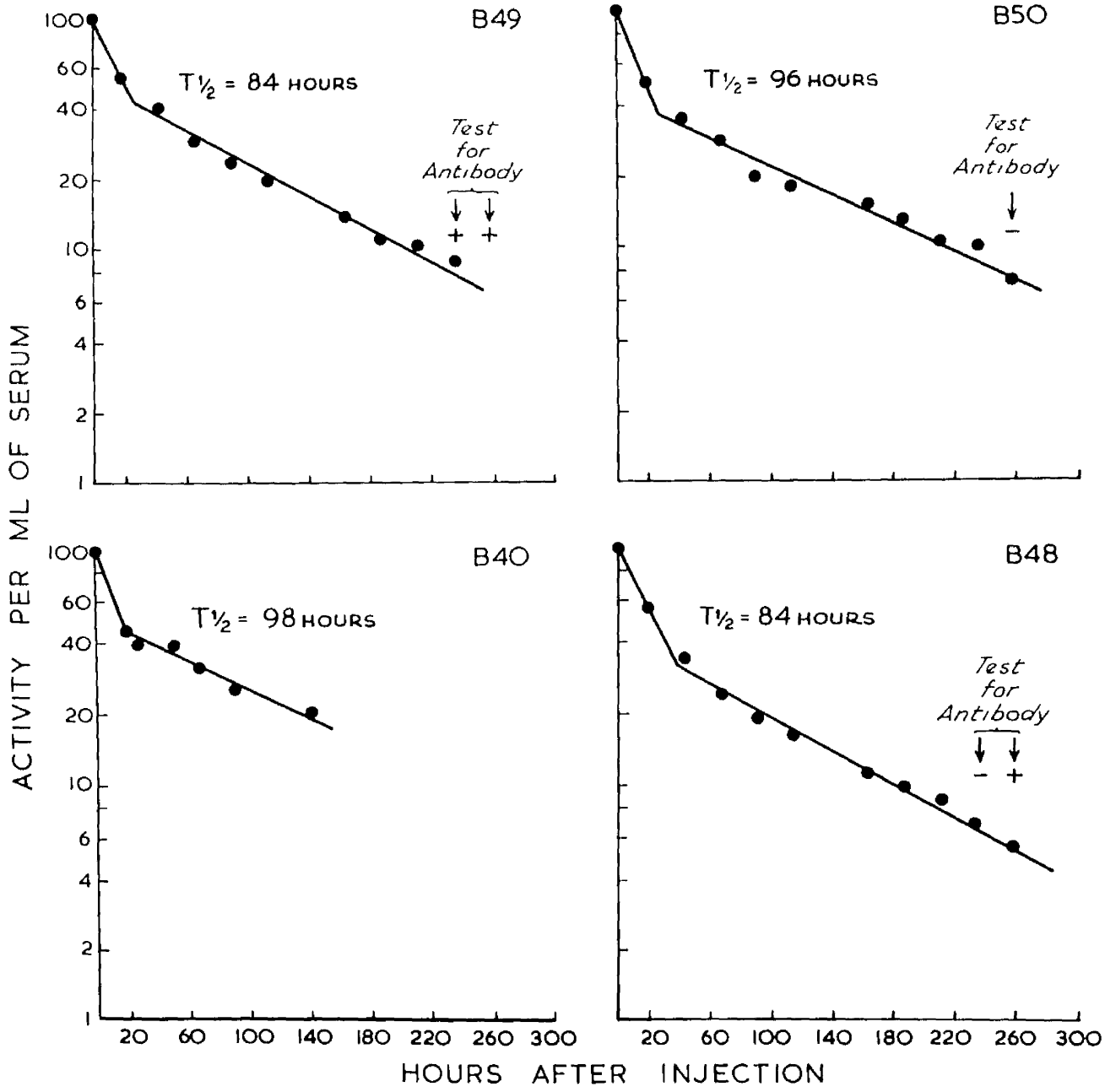
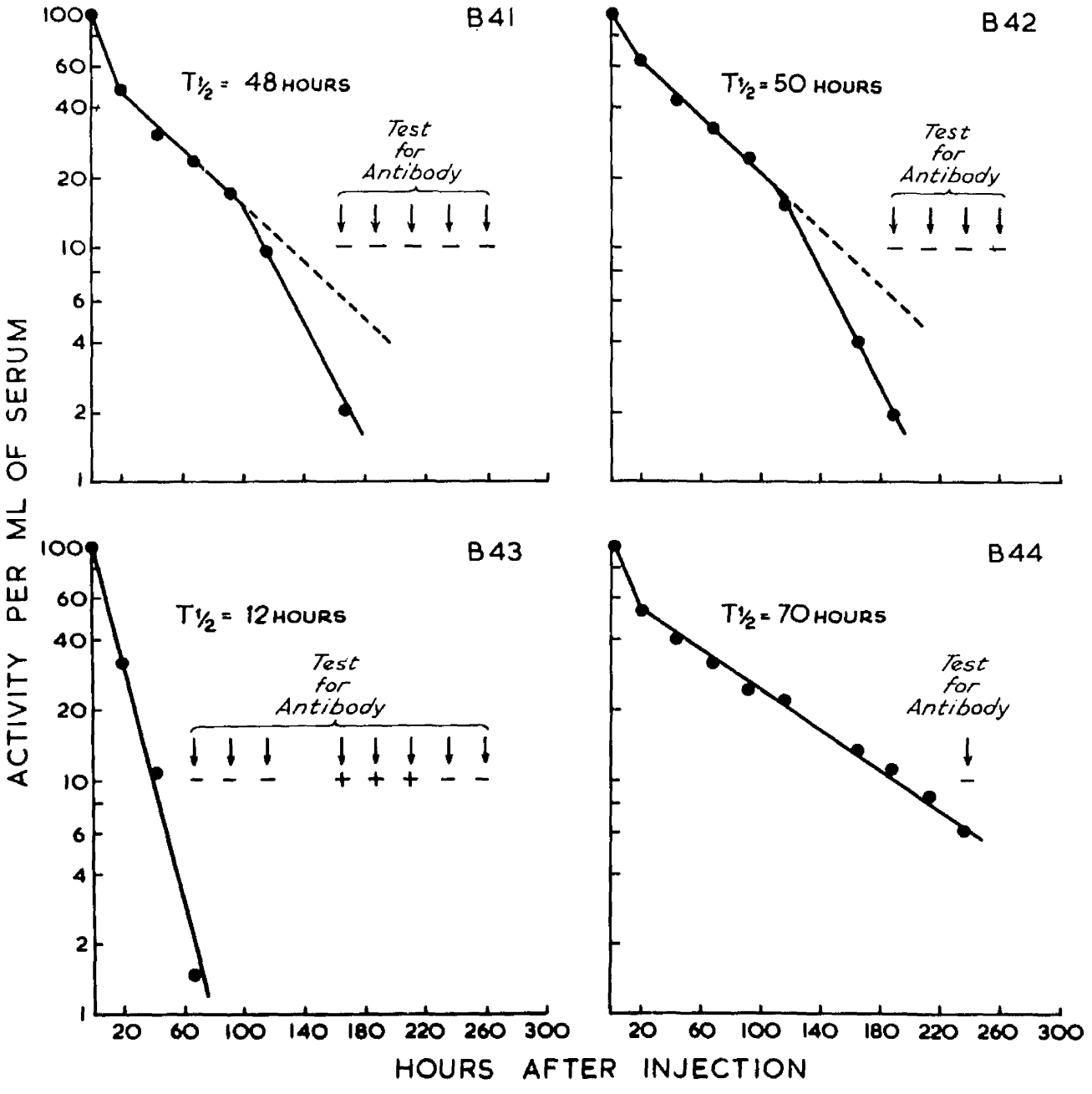

Fractionation of Armour's BGG
on Deae Sephadex A50
0·9
0·7
0·5
0·3
0·1
Extinction at 278 mμ.
β-GLOBULIN
0·2
0·1
Molarity of NaCl
100
200
300
400
500
Eluate Volume (ml.)

Fig. 34 Removal of I^{131}-BSA from the circulation of four normal cats.



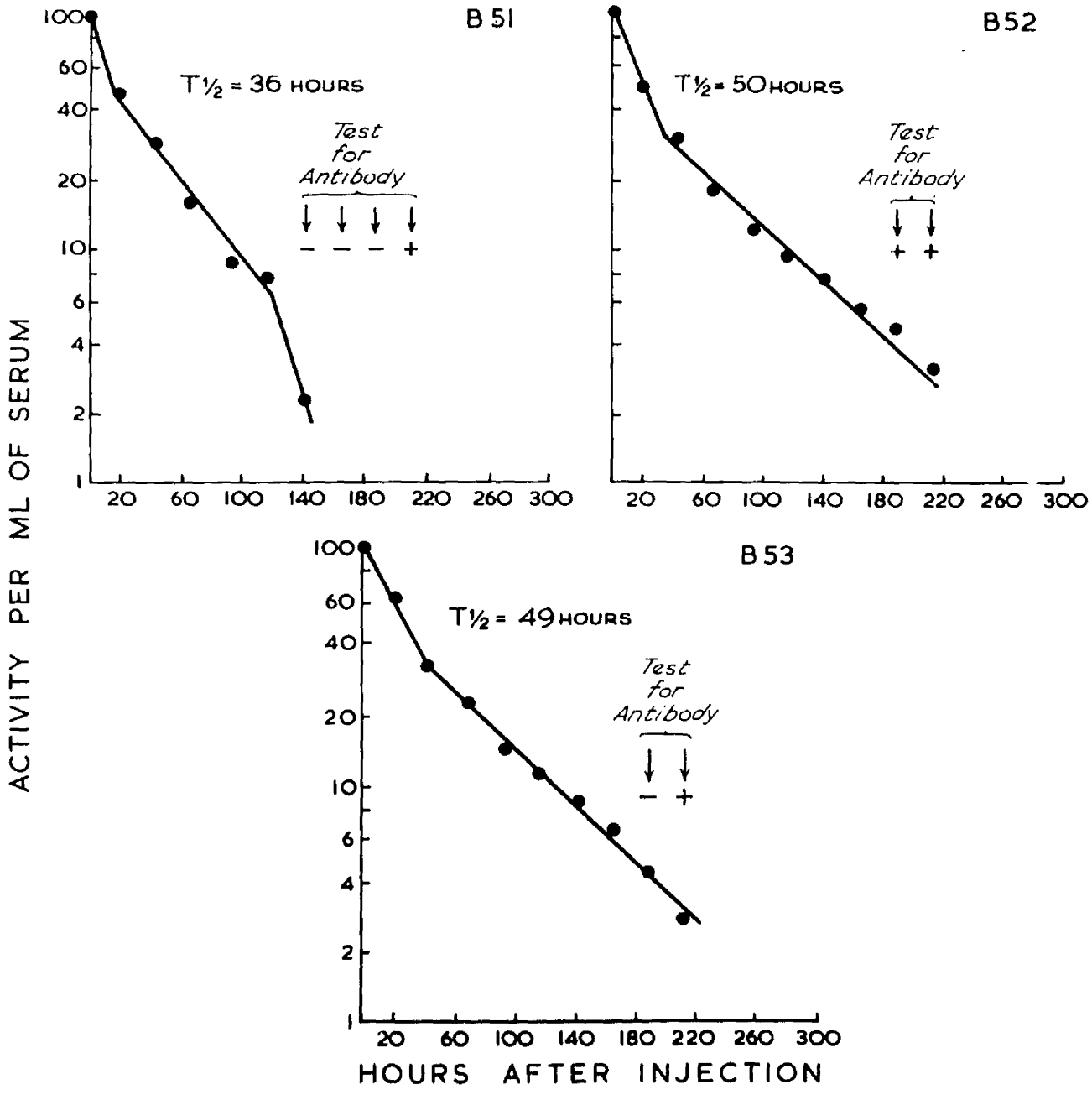
Removal of I^{131}-BSA from the circulation of 4 normal cats.

Fig. 35 Removal of I^{131}-BSA from the circulation of four cats previously immunized with BSA in complete Freund's adjuvant.



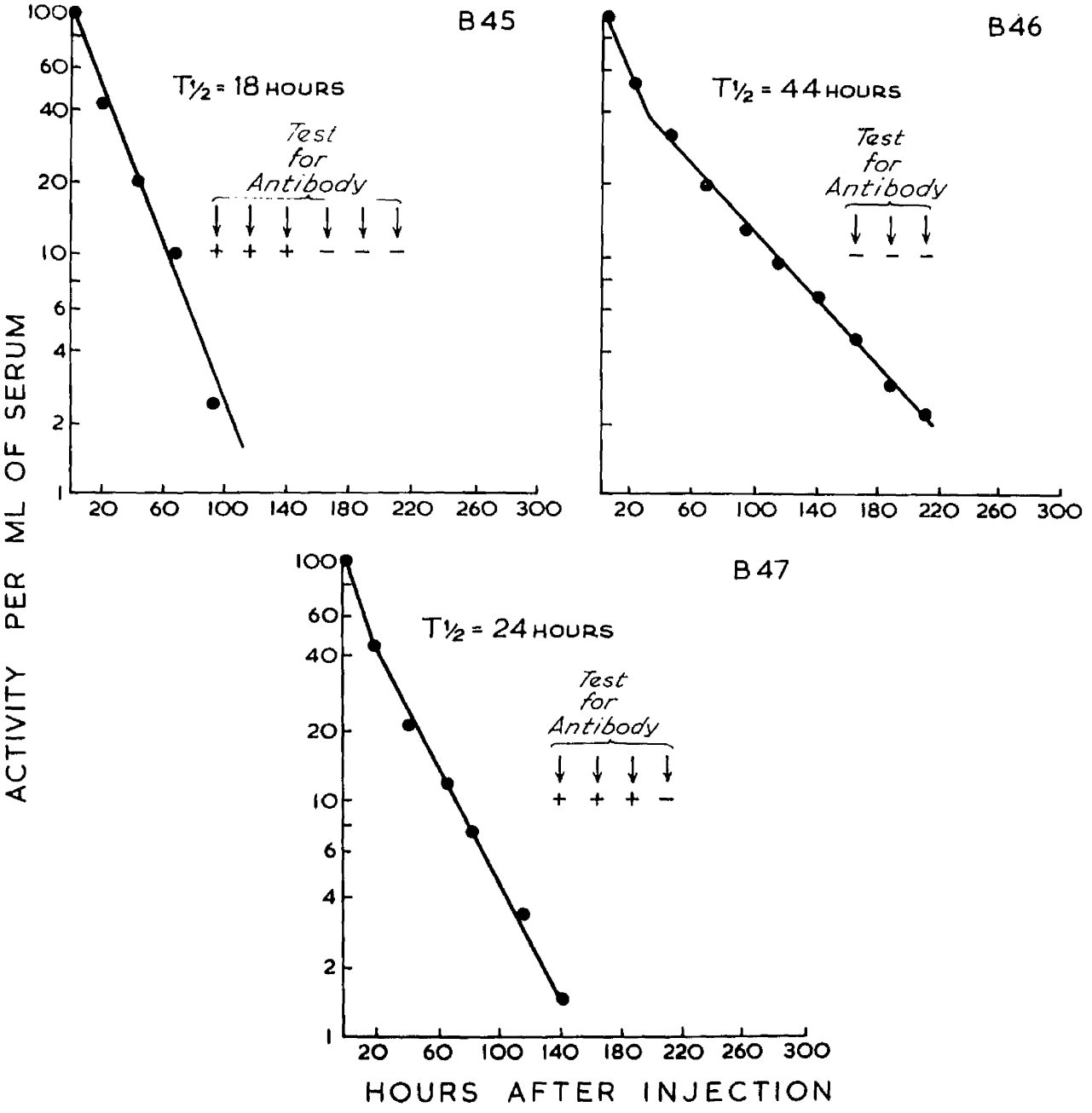
Removal of I^{131}-BSA from the circulation of 4 cats previously immunised with BSA in complete Freund's adjuvant.

Fig. 36 Removal of I^{131}-BGG from the circulation of three normal cats.



Removal of I^{131}-BGG from the circulation of 3 normal cats.

Fig. 37 Removal of I^{131}-BGG from the circulation of three cats previously immunized with BGG in complete Freund's adjuvant.



Removal of I^{131}-BGG from the circulation of 3 cats previously immunised with BGG in complete Freund's adjuvant.

Fig. 38

(a) Immunoelectrophoresis of cat-anti-BGG showing the γ-globulin electrophoretic mobility of the cat antibody. The trough was charged with BNS.

(b) Double diffusion of BNS (Ag) with cat-a-BGG serum (W), redissolved precipitate from half-saturation of $(NH_4)_2SO_4$ of cat-a-BGG (P) and the supernatant of the same precipitate (S). One well was charged with rabbit-a-BNS (R).

CAT ANTISERUM

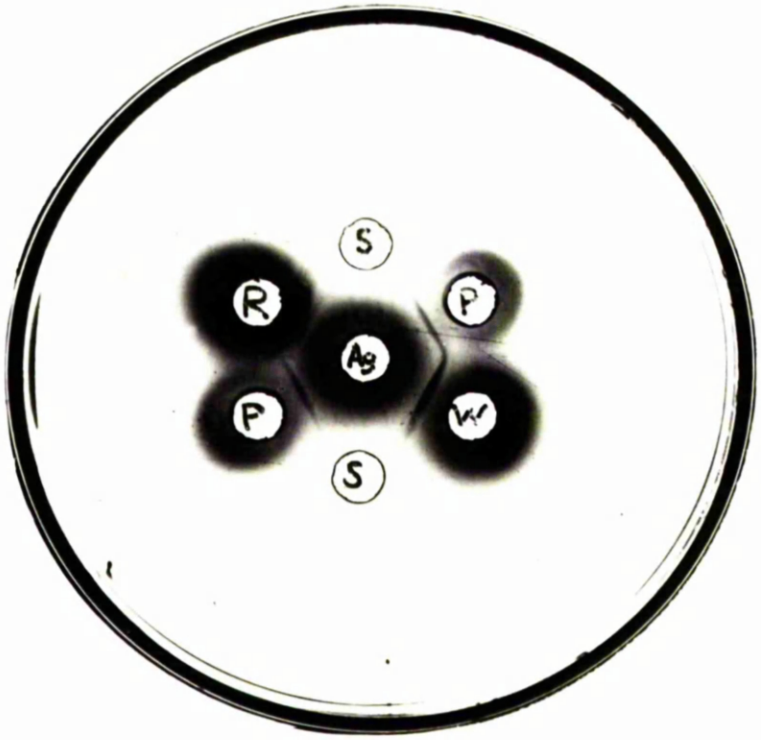


Fig. 39 I^{131}-BSA binding by the sera of four cats immunized with DNS.

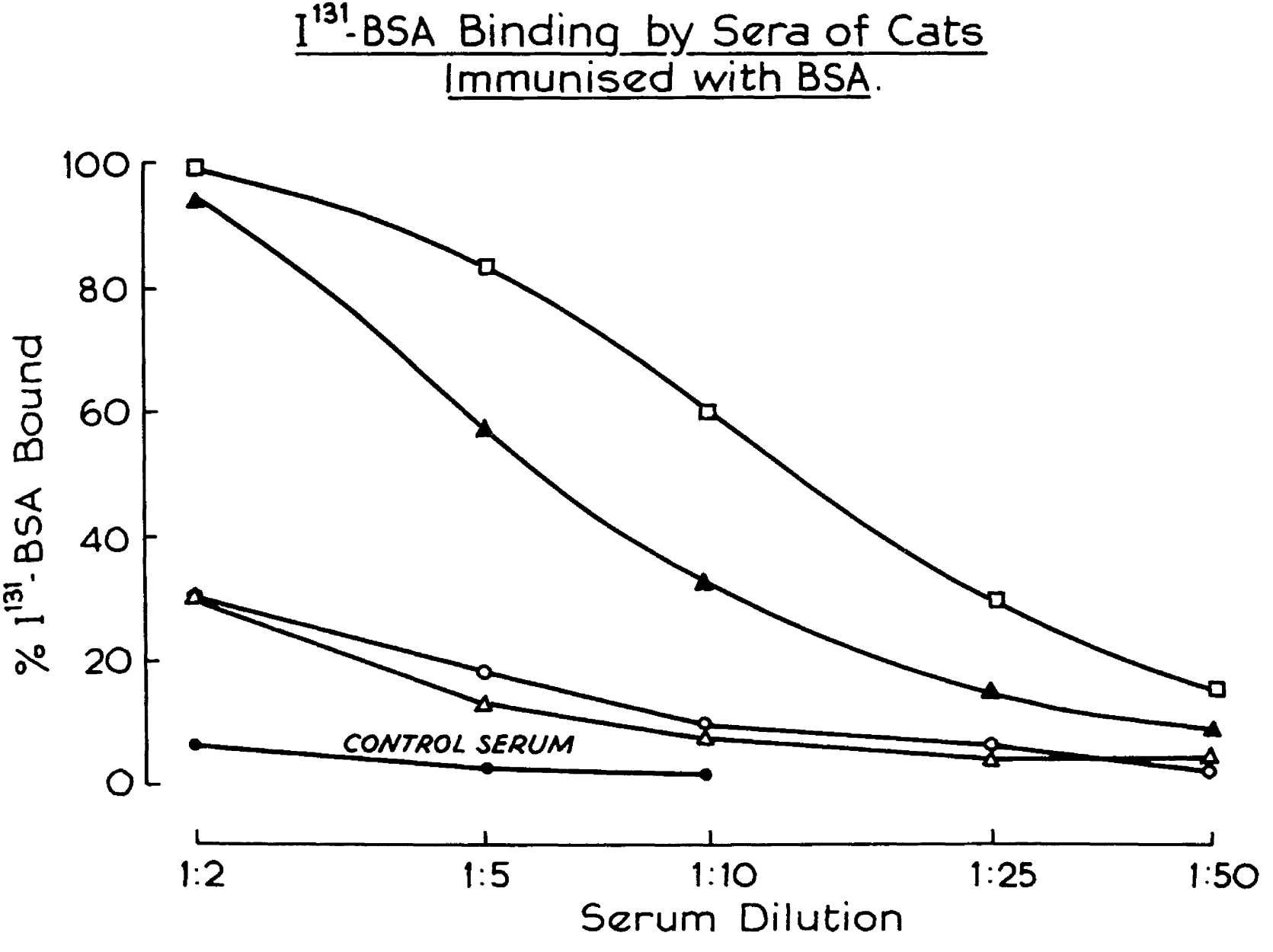

I[131]-BSA Binding by Sera of Cats Immunised with BSA.
% I[131]-BSA Bound
100
80
60
40
20
0
CONTROL SERUM
1:2
1:5
1:10
1:25
1:50
Serum Dilution

Fig. 40 Immunoelectrophoresis of the last elution peak against rabbit-a-EMS.

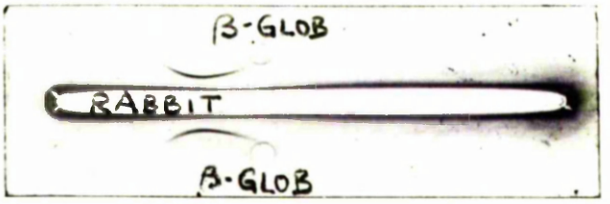
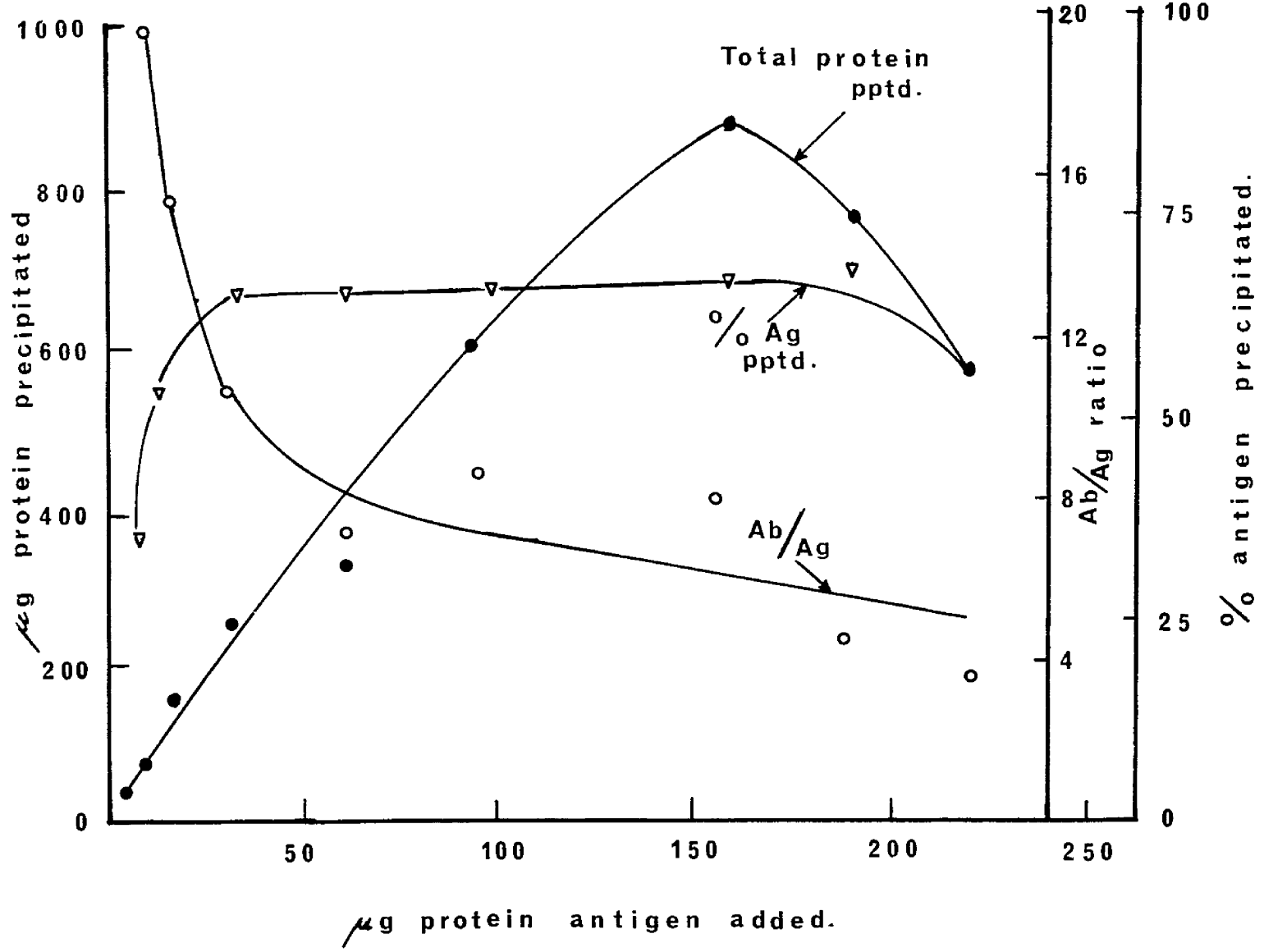

B-GLOB
RABBIT
B-GLOB

Fig. 41 Quantitative precipitin reaction between bovine β-globulin and a pool of cat antiserum.

Quantitative Precipitin Reaction between

Bovine β-globulin and Cat Antiserum (214+220+334).



THE HISTOLOGICAL RESPONSE OF THE CAT TO SOME FOREIGN PROTEINS

Introduction

Many skin diseases of man originate from sensitivity to foreign material and, broadly speaking, two types of reaction occur. An immediate allergic urticaria may result from inhalation or ingestion of or contact with substances such as feathers, horse dander and cosmetics. Other common causes of this type of reaction include penicillin, aspirin, insect-bites and injection of vaccines (Rowell, 1965). Contact dermatitis is also a common form of skin disease and is of delayed type dependent upon integrity of the lymphatic system. Sensitivity of like type is also associated with a wide variety of industrial chemicals, e.g. chromate, turpentine, varnish, resins and formalin as well as cosmetic and soap preparations (Wilkinson, 1965).

Among domestic animals, however, only a few skin diseases of allergic origin have yet been reported. One of those disorders is "Queensland Itch" which is a disease of horses caused by the development of cutaneous hypersensitivity to the bites of a species of sand-fly, Culicoides robertsi (Riek, 1954), while another example is afforded by atopic dermatitis of the dog that is associated with ragweed pollen (Patterson, et al. 1963). In general veterinary

practice there is experienced a high incidence of eczema in the cat and, although the aetiology of the condition remains essentially unknown, there is some circumstantial evidence implicative of an allergic reaction to flea-bites (Jennings, 1953) or dietary factors, such as fish (Kral, 1958).

Experimentally, skin reactions of hypersensitive nature have been extensively studied in the guinea-pig and rabbit whereby the conditions governing their induction in those species have been well established. In the rabbit, Leskowitz (1960) has produced both immediate and delayed types of hypersensitivity to defined protein antigens by the use of antigen incorporated in complete Freund's adjuvant. In the case of animals with circulating antibody, the preceding author achieved differentiation between immediate and delayed reactivity by means of cutaneous tests with specific antigen-antibody precipitates which, in contrast with antigen alone, were found not to produce Arthus-type phenomena after intradermal injection.

The ensuing work was designed to effect similar observations on the cat based upon the Leskowitz system of immunization and skin-testing.

Materials and Methods

Animals

Hybrid adult cats were used and were kept in single cages on a

standard diet of tinned meat, milk and water.

Antigens

The animals were immunized with bovine normal serum (BNS) or with commercial preparations of bovine serum albumin (BSA) or bovine gamma globulins (BGG) Fraction II, (Armour Ltd.). The same antigens were employed in cutaneous tests either as solutions in sterile 0.15M NaCl or, in the case of BGG and BNS in experiment II, as re-suspended specific precipitates of the antigens and homologous rabbit antibodies. Preparation of precipitates involved addition of BGG or BNS to rabbit anti-BNS serum in amounts known to ensure excess of antibody. After incubation at 37°C for 30 minutes followed by refrigeration overnight, precipitates were centrifuged, washed and re-suspended in cold saline solution. Samples were analyzed for content of protein by means of the biuret reaction as recommended by Kabat and Mayer (1961).

Immunization

Details of relevant procedures are given in Table 13.

Skin Tests

At intervals after immunization, 0.1 ml. of antigen or of sterile saline was inoculated intradermally into previously prepared sites on the thorax or the flank. Prior to each test, samples of blood were taken for serology and 1.0 ml. of a 2.5% solution of Evans blue was given by intravenous injection. In experiment I,

Table 13

IMMUNIZATION AND TESTING PROCEDURES

Experiment	Cat No.	Immunization Procedure	Days of Skin Tests
I	41-44	BSA in FA, 15 mg./kg. by i.m., s.c., i.d., d.p. on days 1, 6, 10, 14.	22, 29, 36, 43.
	45-46	BGG in FA, as above	22, 29, 36, 43.
	47	BNS, 1.0 ml. s.c. on days 1, 3, 6, 10.	22, 29, 36, 43.
II	61-65	BGG in FA, 15 mg./kg. by i.m., s.c., d.p. on day 1.	5, 7, 9, 11, 14.
	67-69	BNS in FA, as above.	7, 10, 14.

i.m. = intramuscular
s.c. = subcutaneous
i.d. = intradermal
d.p. = digital pad
FA = Freund's adjuvant

concentrations of 1, 30 and 100 μg. of BSA antigen-N per 0.1 ml. were employed. During the second experiment antigen solutions were made to contain 1, 10 and 100 μg. of BGG-N per 0.1 ml. or 2, 20 and 200 μg. of BNS-N per 0.1 ml., and suspensions of specific precipitates were adjusted to include 1 μg. and 10 μg. of BGG-N per 0.1 ml. or 2 μg. and 20 μg. of BNS-N per 0.1 ml. Sites of inoculation were examined frequently during the first six hours and again at 24 and 48 hours for evidence of blue coloration, oedema, erythema and induration. Reactions were classified as "early" if they occurred within four hours of injection of antigen or as "late" if they did not become manifest until after four hours. The terms, "early" and "late" were preferred to the expressions, "immediate" and "delayed", because the latter have established immunological connotations. All test antigens were also injected into non-immunized cats but yielded consistently negative results.

Histopathology

Samples of skin from areas of positive reaction were removed by means of the biopsy instrument described by Evans et al. (1957) and were fixed in picro-formol solutions for 24 hours after which they were processed and sections cut at 5 microns were stained by haemalum and eosin, periodic acid-Schiff, toluidine blue (pH = 4.0) and phosphotungstic acid-haematoxylin.

Passive Cutaneous Anaphylaxis (PCA) in the Guinea-pig and Cat

PCA reactions in albino guinea-pigs of 400 gms. weight were

stimulated by intradermal inoculation of 0.1 ml. cat antiserum followed 4-5 hours later by intracardiac injection of 0.5 ml. of 1% Evans blue. Thirty minutes were allowed for the development of non-specific blue coloration at the prepared sites whereupon 70-100 μg. of antigen were administered intracardially. In each animal, control sites were injected intradermally with saline and cat normal serum. After inoculation, all sites were observed for 45 minutes, any areas of blue coloration measured in millimetres and the animals sacrificed. Samples of skin from positive, negative and control sites were taken for histological examination and were processed as already described for feline skin. A similar procedure was adopted for PCA reactions in the cat except that in each case the antigen comprised 70 μg. BNS and 50 μg. BCG.

Results

Direct Skin Testing of Sensitized Cats

In experiment I (Table 14) there was not any response to skin tests in the instance of cats stimulated by repeated injections of BSA in Freund's adjuvant although one animal was found to possess detectable amounts of circulating antibody. Of two cats given repeated immunizing injections of BCG in Freund's adjuvant only one (No. 45) exhibited an early reaction which became apparent within 15 minutes, was manifested in local oedema and erythema but faded

in intensity and disappeared within a few hours. In both cats, subsequent tests were negative although precipitating antibody was found to be present in cat No. 45 on each of the test days. In cat No. 47 (immunized with BNS) a positive reaction was elicited at 48 hours following testing on day 29 and at 24 hours after testing on day 36 and on each occasion the locus of the test exhibited an oedematous plaque-like lesion, 2 cm. in diameter and covered by a fibrinous exudate. Precipitating antibody was detected in this animal on all four occasions of testing.

In Experiment II, for a period of up to ten days skin tests were negative in all the cats excepting No. 67 in which on the tenth day an oedematous swelling appeared within 15 minutes of the introduction of both diluted and concentrated antigens. That response had disappeared within 4 hours. Of those cats sensitized with BCG, No. 65 manifested on the eleventh day an early response to soluble antigen (Table 15), which reaction developed within 15 minutes of injection and was manifested as an oedematous swelling that, however, disappeared within 4 hours. On days 11 and 14, cats Nos. 61, 62, 63, 64 and 65 exhibited reactions which occurred at the site of injection of precipitate and were apparent as nodular, erythematous lesions 2-4 hours after injection; those changes were still present at 24 hours but had disappeared by 48 hours.

Of the three cats immunized with BNS two (Nos. 67 and 69) gave

Table 14

RESULTS OF SKIN TESTS - EXPERIMENT I

Cat No.	Immunized with	Day of Skin Test								Circulating Antibody
		22		29		36		43		
		E	L	E	L	E	L	E	L	
41-44	BSA	-	-	-	-	-	-	-	-	Present in 43 only on day 22.
45	BGG	+	-	-	-	-	-	-	-	Present on day 22, 29, 36, 43.
46	BGG	-	-	-	-	-	-	-	-	Negative
47	BNS	-	-	-	+	-	+	NT		Present on day 22, 29, 36, 43.

E = early - 0-4 hrs. after intradermal antigen
L = late - 4-24 hrs. after intradermal antigen.
NT = No test

Table 15

RESULTS OF SKIN TESTS - EXPERIMENT II

Cat No.	Immunizing antigen	Soluble				Precipitate				Circulating antibody
		Day of Test				Day of Test				
		11		14		11		14		
		E	L	E	L	E	L	E	L	
61	BGG	NT		-	-	NT		+	+	-
62	"	NT		+	-	NT		+	+	-
63	"	NT		+	-	NT		+	+	-
64	"	-	-	NT		-	+	NT		-
65	"	+	-	NT		-	+	NT		+ on days 9, 14.
67	BNS	NT		+	-	NT		+	+	+ on day 14.
68	"	NT		-	-	NT		-	+	+ on day 14.
69	"	NT		+	-	NT		+	+	+ on days 7, 14.

E = early - 0-4 hrs. after intradermal antigen
L = late - 4-24 hrs. after intradermal antigen
NT = No test

on the fourteenth day a positive reaction to soluble antigen, which manifestation was of the early type, becoming apparent within 15 minutes of injection and having vanished within 4 hours. To BNS precipitate the same two cats developed both an early and a late response on day 14 while cat No. 68 exhibited only a late response.

Passive Skin Testing of Guinea-pigs and Cats

Guinea-pigs

Prior to the injection of antigens non-specific blueness did not occur at the prepared sites nor did it develop within 45 minutes at sites injected with either normal cat serum or saline. A positive reaction was not obtained with sera from cats immunized with BSA whether, or not, those sera contained precipitating antibody. Oedematous blue patches, 20-30 mm. in diameter, were regularly obtained at sites injected with cat antisera to BGG and BNS (Fig. 42). Reactions of that type were forthcoming with sera containing precipitating antibody even after dilution to as much as 1:80 although not all such sera gave a positive response.

Cats

The only positive changes were associated with antiserum to BGG (Fig. 43). Antisera to BNS, although giving positive PCA reactions in the guinea-pig, failed to elicit any reaction.

Direct Skin Tests in Cats - Histopathology

As appreciable in skin biopsies taken 30 minutes after injection, the histological features of early reactions consisted of degeneration

of the epidermal cells shown by loss of staining affinity and by increased fluid content of the cells of the stratum germinativum. Massive dermal oedema gave rise to swelling and separation of collagen fibres, which abnormality was most apparent just below the epidermis (Fig. 44). Veins and capillaries contained large numbers of neutrophils many of which had migrated into the surrounding tissues (Fig. 45). Eosinophils were not numerous. There appeared to be a fall in the number of mast cells present and those which were to be seen showed evidence of degranulation.

In the case of the late-type reaction, sections of biopsies procured at 24 hours presented signs of epidermal degeneration consisting of loss of staining affinity and increased fluidity of the cells. The upper dermis was oedematous and hyperaemic but exhibited little cellular reaction whereas in the corium there was a massive neutrophilic infiltration (Fig. 46) which obscured most of the blood vessels but a few capillaries were seen to contain large numbers of the latter cells. Many of the neutrophils in the tissue-spaces had died and their nuclear remnants were abundant. In addition there were many macrophages together with a few eosinophils. Mast cells were present to about the same number as in normal skin but were partly degranulated. In sections stained by phosphotungstic acid-haematoxylin the large amount of fibrin found in the tissue-spaces was indicative of the damage done to the walls of blood-vessels.

In cat No. 47, the atypical response, which developed at 24 and 48 hours after injection, consisted of necrosis of the epidermis and dermis attended by intense capillaritis in the upper corium with associated neutrophilic infiltration (Fig. 47). In the deep dermis and the subcutis there was severe oedema and vasodilatation and the blood-vessels of the area were packed with neutrophils.

Passive Skin Tests in Guinea-pigs and Cats - Histology

Guinea-pigs

Guinea-pigs PCA reactions in the guinea-pig were characterized by oedema of the epidermis and dermis. Throughout the latter part of the skin, there was also dilatation of the blood-vessels and lymphatics and many of the small veins and capillaries were crammed with leucocytes which were mainly of eosinophilic and neutrophilic type. Many eosinophils were also to be found in extravascular locations. In the subcutis, massive eosinophilic accumulations were conspicuous around the blood-vessels and emigration of those cells through the walls of veins was to be observed. Mast cells were not seen in sections stained by toluidine blue.

Cats

The PCA response in the cat consisted of severe oedema of the dermis and epidermis. Although depleted in number, most of the mast cells were normal in appearance but a few showed some degree of degranulation. In the deep cutis and the subcutis the most obvious

changes were vasodilatation and a polymorphonuclear-eosinophilic invasion which was, however, less massive than that found in the guinea-pig.

Discussion

The results of the preceding experiments clearly indicate that it is possible to elicit immunological reactions to certain foreign proteins in feline skin. Thus, although consistently negative results were obtained in cats immunized with BSA, positive dermal reactions were evoked in 6 out of 7 cats sensitized with BGG and in all four cats similarly treated with BNS. Following a single dose of BGG (Cats Nos. 61-65) or BNS (Cats Nos. 67-69) in Freund's adjuvant injected by various routes, cutaneous reactions were forthcoming as early as the 11th and the 10th day, respectively, in each group and were still to be evoked on the 14th day. Repeated injections of antigen in Freund's adjuvant were less serviceable in the establishment of cutaneous hypersensitivity as determined by tests carried out during the three weeks after immunization. There was not any obvious relationship between the presence of circulating precipitating antibody and excessive susceptibility of the skin inasmuch as skin reactions failed to develop not only in Cat No. 43 possessed of antibody to BSA but also sometimes in Cats Nos. 45 and 47 that had developed circulating antibody to BGG and BNS. For that

observation, a possible explanation may lie in the presence of blocking antibody inhibitory of the combination of antigen and skin sensitizing antibody. On the other hand, positive dermal reactions were obtained from cats in which antibody was not detectable by means of agar gel techniques, as in the instance of Cats Nos. 61-64, which finding may be a reflection of the greater efficacy of local cutaneous anaphylaxis in the detection of antigen (Kabat and Mayer, 1961).

The time taken by lesions to develop after testing together with the histological features point to two main types of reaction viz. (a) local cutaneous anaphylaxis which appeared within minutes of injection of antigen and passed off within 4 hours and (b) the Arthus-type reaction. Histologically, the salient features of local cutaneous anaphylaxis were oedema of the dermis and a marked perivascular neutrophilic response combined with a fall in the number and degranulation of the mast cells. Arthus-type reactions were characterized by a neutrophilic reaction so massive that it obscured most of the blood vessels but there was not any appreciable decrease of the mast cell population. In only one cat was there observed a response which did not comply with the above descriptions but took the form of an acute necrotizing dermatitis that resembled cases of allergic vasculitis occasionally encountered in man (J. D. Milne, personal communication).

Both local cutaneous anaphylaxis and Arthus-type reactions in

the cat are broadly similar to those experienced in other species though, in the case of the former condition, only a few eosinophils were to be noted compared with the marked eosinophilic immigration which occurs in the like response in the guinea-pig (Fisher and Cooke, 1957). While the pharmacological mediator of feline cutaneous anaphylaxis is still unknown, the degranulation and depletion of mast cells tend to suggest that release of histamine may be involved.

As the guinea-pig is the most suitable animal in which to study cutaneous sensitivity, it is fortunate that the cat is a Forssman-positive species thus eliminating any risk of non-specific reactions of that type happening in passive transfer studies. While passive transfer of sensitivity to BCG and BMS was regularly obtained in the guinea-pig by use of cat antisera, that technique was less successful when applied to the cat, in which species a positive response was forthcoming only with antisera to BCG. The reason for that disparity remains obscure but may be ascribable to qualitative differences in the fixation of cat antibody to the skin of the two species. In both species of animal, the reaction took the form of a local cutaneous hypersensitivity and varied only in respect of the considerable accumulation of eosinophils and the greater severity of the lesions in the guinea-pig. Having regard to the relatively greater number of mast cells to be encountered in feline skin, it is somewhat surprising that the PCA reaction was so mild and, clearly, further work

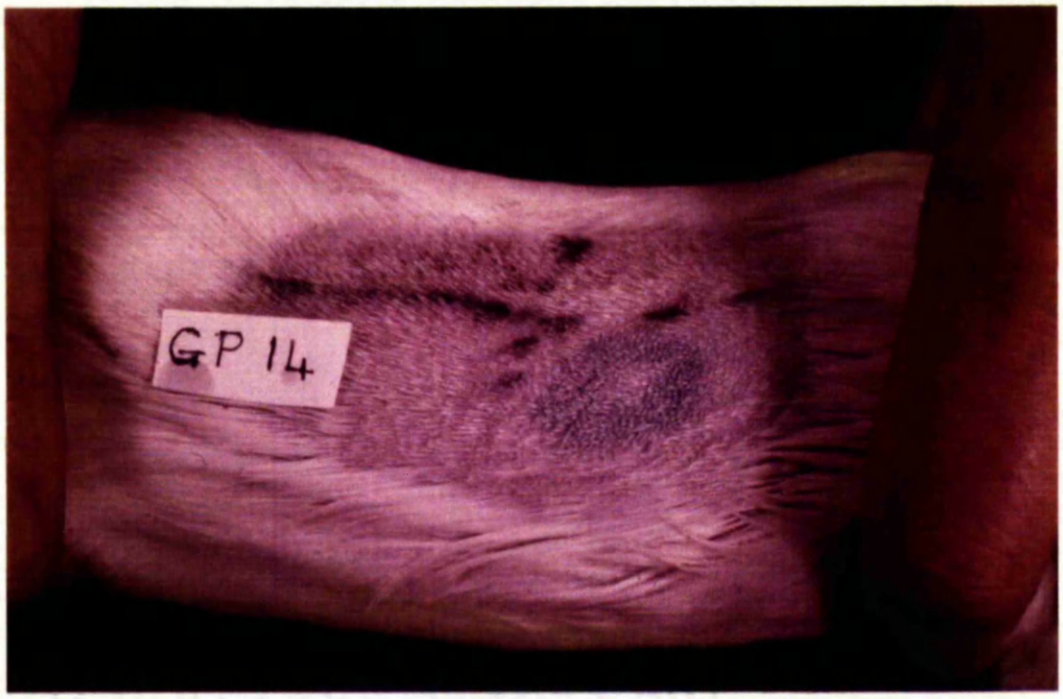
is required to determine the degree to which the mast cell is involved in direct and passive skin responses in the cat. Of singular interest is the fact that in feline eczema both the mast cell population and histamine content of the skin show a four-fold increase.

Despite the use of sensitizing procedures known to produce delayed hypersensitivity in rabbits and guinea-pigs, that type of reaction was not elicited in any of the cats. The reason for such failure was not determined but may reside in a species difference inasmuch as the tuberculin reaction, a typical delayed response, is difficult to elicit in cats suffering from tuberculosis (Francis, 1958).

In conclusion, the work has established that cat skin may afford a site for the production of immunological reactions to foreign proteins and associated experimentally induced lesions. Through further study of the pathogenesis of experimental cutaneous hypersensitivity in the cat, it is hoped to throw some light on the aetiology and pathogenesis of feline eczema.

Fig. 42 PCA reaction in the guinea-pig.

Fig. 43 PCA reaction in the cat.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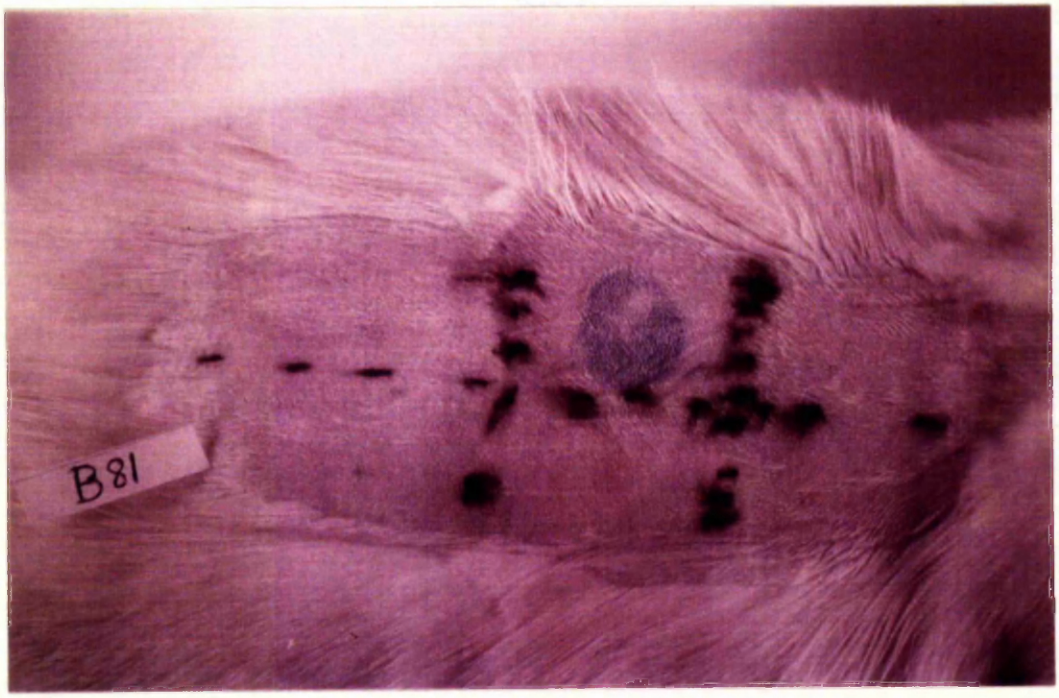
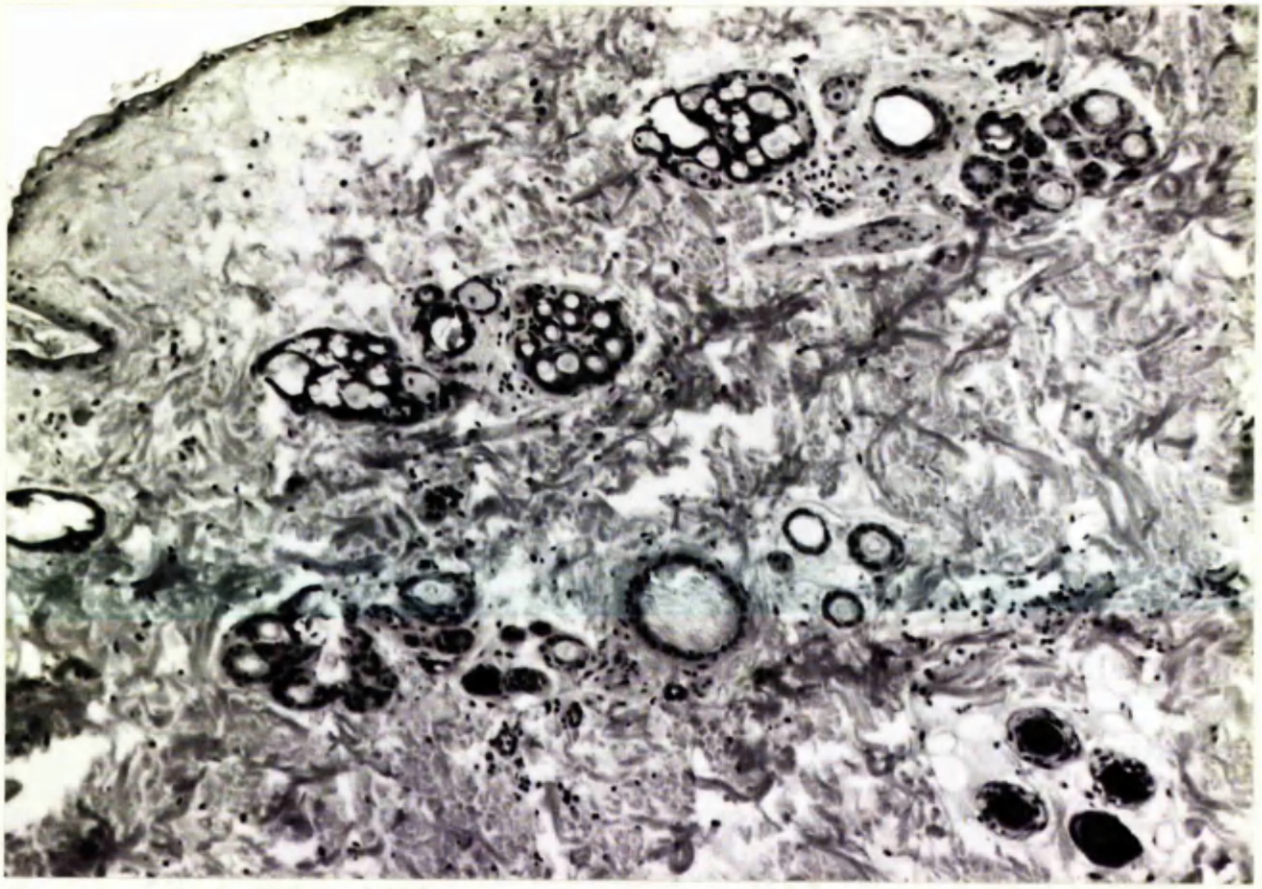

B81

Fig. 44 Histology of PCA reaction in the cat. Note dermal oedema and separation of collagen fibres. Haemalum and eosin, x60.

Fig. 45 Histology of PCA reaction in cat. Note perivascular neutrophil response. Haemalum and eosin, x450.



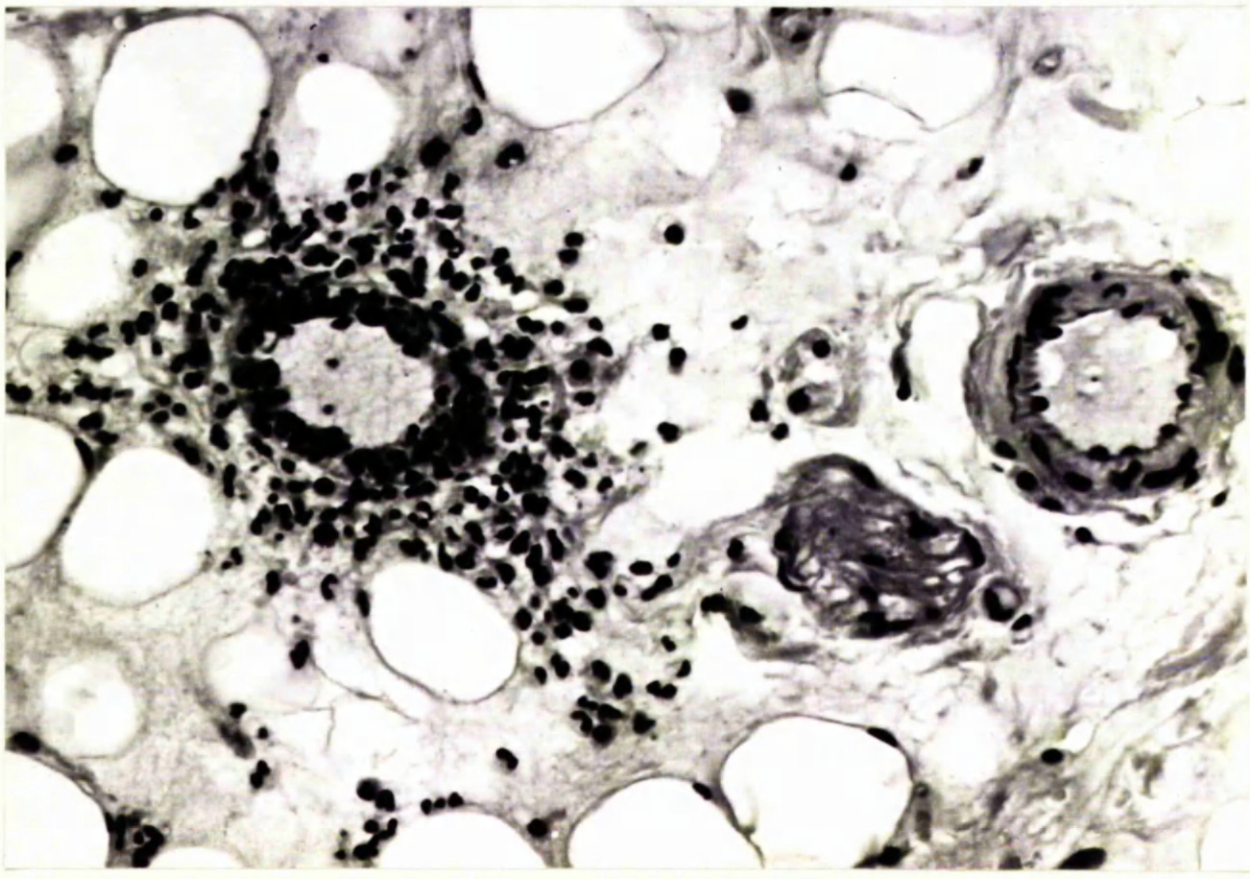
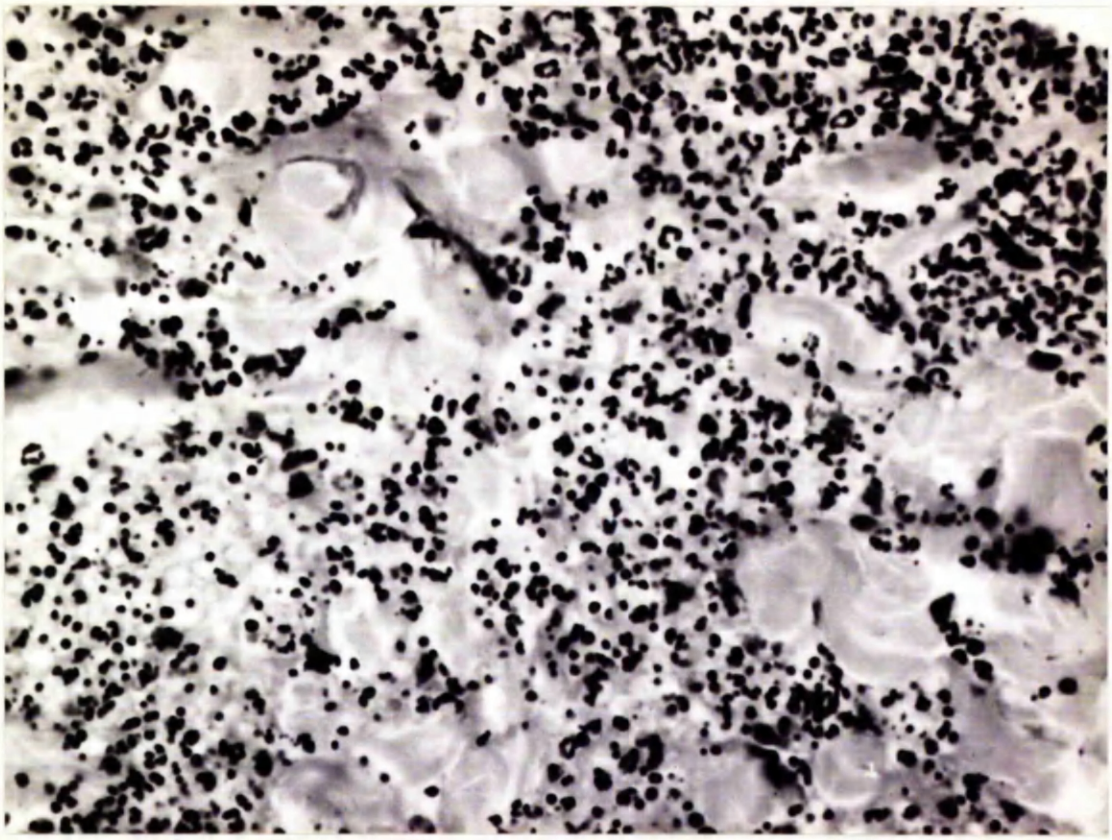
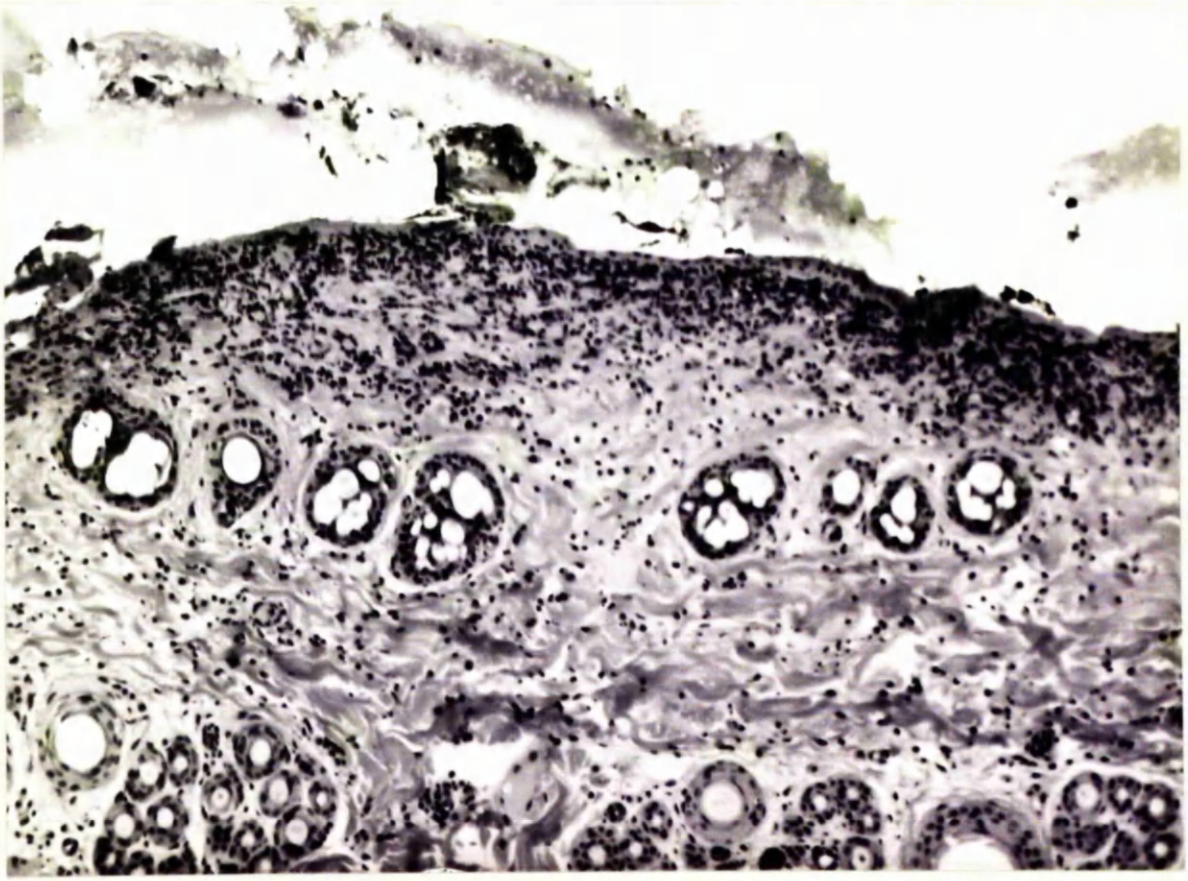


Fig. 46 Arthus-type reaction in cat with intense neutrophil response. Haemalum and eosin, x450.

Fig. 47 Acute necrotizing dermatitis in cat No. 47. Note epidermal necrosis and marked subepidermal neutrophil response. Haemalum and eosin, x110.





ANAPHYLAXIS IN THE CAT

Introduction

Although anaphylaxis has been established in many species of animals since the original experiments of Portier and Richet (1902), hitherto there has not been any record of the induction of that condition in the cat. Indeed, according to Wilson and Miles (1964), the cat is peculiarly resistent to anaphylactogenesis and in the only paper (Akcasu, 1963) on feline immune responses to foreign proteins discovered in the available literature cats are declared to be wholly insensitive to such stimuli. The ensuing section furnishes a record of the occurrence of anaphylaxis during a series of experiments in which the serological and dermal reactions of certain cats to parenterally administered foreign proteins were observed.

Materials and Methods

Immunological Procedures

To determine the susceptibility of the cat to heterologous serum, a 4 kg. male, No. B47, was made to receive four sensitizing injections of 1 ml. of bovine normal serum (BNS) that was given subcutaneously on days 1, 3, 6 and 10 of the experiment. Blood samples were taken on days 22 and 29 and the resultant sera were tested for precipitating antibodies by means of double diffusion in agar gel as well as

by immunoelectrophoresis. A 'shocking' dose of 5 ml. of BNS was given intravenously on the 29th day to Cat No. B47 and also to a non-sensitized control cat.

A second case of anaphylaxis, accidentally induced, was observed in a 3.1 kg. male cat (No. B66) that had been immunized with bovine gamma globulins (BGG) (fraction II from bovine plasma, Armour Pharmaceutical Co., Ltd.). The immunological history of this animal was as follows: On days 1, 6, 10 and 14 an amount of antigen equivalent to 15 mg./kg. suspended in complete Freund's adjuvant (Difco) was injected by four routes at eight sites as shown in Fig. 48 That procedure resulted in the production of precipitating antibody to one of the three components of BGG (Fig. 49). Approximately 20 mg. of ^{131}I-trace labelled BGG was given intravenously on the 63rd day during an antigen elimination experiment and, thereafter, the cat was rested until day 320 when 15 mg./kg. of BGG in Freund's adjuvant was injected via the paths already described. Once more precipitating antibody was elaborated. The final, and fatal, injection of 20 mg. of BGG in 0.15M NaCl was administered on day 441.

Histological Observations

Portions of tissue taken from all organs of the body were fixed in corrosive-formol solution. After embedding in paraffin-wax, sections were cut at 5 microns and stained by haematoxylin and eosin, by periodic acid-Schiff (Lillie, 1950) and by toluidine blue as well

as by aldehyde-fuchsin for mast cells (Gomori, 1950).

Results

Clinical

Within one minute of the intravenous injection of 5 ml. of BNS Cat No. B47 exhibited a marked response, the main features of which were vigorous scratching of the head, distressed respiration, salivation, vomiting, incoordination and general collapse. The animal remained in a state of acute depression for 15 minutes and then gradually recovered. Twelve hours later it appeared quite normal. In contrast with these findings, the non-sensitized control cat did not manifest any signs of disease either immediately after injection or during the following 5 hours.

In the other observed case of anaphylaxis (Cat No. B66) indications of illness were again exhibited within one minute of intravenous injection of antigen but were more serious. The animal passed into a state of acute collapse, respiration was distressed and copious quantities of blood-stained frothy fluid escaped from the mouth and nostrils. Three minutes later the animal became comatose and died. Although formal controls did not exist for this animal, it may be emphasized that primary intravenous injection of BCG into normal cats has never been found to give rise to any clinical disorder.

Post-mortem Findings

All the superficial lymph-nodes of Cat No. B66 were enlarged as might be expected having regard to the number of subcutaneous and intramuscular injections of antigen that had been administered to the animal. In the thorax there was present approximately 5 ml. of sanguineous fluid. The anterior portions of the left and right apical lobes of the lungs were greatly enlarged and pale in appearance as a result of severe emphysema, while the cardiac and diaphragmatic lobes were markedly haemorrhagic (Fig. 50). There was severe pulmonary oedema and the trachea and major bronchi were filled with blood-stained fluid similar to that which exuded from the cut surface of the lung.

The liver was of dark green colour resembling that indicative of haemochromatosis. The stomach contained 5-7 ml. of blood-stained fluid (probably, swallowed just before death), but was otherwise normal in appearance. Significant lesions were not noticeable in the other organs of the body.

Histological examination of the lungs revealed massive haemorrhage into the alveoli. Severe emphysema was observable particularly in the apical lobes and to a lesser degree elsewhere in the pulmonary tissue. In those alveoli and bronchioles where haemorrhage was less conspicuous there was marked accumulation of oedematous fluid. The arteries and arterioles were packed with leucocytes, mainly of

neutrophilic type (Fig. 51), a finding that has been described in anaphylaxis of other animals (Gladstone, 1962) and due to which there is a concomitant peripheral leucopenia. Sections stained by toluidine blue and by aldehyde-fuchsin proved negative for mast cells. There was some evidence of broncho-constriction in that the lumina of the bronchioles were less patent than normally.

Discussion

That the phenomena described above pertain to anaphylaxis is indicated by the rapid onset of clinical manifestation relative to injection of antigen as well as by the similarity of those signs and of the post-mortem findings to those observable in other species, notably the guinea-pig. Further evidence that the reaction has an immunological basis lies in the lack of response of non-sensitized cats to the intravenous injection of BNS and BCG.

A condition closely resembling anaphylaxis was described by Brodie (1900) in respect of cats that had been given a single intravenous injection of heterologous or homologous serum. The phenomenon was analysed by Gilding and Nutt (1944) who concluded that the observed effects of decreased heart-rate, fall of blood pressure, altered respiration and increased peristalsis were due to a vagal reflex and were largely abolishable by exercise of vagotomy or by administration of atropine. In our experience, primary intra-

venous injection of 2-5 ml. of BNS has been without clinical effect in 5 out of 6 cats tested and only mild transient depression was to be observed in the case of the solitary affected animal. Furthermore, a single intravenous injection of 20 mg. of EGG did not adversely affect 7 cats tested.

Both the gross anatomical and histopathological findings strongly suggest that, in the cat, the lung is the organ of the body most susceptible to anaphylactic shock. The changes observed, namely, haemorrhage, oedema, emphysema and leucocytosis, closely correspond with those described in the guinea-pig (Wilson and Miles, 1964). Although feline pulmonary tissue is normally rich in mast cells (Riley and West, 1953), the latter units were totally absent from the lung of the affected animal, which may signify that release of histamine is involved in feline anaphylaxis. Without further study, however, it is impossible to define the principal pharmacological mediator as cat lung is some 200 times more sensitive to serotonin than it is to histamine (Austen and Humphrey, 1963).

Considerable further investigation is needed before a proper comparative assessment of the induction and pathogenesis of anaphylaxis in the cat becomes possible. Inevitably, passive transfer studies must be undertaken and in this respect it is noteworthy that the feasibility of passive cutaneous anaphylaxis in the guinea-pig with cat antiserum to human C-reactive protein has been demonstrated by Gotschlich and Stetson (1960).

Fig. 48 Sites of injection of bovine gamma globulin in cat B66.

Antigen injection sites

Ag dose level : 15 mg/kg

Ag dose volume : 1·0 ml.

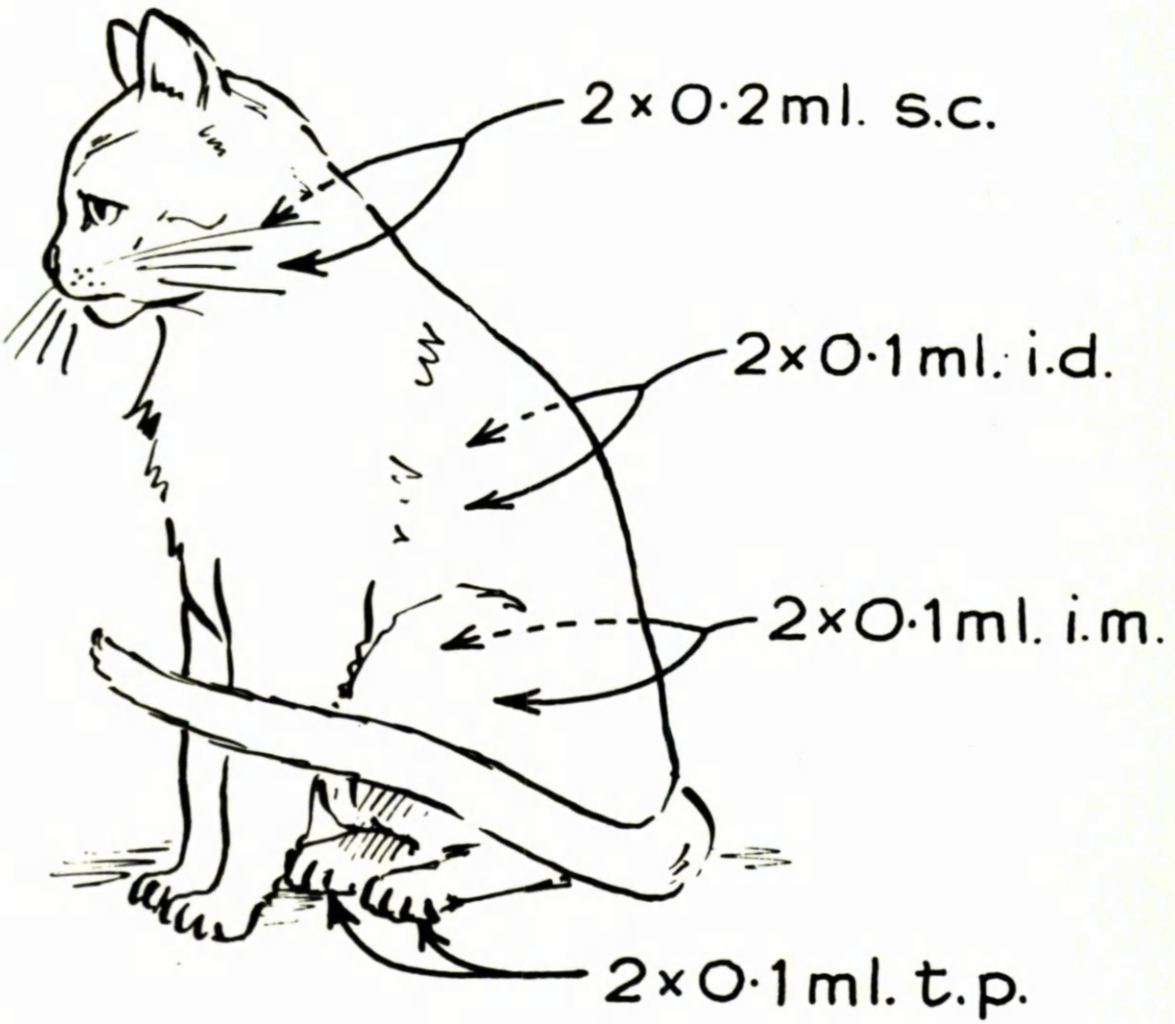
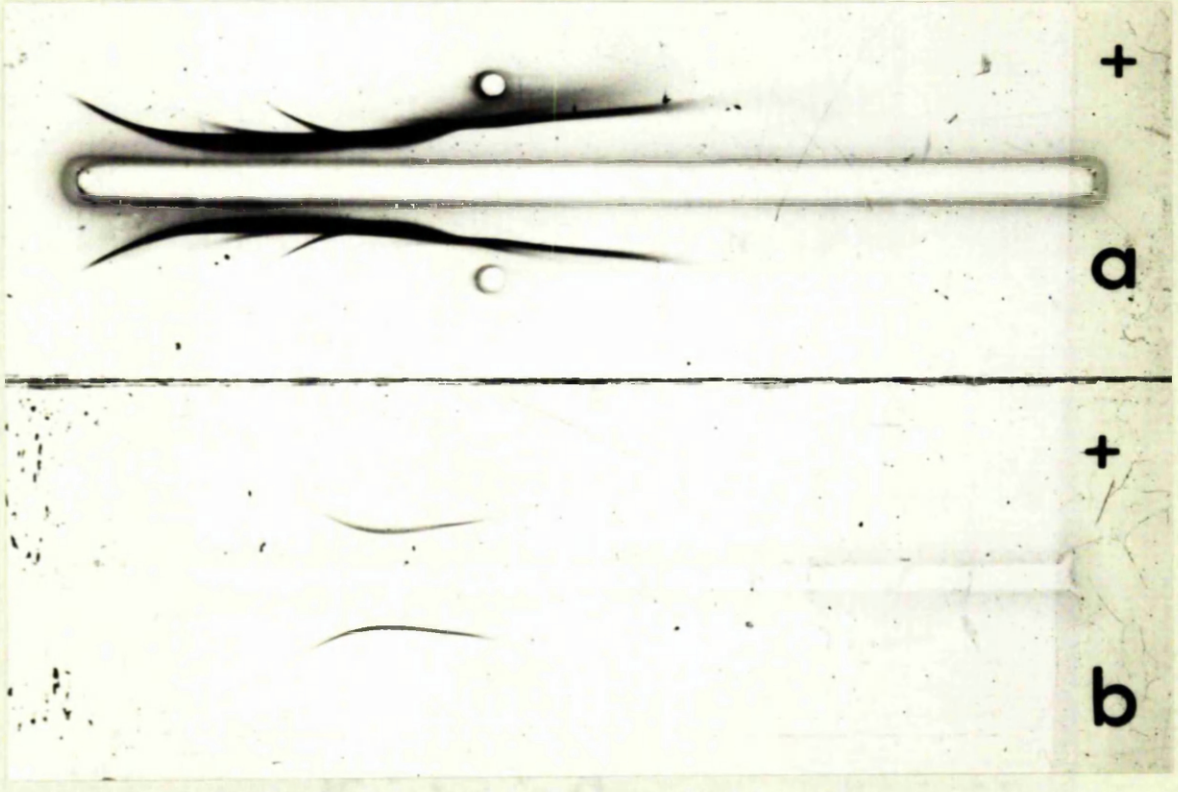


Fig 49 Immunoelectrophoretic analysis of BCG showing precipitation of three components by rabbit antiserum (a) and of one component by cat antiserum (b).


+
a
+
b

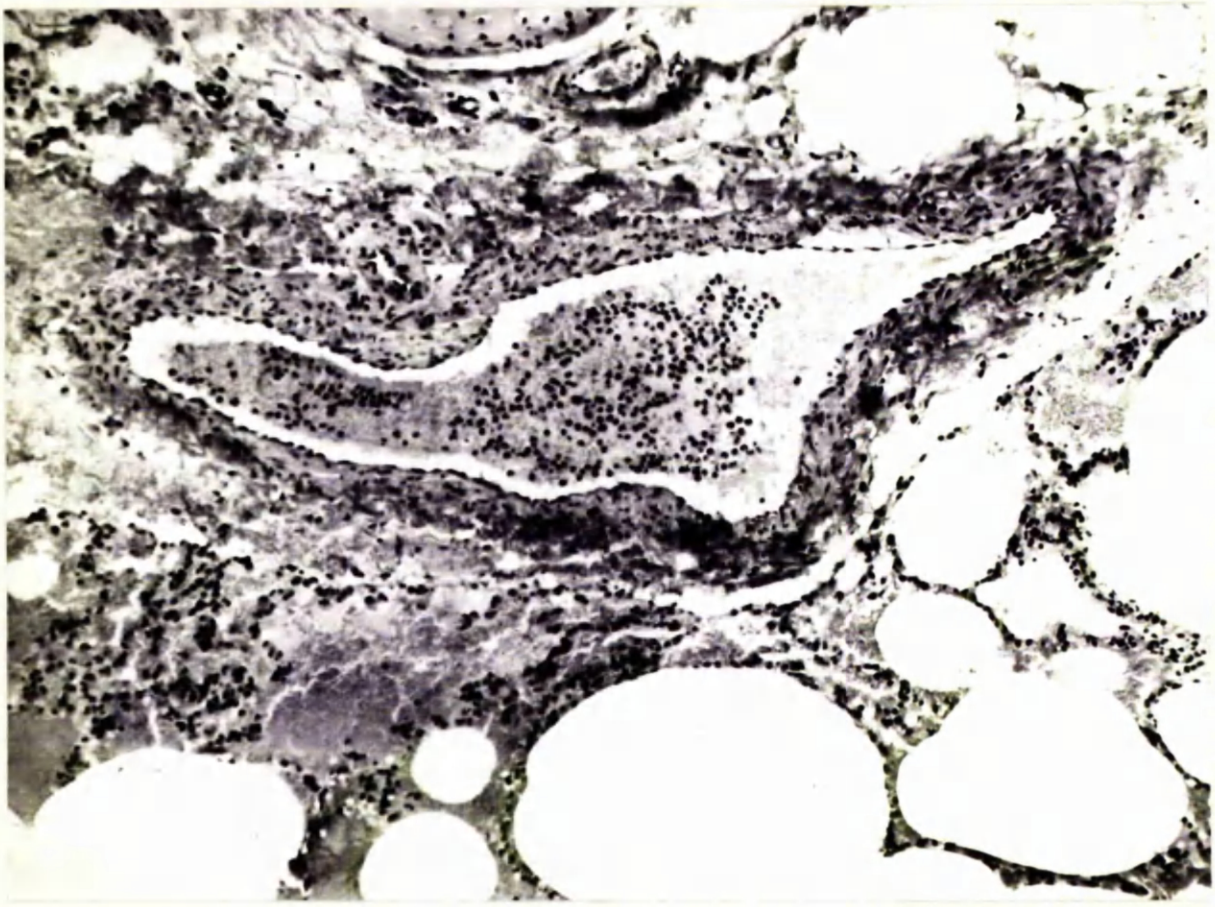


Fig. 50 Cat lung showing emphysema, haemorrhage and oedema as a result of anaphylactic shock.

Fig. 51 Cat lung in anaphylaxis. Accumulation of leucocytes in artery. Emphysema and haemorrhage also present. Haemalum and eosin, x110.

SUMMARY AND CONCLUSIONS

Structure of Feline Skin

The results of this work serve to show that while the skin of the cat generally resembles that of other mammals, it is possessed of its own peculiar properties. One of the latter is eruption of the guard hair through a single follicular opening on to the surface of the epidermis. In that particular respect, the results obtained differ from those of Creed (1958) and of Strickland and Calhoun (1963) as well as from the description of the emergence of canine hair given by Lovell and Getty (1957). The distribution and size of the sebaceous glands conform with published descriptions but disagree with the findings of Mathis (1935) in that another secretory system was not to be found. The presence of phospholipid in feline skin as described by Montagna (1962) was not confirmed, which outcome may have been due to the more specific technique employed in this study since Dunnigan (1964) has shown that the acid-haematin method stains hydrophobic lipid even after pyridine extraction and is not specific for phospholipid. In the present study, although control sections of human atherosclerosis proved consistently phospholipid-positive, feline sebaceous glands gave a negative result when stained by the technique of Menschik (1953).

The morphology of the apocrine glands was found to be similar to

that described by Strickland and Calhoun (1963). The need for further research on the structure and function of these organs is shown by the finding of Munger (1965) that the apocrine glands of both the interdigital skin and the ear of the cat have an eccrine form of secretion. Although feline sudoriferous glands respond to stimulation by heat they appear to be too small to play a significant part in the regulation of body temperature. The quantitative results pertaining to histamine and mast cells are in agreement with those of Riley (1959). The mast cells of the cat were found to differ from those of the dog in that they were PAS-positive as well as metachromatic when stained with toluidine blue. The function of the mast cell in healthy tissues is still unknown apart from the fact that it forms a reservoir of readily available histamine and heparin. With regard to the latter substance, a study of the heparin content of feline mast cells would be of peculiar interest since only small amounts of the material are to be found in mastocytomata of the cat as compared with similar tumours of the dog and cow (Riley, 1959).

Response to Corticosteroids

The parenteral administration of corticosteroids produced a marked effect on cat skin which appeared to be directly related to the dose employed. The atrophy of the epidermis and of the pilosebaceous units which occurred may be due, as in the rat, to a depression of mitotic activity (Ghadially and Green, 1957).

Further work is also required in order to ascertain whether the cat skin becomes refractory to prolonged corticosteroid therapy as apparently happens in the mouse (Morill and Herman, 1961). Large amounts of betamethasone were required to produce degranulation of the mast cells and a fall in the histamine content of cat skin. The mechanism of these changes remains obscure and demands further investigation.

Feline Eczema

The histopathological changes in the skin in miliary eczema are typical of those of an acute or a chronic dermatitis. However, as compared with the human disease, vesiculation was not a feature of the condition encountered in this investigation. The great increase in the mast cell population and histamine content of the skin may be related to the subepidermal fibrosis which was so prominent in chronic eczema of the cat. Evidence for this theory may be forthcoming from studies of the rate of healing of feline eczematous lesions in which the mast cells have been degranulated by histamine liberators.

Chronic cases of eczema were characterized by marked acanthosis and parakeratosis. The precise causation of these changes is not yet known but may be connected with increased rate of epidermal mitosis associated with more rapid migration of affected cells from the basal layer to the horny surface whereby insufficient time is available for complete keratinization. An investigation of psoriasis

in man (Van Scott and Ekel, 1963) has shown that enlargement of dermal papillae leads to lengthening of the basal layer with resultant increase in the number of germinative cells per unit length of the superficial epidermis. In addition, mitotic potential is imparted to a basal zone approximately three cells thick so that a greater population of germinative cells passes to the surface in three to four days instead of the twenty-seven days which is the approximate time taken by normal epidermal cells to undergo keratinization.

The aetiology of feline eczema continues to be problematical and many of the animals examined did not suffer from any obvious infestation by fleas or other ectoparasites. Again, correlation between the incidence of the condition and either sex or diet was not establishable. However the work in the dog on ragweed allergy (Patterson et al., 1963), that on the response of guinea-pigs to flea antigens (Hudson, et al., 1960) and investigations on "Queensland itch" in the horse (Riek, 1953) have confirmed that allergic reactions occur in domestic animals and, probably, eczema of the cat is a manifestation of this type of response.

Although betamethasone therapy has been shown to be of value in the treatment of eczema in the cat, the tendency for the condition to recur after such treatment indicates that its main action is towards suppression of the inflammatory response rather than removal of the cause. The failure to produce a significant decrease in the histamine content and the mast cell population of eczematous skin may be attribut-

able to the small amounts of betamethasone administered since in the case of normal skin a very high dosage was required to produce degranulation of the mast cells and depletion of histamine.

Immunology

In the investigation into the serological response to foreign proteins, the cat was shown to form precipitating antibody to purified protein antigens, such as BSA and EGG, as well as to heterologous sera, such as RNS and RNS. The reaction to BSA was but feeble and that to EGG, although stronger, proved to be referable only to a minor β-globulin component of the antigen. Cats immunized with bovine or rabbit serum were rendered fairly sensitive to the homologous α- and β-globulins but precipitins were not produced to either albumin or γ-globulin. Cat anti-bovine serum exhibited only a weak binding capacity for BSA. The general characteristics of the quantitative precipitin reaction between bovine β-globulin and specific cat antiserum were similar to previously described systems but cat antiserum apparently contains some non-precipitating antibody. The half-life of I^{131}-BSA and I^{131}-EGG in the cat were found to be 3.8 and 2.8 days, respectively, and, as a rule, accelerated immune clearance was to be observed only in previously sensitized cats. Further work in this field is required to investigate more fully the precipitin response of the cat to a wider range of antigens and to study the properties and behaviour of cat antibody before a proper assessment of the

immunological capacity of the species may be made.

In respect of gross and histological features, cutaneous hypersensitivity to bovine serum proteins took the form of either local cutaneous anaphylaxis or an Arthus-type reaction and the most convincing responses followed sensitization with BNS and BGG. Negative results were constantly obtained with BSA. In the guinea-pig, PCA reactions were demonstrated by use of cat antisera to BNS and BGG but in normal cats only with the agency of antiserum to BGG was such sensitivity established. The feline reaction was less intense than that of the guinea-pig and involved degranulation of mast cells but did not show the marked eosinophilic response so characteristic of the cavy.

The failure to produce delayed type hypersensitivity in the cat by the injection of purified protein preparations was disappointing and emphasizes the need for further apposite work embracing both pure proteins and more complex antigens, e.g. extracts of fleas and of fish, as well as chemicals, such as dinitrochlorbenzene which has been shown to produce contact hypersensitivity.

In the instance of two cats, administration of bovine serum evoked anaphylactic shock, the main clinical features of which were acute respiratory embarrassment and collapse. Pulmonary emphysema, haemorrhage and oedema were the principal post-mortem findings while histological examination showed the arteries to be packed with leucocytes

and the pulmonary tissue to be devoid of mast cells, which observations suggest that the lung is the site of anaphylactic crisis in the cat.

Finally, it may be asserted that the skin of the cat generally resembles that of man and other domestic animals both in structure and its response to injury. Much work, however, remains to be done to elucidate (a) the role of the mast cell in both normal and eczematous skin and (b) the susceptibility of that tissue to a wide range of antigens before any definitive statement may be made as to whether, or not, feline eczema ranks as an immunological response.

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ADDENDUM

The following publications have arisen from this work:

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