ON THE EOSINOPHIL LEUCOCYTE

by

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C O N T E N T S

THE HISTORY OF THE EOSINOPHIL CELL

Origin and Site of Formation

It is over a hundred years since Wharton Jones (1846) first described in fresh blood the "coarse granular corpuscle" and commented upon its amoeboid nature. Although his observations were fairly soon confirmed (Förster, 1861; Schultze, 1865), it was not until Ehrlich in 1879 described his staining technique that the first step was taken to unravel its mysteries. Ehrlich demonstrated the great affinity of these coarse granules for eosin and later (1898) came to regard the eosinophil leucocyte as having an entity distinct from the neutrophil leucocyte, and that it was formed in the bone marrow from a non-granular cell which acquired those granules as it matured. Many years were to elapse before this view became universally accepted.

In 1892, Müller observed mitosis in an eosinophil cell of mammalian blood, thus establishing that homoplastic regeneration could occur. In the same year Van der Stricht, confirming this, further asserted that the eosinophil did not develop from a non-granular cell and suggested that it was a transformed neutrophil. This was supported by Thayer (1897), Brown (1897), Klein (1899) and others, but general opinion at the time was undecided (Muir, 1898).

That the granules represented exogenous material which has been phagocytosed was a popular view, and there was no lack of suggestions as to the nature of this material. Tettenhamer (1893) thought it might

be derived from the degenerating nuclei of maturing red cells. Sacharoff (1895) regarded the granules as nucleoli discarded from haemocytoblasts and phagocytosed, while Thayer suggested that the granules were toxic substances absorbed from muscle infested by Trichina. The theory which had most adherents, however, was that put forward by Weidenreich.

Weidenreich (1901), while studying haemolymph nodes in the sheep and rat, remarked on the presence there of eosinophils, which incidentally he regarded as being quite different from the eosinophils of the blood, associated with degenerating and fragmented erythrocytes. As on closer examination he found it difficult to distinguish between the eosin staining red cell fragments and the granules of the eosinophil cells, he concluded that they were identical and suggested that the tissue eosinophil was formed from a non-granular cell by the phagocytosis of fragments of, or even of whole red blood cells. Sabin (1905) agreed with Weidenreich that the differentiation of red cell fragments and eosinophil granules was difficult, while on the other hand Ascoli (1904) felt that he could always distinguish one from the other. In an attempt to prove Weidenreich's theory, Stschasnyi (1905) injected guinea pigs with the red cells of sheep, rats and other animals. His findings were much the same as Weidenreich's, and he confessed that he could not produce any intermediate stages. Undaunted, Weidenreich (1910) performed a set of similar experiments and claimed to have demonstrated the actual phagocytosis of erythrocyte material with formation of tissue

eosinophils. This view held much sway for a time, but by 1914, work done on the eosinophil had reached such proportions that Schwarz in the same year quoted 2,693 pertinent references!

Meantime, a search was being made for the site of origin of the eosinophils of the blood. While Ehrlich, Muir (1901) and Maximow (1906) considered that under normal circumstances the bone marrow was the only place where eosinophils were formed, Dominici (1909) considered that there were in addition, extra-medullary foci of formation. Browning (1905) remarked upon the formation of eosinophils in the foetal thymus but although no actual extra-medullary formation was reported in adult tissues, numerous descriptions of localised eosinophilia were published. Teichmüller (1898) stressed the abundance of eosinophils in the lungs; Dudgeon (1905) found them in the adult thymus; Downie (1911) discovered "a gold mine for eosinophils" in the lung of Amplyopia, but eventually in 1925, Doan, Cunningham and Sabin, in their supra-vital studies on the blood forming organs of the pigeon and the rabbit, showed that all blood cells were derived from the reticulo-endothelial system. Biggart (1932) and Turnbull (1936) considered that after birth, haemopoiesis was confined to the bone marrow under normal circumstances. The work of Gilmour (1941) on embryonic and foetal tissues completed the picture of blood formation as it is known to-day.

Nature of the Granules

Perhaps it has been the bright red colour which Ehrlich gave to the eosinophil granules which has attracted the haematologist, but there certainly has been no lack of work done, or speculation upon their nature and function.

Sherrington (1894) attempted to demonstrate iron in the granules and although he was unsuccessful he did find that the granules contained a trace of phosphorus. Barker (1894) succeeded where Sherrington failed, and by a test for "masked" iron demonstrated its presence in the granules. Whereas some workers regarded the granules as being absorbed toxic material (Thayer, Brown), others suggested that they might have some nutritional significance. Habershon (1905) thought the granules contained glycogen or glycogen-like material; Rous (1908) thought they were of a protein nature; and Berg actually demonstrated the presence of tyrosine in them.

Cesaris-Demel (1909) stated that occasional leucocytes contained a few lipoid granules and regarded these as being phagocytic in origin, or as possibly representing cytoplasmic degeneration. Savini (1924) however, found lipoid granules to be much more common, so much so that he considered lipoid to be a constant physiological constituent of the granular leucocyte. This was confirmed by Bacsich (1935) who, using Sudan III stain, demonstrated the lipoid nature of leucocytic granules. After Lison (1934) introduced Sudan Black stain, Sheehan (1939) used it

with Romanowsky stains, and succeeded in staining leucocytic granules, though as Discombe (1946) pointed out, the localisation of the dye in the granules might well be brought about by the presence of an oxidase.

Meantime, Boyd (1935) brought forth the interesting observation that whenever there was increased activity of the granular cells as in the leucocytoses of infections, there was an increase in the phospholipid content of the granular cells. In cases of infection, where the phospholipid content was low, the patient fared badly. Boyd suggested that the level of phospholipid content of the granular cells might be a useful index of their activity. Code (1937) devised a method of estimating histamine in the blood physiologically and set out (1937a) to find which constituents of blood contained most histamine. He decided that probably most histamine was contained by eosinophil or basophil cells.

Thorell (1944) demonstrated desoxyribonucleic acid in leucocyte granules and this was confirmed by Gardikas and Israels (1948) and Turchini and Khau van Kien (1949).

Recently (1950), Bloom and Wislocki, using a Sudan Black staining method, showed that leucocytic granules do contain phospholipids and that in particular, it would appear that eosinophil granules have a coating of phospholipid, the centre containing protein and more lipid material. This would seem to agree with most observations hitherto made on the nature of the eosinophil granules.

Function of the Eosinophil

One of the earliest speculations on the function of the eosinophil was made by Heidenhain in 1888. In a study of the intestine of animals, he noticed that there was a reduction in the number of eosinophils in the mucosa when the animals were starved. The suggestion was offered that the eosinophil might be of nutritional significance. Tanszk (1896) examined the blood of "Succi - the fasting man" and noted that during his fasts there was a fall in the level of circulating eosinophils. Studies similar to Heidenhain's were made by Opie (1904) who, besides confirming Heidenhain's work, remarked that when the animals resumed feeding and their body weight began to rise, the number of eosinophils in the mucosa was reduced. Four years later, Rous, making differential cell counts of the lymph in the thoracic duct of dogs, found that the eosinophil was a common constituent of lymph, and that when food was withheld from the animal the proportion of eosinophils fell. Conversely, when feeding was resumed, the proportion of eosinophils rose, especially after a meal rich in protein rather than after one rich in carbohydrate. These findings, paralleling those of Heidenhain and Opie, led Rous to conclude that the intestinal mucosa was, as far as the lymph was concerned, the source of eosinophils, and that the granules were probably protein material. This association of eosinophil and protein has since been a common observation.

It was known early on that the eosinophil leucocyte has phagocytic

powers. This was demonstrated by Mesnil (1895) who showed how it could digest bacteria, and further, that it exhibited a positive chemotaxis. This has been amply confirmed by Weinberg and Seguin (1914); McDonald and Shaw (1922) and by Ingraham and Wartman (1939). In 1915, Weinberg and Seguin incubated an eosinophil-rich exudate with hydatid fluid at 37°C and found that after 1 hour the eosinophils had lost their phagocytic power and the hydatid fluid its toxicity. Their suggestion that the eosinophil had a protective function has been offered time and again. Chauffard and Boidin thought so in 1907 and in 1932, Biggart, commenting on the constant presence of eosinophils in tissues in which there was foreign protein, came to the same conclusion, as also did Campbell, Drennan and Rettie in 1935.

When Code showed that eosinophils were rich in histamine, a new interest in the part played by this cell in hypersensitivity and related phenomena was aroused. There had been a long association of eosinophilia with allergic conditions, and as early as 1912, animals surviving anaphylactic shock had been observed to have an eosinophilia (Schlecht and Schwenker). Recently Godlowski (1948) carried out an interesting experiment. He produced an eosinophil-rich exudate in the peritoneal cavity of a guinea pig by repeated injections of horse serum. When he poured some exudate over a guinea pig uterus which was already sensitised to the same horse serum, he produced an anaphylactic reaction. This could only be done if a common antigen were used to produce the exudate and to sensitise the uterus. Godlowski considered that some

anaphylactoid property had been transferred by the eosinophil. Samter (1949) could prevent anaphylaxis in sensitised guinea pigs by administering anti-histamine drugs before the shocking dose, but he could not prevent the accompanying eosinophilia. Samter was impressed by the frequent association in his animals of eosinophilia of the lung, and a high level of eosinophils in the blood. He felt that this might be significant. It would appear that some part is played by the eosinophil in antigen-antibody reactions, and Dalton (1949) suggested that the eosinophil actually took part in it, releasing histamine into the tissues as the antigen was neutralised. Studying the eosinophil on a warm stage, under the phase-contrast microscope, Squires (1951) has commented on the interesting behaviour of the granules. During the slow amoeboid movement of the cell, the granules always precede the nucleus and when he introduced small particles of protein into the path of the cell, Squires found that the granules became actively motile within the cytoplasm, as if excited by the presence of protein. Whatever the significance of these movements, there seems little doubt that the eosinophil in some way deals with toxic, probably protein material which may or may not be associated with immunological reactions. More than this we cannot say.

EOSINOPHILIA

Clinical Eosinophilia

Any rise in the level of circulating eosinophils above 4 per cent of the total white cell count (Kennan, Shipp and Hetherington, 1937), or an absolute level of 240 cells per cubic mm. (Discombe, 1946a), is usually regarded as constituting an eosinophilia. This is associated regularly with some clinical conditions and irregularly with many others. In a monograph on the subject, Pessoa and Meira (1935) assessed the number of pathological states associated with eosinophilia of the blood to be 56, and no doubt it stands at a higher figure to-day.

The association of eosinophilia with parasitic diseases began with the observations of Brown and Thayer in trichinosis and in a short time, as reports came forward, this became one of the most constant associations of eosinophilia. Taliaferro reviewed in 1940 the immunology of infection with parasitic worms. He ascribed to the eosinophil only a minor role in the body's reaction to the parasite, considering that the elaboration of antibodies was of greater importance, but although these antibodies are quite complex (Olivier-Gonzalez, 1943), the eosinophilia is sometimes a most striking feature of such cases. Kirk (1942) reported that the proportion of eosinophil leucocytes in the blood may reach 88 per cent. In 1945, Lavier correlated many of the clinical and immunological features of helminthiasis.

Ehrlich knew in 1898 that asthmatics and sufferers from pemphigus

sometimes exhibited eosinophilia and it was frequently observed in many other allergic and skin conditions. An enumeration of conditions in this, the greatest and most varied group, presents a formidable list.

Eosinophils had been observed by Ehrlich to be increased in cases of myeloid leukaemia, but it was not until the next century that cases of leukaemia, in which the eosinophil cell predominated, began to be reported. After Stillman described his cases in 1912, numerous case-reports appeared (Shapiro, 1919; Giffin, 1919; Aubertin and Giroux, 1921).

It was Klinkert (1911) who first described that interesting phenomenon, familial eosinophilia, and cases continue to be reported from time to time (Armand-Delille, Hurst and Sorapore, 1930; Stewart, 1933; Gray and Shaw, 1949).

In a study of tuberculosis in Switzerland, Löffler (1936) reported a clinical condition which now bears his name. These were cases of a minor respiratory illness, in the course of which transient shadows were seen in X-ray pictures of the lung. There was always an eosinophilia of the blood, varying from 7 per cent to 35 per cent, and Löffler regarded the X-ray shadows as eosinophil infiltrates. The occurrence of this syndrome, or one closely resembling it, was reported in India by Frimodt-Möller and Barton (1940) and later by Weingarten (1943), and many cases of tropical eosinophilia have been cited since (Viswanathan and Natarajan, 1945; Wilson, 1947; Telles, 1947).

Apley and Grant (1944, 1945) regard these two syndromes as variations of one condition.

In 1940, Lichtenstein and Jaffe and, simultaneously, Otani and Ehrlich, described a lesion of bone characterised histologically by sheets of histiocytes with scattered aggregations of eosinophil cells. This lesion, eosinophilic granuloma of bone, and its clinical features have been widely reported since. It does not appear to be associated with eosinophilia of the blood.

Other conditions, many no doubt possessing an allergic basis, are sometimes accompanied by eosinophilia. These include such diverse states as subacute and chronic infections, post febrile states, Hodgkin's disease and cancer (Muir, 1898; Ehrlich), leprosy (Milian, 1901), tuberculosis (Dominici, 1900; Gill, 1940), and acute rheumatism (Friedman and Holtz, 1935). In therapeutics eosinophilia occurs from time to time either intentionally, as in the Nirvanol treatment of chorea (De Rudder, 1926; Whitaker, 1930), or as a side effect, as in treatment by digitalis (Smith and Benner, 1931-2; Romano and Geiger, 1936), insulin (Lawrence and Buckley, 1929), penicillin (Lyons, 1943) and streptomycin (Farrington, 1947), and in the treatment of pernicious anaemia with raw liver (Minot and Murphy, 1927), etc., etc.

Experimental Eosinophilia

(a) Extracts of parasitic material

As the association of eosinophilia and parasitic helminths is one of the most constant, it is not surprising that investigators along the years have turned to such material as a means of studying eosinophilia. Following Thayer and Brown's observations, Milian (1901) demonstrated the eosinophil reaction as it occurred in sarcosporidiosis of beef muscle. Chauffard and Boidin (1907), studying hydatid cysts, found a pericystic reaction which they regarded as neutralising the cyst fluid as it permeated the cyst wall. Only when this barrier failed, they concluded, did the patient become sensitised to the antigen.

Working with ascaris, Leroy (1910) collected aseptically, coelomic fluid from these worms and injected it intravenously into dogs. This was toxic but only so in large doses, when it killed the dogs in 30 minutes to 1 hour. Leroy then prepared fractions of this fluid, depending on its solubility in water and in 95 per cent alcohol, and reported the greatest potency in water-soluble and alcohol-insoluble parts. These were, he pointed out, the fractions most likely to contain protein.

Weinberg and Julien (1911) instilled ascaris coelomic fluid into conjunctival sacs of horses producing a severe oedema, with, in some, dyspnoea, diarrhoea, and even collapse. Those horses which did not react were found to be infected with ascaris. In 1913, Weinberg and Seguin sectioned the eyelids of the reacting horses, discovering in

them eosinophilic infiltration. By further experiments they showed that small quantities of this toxin could be neutralised by serum from ascaris-infected horses. Herrick (1913) successfully produced an eosinophilia of the blood in animals by injecting aqueous extract of ascaris, but only after repeated injections. When he boiled his extract and injected the supernatant fluid, no such changes were produced. Herrick concluded from this that the effect was due to protein.

Shinamaru and Fujii (1916, 1917) isolated from dried ascaris material a toxic substance they called "Ascaron" which could produce anaphylactoid shock in animals. From now on, worm extracts were used increasingly in experimental work. Emery and Merrick (1929) and Essex, Markowitz and Mann (1931) used them in experiments on respiration and the circulation. In his immunological studies, Campbell (1936, 1938, 1938a, 1939, 1942) also used them, and isolated an insoluble keratin which was toxic on a single injection (1942), but no blood changes were recorded. In 1944, Olivier-Gonzalez used an isolated polysaccharide to inhibit iso-agglutinins of human blood.

Machebouf and Mandoul (1939) extracted from ascaris a substance toxic in one dose, which they regarded as being probably a toxic amine. On further analysis (1939a), this extract was found to be soluble in 50 per cent alcohol and thermolabile. Mandoul (1939) pointed out that such substances are present in the tissue of the worm and because they are densely bound can only be extracted by precipitation by a chemical reagent such as trichloracetic acid or by digestion with pepsin.

Rocha e Silva and his co-workers (1946, 1946a, 1946b, 1946c) attempted to identify these toxic substances but succeeded only in supplying a long list of reactions to chemical tests, concluding that the active material was a proteose of high molecular weight. Deschiens (1948), in a review of ascaris extracts and their properties, suggested that in chronic worm infestation the polypeptide fraction was probably a precursor of histamine, or at any rate closely related to it. The polysaccharides and glucolipids he considered, possessed in addition an eosinophilogenic property. Sprent recently (1950) found that the removal of protein from ascaris extracts removed their anaphylactogenic property in non-sensitised animals. In sensitised animals he failed to produce shock by a variety of substances, including bacterial polysaccharides, normal homologous tissue, hyaluronidase, and other animal materials. He concluded that the anaphylactogenic activity required both protein and polysaccharide fractions.

(b) The Autonomic Nervous System and the Eosinophil

In 1910, Bertelli, Falta and Schweeger carried out experiments on dogs and rabbits in which these animals were given adrenaline, pilocarpine and atropine, and the effect of these drugs on the blood eosinophils observed. Pilocarpine injections were followed by a rise in eosinophils, while adrenaline and atropine were followed by a fall. Their deduction, that the eosinophils were under nervous influence, was supported by Eppinger and Hess (1917), who reported eosinophilia as a frequent finding in cases of vagotonia. Camp (1927-8) injected various drugs into animals, paying attention to any changes which might take place in the blood picture. Some drugs, he noticed, produced a transient rise in lymphocytes followed by an increase in neutrophils as the lymphocyte level subsided. Although of a varied chemical nature, the drugs producing this change were all parasympathetico-mimetic in their action. Depressors of the parasympathetic nervous system such as adrenaline, atropine and calcium, resembled each other in their action, atropine being able to check completely the action of pilocarpine on the blood. The effect of direct stimulation of the vagus was observed by Hajos, Nemeth and Enyedy (1926) who applied faradic current to the vagi of rabbits for periods varying from 30 minutes to 8 hours. After an hour the rabbits became extremely dyspnoeic and in 8 out of 10 there was a rise in the blood eosinophil level.

Following this came some interesting observations on the human subject. Wieck (1931), describing a case of eosinophilia of obscure

origin, told how he brought about a fall in the eosinophils by treating the patient with adrenaline. Pilocarpine, he observed, produced a rise. In a case of digitalis eosinophilia, Smith and Benner could not produce any change in the eosinophil level using pilocarpine but did lower it by atropine and adrenaline injections. Later, Romano and Geiger, reporting a similar case of digitalis eosinophilia, injected the same drugs and produced identical results.

Acetylcholine was used by Granzner (1938) to produce an eosinophilia in guinea pigs but only on intra-cardiac or intra-peritoneal injection. Subcutaneous administration, he reported, had no effect. The eosinophilogenic effect of acetylcholine could, he showed, be prevented by atropine. In 1943, Campbell found that acetylcholine could enhance the eosinophilic effect of a second dose of keratin given to guinea pigs, but had no effect on normal animals.

The picture of the nervous control of the eosinophil was complicated by Vogt's (1944) observation that injections of adrenaline increase the output of adrenal cortical hormone. Forsham and others (1948) showed how this hormone and its stimulator, adrenocorticotrophic hormone, produced an eosinopenia. So constant is this change that it is used as a test for adrenal cortical function after injections of A.C.T.H. (Thorn and others, 1948) or adrenaline (Thorn and Bayles, 1949). This effect is also used to determine the adrenocorticotrophic activity of chemical compounds (Spies and Stone, 1949), though it has been exhibited by anti-diffusive substances such as sodium salicylate and sodium gentisate (Bertolani, Lorenzini and Bonati, 1951; Meade and Smith, 1951).

(c) The Eosinophil and Dyspnoea

Pescatoria (1930) considered that all eosinophilia was characterised by dyspnoea and regarded carbon dioxide as the essential stimulus of eosinophil formation. By constricting the extremities of rabbits, he produced a localised tissue anoxia, and when he released the constriction the level of eosinophils in the blood rose. Banerji (1925) noted an eosinophilia in a rabbit whose trachea had been obstructed, so later (1933) he set out to observe the effect on the blood eosinophils of exposure to an atmosphere of carbon dioxide. Several rabbits were treated in this way, and he reported a small, but not very significant rise in eosinophils. Banerji suggested the possible importance of acid-base balance since in the treatment of chorea by Nirvanol, the patient's urine becomes acid immediately prior to the development of the eosinophilia.

Chillingworth, Healy and Haskins (1934) dismissed the chemical aspect and held that the essential stimulus was over-distension of the pulmonary alveoli. By means of a ball-valve mechanism inserted into the trachea, they set up this stimulus in dogs and produced an eosinophilia which lasted until the ball-valve was removed. The eosinophilia then subsided, while the emphysema produced in the process remained. This procedure does not exclude participation of the vagus just as the experiments of Hajos, Nemeth and Enyedy, whose animals were extremely dyspnoeic after an hour of vagal stimulation, do not exclude the pulmonary factors considered above.

(d) The Eosinophil in Allergy and Anaphylaxis

The production in the animal of an allergic reaction with its exudates and eosinophils by the repeated injection of an antigenically potent material, is a well recognised procedure (Biggart; Campbell, Drennan and Rettie, and others). This has been used by many workers to provide material for studies in eosinophilia, allergy and anaphylaxis (Weinberg and Seguin, 1915; Godlowski). Campbell (1936) sought to separate anaphylaxis and eosinophilia experimentally by producing eosinophilia by one single injection of material. This he could not do until he used a keratin derived from ascaris which, however, as it was insoluble, could still permit of antigen-antibody interaction (1942).

Histamine was injected into horses by Akerblom and Sjoberg (1938) producing a rise in blood eosinophils. The Giuseppes (1938), on the other hand, found histamine to be inactive in this respect. The apparent discrepancy may possibly be explained by the fact that Akerblom and Sjoberg used a "langdauernder" (long lasting) preparation of histamine. Campbell (1943) injected histamine and other drugs into guinea pigs and showed that, whereas histamine was not in itself an eosinophilogenic agent, it could enhance the eosinophil response to a second dose of antigen in sensitised animals. He failed to find any eosinophilogenic substance in the blood of shocked animals. Stressing the association of anaphylaxis and eosinophilia, he remarked that the substances which diminished anaphylaxis, for example adrenaline,

also diminished eosinophilia. With this, Samter did not agree, as he prevented anaphylaxis in guinea pigs by anti-histamine drugs while the eosinophilia appeared as usual. This is an interesting finding, especially in view of the fact that anti-histamine drugs resemble adrenaline in their action.

(e) Other Methods

From time to time cases are reported where an eosinophilia has been deliberately produced by "unorthodox" means. Among such are the production of eosinophilia by the prolonged administration of potassium chloride (Dominici, 1900); by feeding dogs on copper sulphate (Vacca, 1929); and diets of raw liver in normal humans (Meulengracht and Holm, 1930).

THE SCOPE OF THE PRESENT WORK

The state of our knowledge of the eosinophil leucocyte invited the attention of the author as providing a field in which investigation was required. In particular, two features of the cell have appeared to be of special interest - What is the essential stimulus of formation of the eosinophil? And what is its true function? It is towards an answer to these two questions that the present work is directed.

Technical methods in the field of endocrine chemistry being as they are, no excursion was made in that direction and it was decided to avoid the fickle and often inconclusive results frequently obtained in allergic and anaphylactic reactions. Although this still leaves a wide field, the present work has been limited to the production of eosinophilia in normal unsensitised animals by a variety of materials of known and unknown chemical nature and of varied physiological activity. The effects of these substances on the blood of these animals and the changes which took place in their tissues have been closely studied in an attempt to achieve, in some small measure at least, the purpose of this investigation.

MATERIALS, ANIMALS AND METHODS

Materials

The materials used for injection into animals were Ascaris extracts and various drugs.

Ascaris extracts

From the review of Ascaris extracts made by Deschiens, it would appear that almost any material derived from Ascaris possesses antigenic properties, the most potent being those extracted after precipitation by trichloracetic acid (Mandoul). Extracts were made on two occasions. On the first occasion, only a small amount of material was prepared. In proving the potency of these extracts, they were soon used up. In order to continue the experiments more extracts were prepared, an almost identical procedure being followed. The exact details of the methods are set out below.

1st preparation. Ascaris suum were obtained from pigs, rinsed in water and placed in 1:5000 merthiolate solution, after which they were sent by post to the laboratory. On receipt, the worms, rinsed again, were chopped into lengths of 2 cms. or so, placed in a mortar and liquid oxygen poured over them. This rendered the pieces of worm very brittle and in this state they were pounded into a fine powder which, when it thawed, became a soft pulp. To this pulp was added an equal volume of 8% trichloracetic acid and the mixture

shaken in a Kahn shaker for 90 minutes. By centrifuging, a milky supernatant fluid was separated off. This fluid was dialysed through cellophane against isotonic saline at 0°C for 48 hours. After dialysis the pH of the extract was found to be 5.2 and this was adjusted to pH 7.2 by N/10 sodium hydroxide. This was called Ascaris extract (1) - Ae.1.

To some of Ae.1 was added an equal volume of absolute alcohol which threw down a white precipitate. The yellow supernatant fluid separated off by centrifugation was distilled in vacuo at room temperature until all the alcohol had been removed. The yellow liquid which remained was called Ascaris extract (2) - Ae.2. This probably contained the polypeptides isolated by Machebouf and Mandoul.

The white precipitate was re-dissolved in water, and re-precipitated by alcohol twice, the final precipitate being washed in alcohol three times, in acetone twice, and dried by aeration. There remained a light, white powder - the glycogen fraction of Machebouf and Mandoul - which was called Ascaris extract (3) - Ae.3.

2nd preparation. Ascaris suum were collected in the same manner and on being received in the laboratory were again chopped into short lengths. These pieces were placed in a "TURMIX homogeniser", and the machine started up. When the material had been reduced to a fine pulp an equal volume of 8% trichloracetic acid was added and the apparatus set in motion once again to ensure a thorough mixing of the

materials. When this mixture was centrifuged, a milky supernatant was obtained as before which, after dialysis and neutralisation with N/10 sodium hydroxide, was treated with absolute alcohol.

The alcohol-insoluble moiety was isolated and dried as before. This was Ascaris extract (4) - Ae.4 - and was probably identical with Ae.3.

The alcohol-soluble fraction was distilled in vacuo at room temperature and on this occasion the distillation was continued until only a few mls. of viscous yellow liquid remained. This was transferred to an evaporating bath in which, by means of acetone, it was evaporated down to a yellow green crystalline substance. This substance answers the description of the polypeptide mixture isolated in a similar manner by Machebouf and Mandoul and was called Ascaris extract (5) - Ae.5.

These extracts were stored at -10°C and used, with two exceptions, within 3 months of preparation.

Drugs

The following were the drugs used in the present series of experiments: Acetylcholine, Adrenaline hydrochloride, "Antistin" (an anti-histamine preparation), Atropine sulphate, Hexamethonium iodide, Histamine phosphate, Physostigmine salicylate, and Sodium nitrite. These were standard pharmaceutical preparations obtained from the hospital dispensary.

Animals

Rabbits and guinea pigs have been the animals most commonly used by previous workers on eosinophilia and related subjects. As most recent work has been done on the guinea pig, it was decided to use this animal so that results obtained might be the more readily comparable. Moreover, the convenience of housing and breeding guinea pigs permitted of larger numbers being available for this study.

The average level of eosinophils in the blood of normal guinea pigs has been estimated by Scarborough (1931) as 500 per cubic mm. and by King and Lucas (1941) as 600 per cubic mm. As the former observations were made on a larger number of animals, it probably comes nearer to the true average. Campbell (1936) and Samter found that some older guinea pigs showed higher levels of eosinophils in the blood which, it has been suggested, might have been due to intestinal parasites. Consequently, these workers used very young guinea pigs for their experiments, in most cases breeding them. In the present work, with a few exceptions, their example was followed. Most of the guinea pigs weighed under 300 grams and several were actually bred for these experiments. Care was taken to ensure that these animals had not been previously sensitised to any materials and once an animal had been injected with an antigenic substance, it was not used again.

All injections were given intra-peritoneally, a procedure easily standardised and which, because of its simplicity, left little room for

mishap. The doses of the extracts were determined largely by trial and error, attention being paid to the experiences of previous observers. Drugs were administered in dosages according to Solimann and Hanzlit (1939).

Methods

It was decided to estimate the number of eosinophils in the blood by one of the direct methods rather than by differential counts. There are several direct methods (Dunger, 1910; Gutstein, 1932; Randolph, 1944), each varying in the composition of the diluting fluid used. The method of Dunger being the most simple was, with a slight modification, used to make all eosinophil counts.

Diluting fluid

Aqueous eosin 1%	5 vols.
Acetone	5 vols.
Distilled water	to 100 vols.

Using sharp scissors, a nick was made in the ear of the guinea pig which bled freely. After wiping away the first drops, as advised by Lucey (1950), blood was sucked up to the 0.1 mark in a white cell pipette. Using Dunger's fluid, this was diluted to the 10 mark and the pipette shaken for 30 seconds only, to avoid causing disintegration of the eosinophil leucocytes (Thorn and others). The chambers of two

haemocytometers were charged and examination made under the microscope after 3 minutes. By this fluid the eosinophils stain red, the other leucocytes remaining colourless. The eosinophils were counted over the whole of the ruled area (improved Neubauer ruling) in the two chambers, and the total number per cubic mm. calculated as follows:

$$\text{No. of eosinophils} = \frac{\text{No. counted in two chambers}}{18} \times 200 \text{ per cubic mm.}$$

For the sake of clarity in the tables and figures, these values have been expressed to the nearest ten.

EXPERIMENT 1

Experimental problem

The extract Ae.1 as prepared, corresponded to the description given by previous workers, but little had been reported of its eosinophilogenic power. Some workers using ascaris extracts had produced a rise in eosinophils in animals but only on repeated injections. By careful observation of the level of eosinophils after a dose of extract it was hoped to discover whether an eosinophilia could be produced in guinea pigs by one injection alone, and if so what the effect of an antihistamine compound would be.

Procedure

9 normal unsensitised guinea pigs were selected. 4 were given 0.25 ml. Ae.1 intra-peritoneally, and 2 more were given the same dose of extract 30 minutes after an injection of 25 mgms. "Antistin".

A control solution was prepared by dialysing 4% trichloracetic acid in the same manner as the original extract and the pH adjusted to 7.2. Of this solution, 0.25 ml. were injected into the remaining animals, 2 of which had been given 25 mgms. "Antistin" 30 minutes earlier.

Eosinophil counts were made before the drugs and extracts were given and then at intervals after the extract was given.

Results

See Table 1 and Figs. 1 (a), (b) and (c).

TABLE 1

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected as follows:

Group 1 — 0.25 ml. Ascaris extract (1)

Group 2 — 0.25 ml. Ascaris extract (1) 30 minutes after injection of 25 mgms. "Antistin"

Group 3 — 25 mgms. "Antistin" + 0.25 ml. control solution

Group 4 — 0.25 ml. control solution

Time after injection	Group 1				Group 2		Group 3		Group 4
	1	2	3	4	5	6	7	8	9
Initial level	50	50	50	80	20	0	10	40	40
3 hrs.	150	340	150	80	100	10	50	20	0
6 hrs.	310	300	260	10	70	0	0	50	0
12 hrs.	680	600	590	70	10	0	10	10	40
18 hrs.	-	-	300	220	-	-	-	-	-
24 hrs.	80	220	340	120	20	30	10	40	0
48 hrs.	20	30	50	10	-	-	-	-	40

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

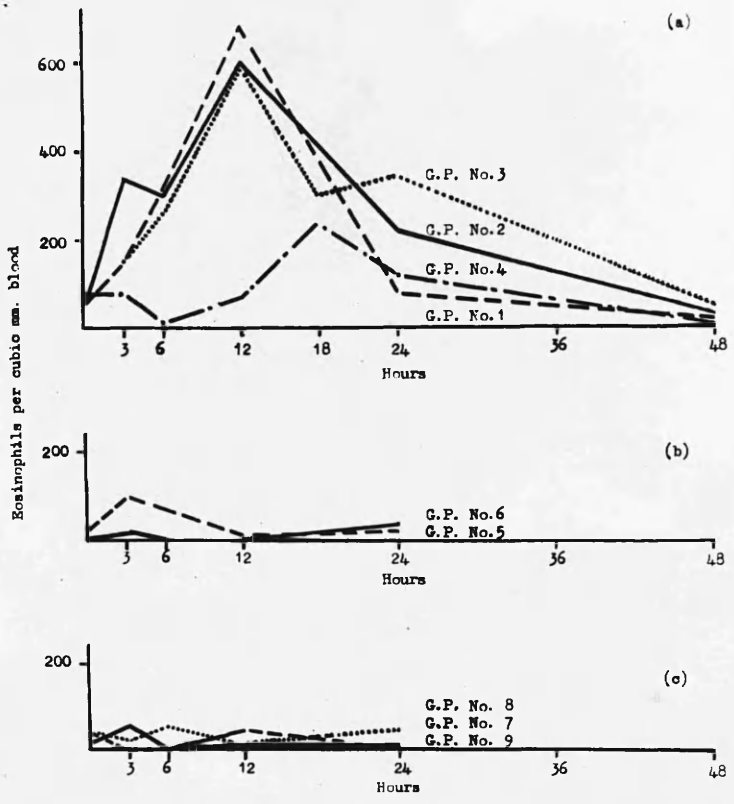


Fig. 1 (a) Normal guinea pigs injected with Ascaris extract (1).
(b) Normal guinea pigs injected with Ascaris extract (1) 30 minutes after being injected with "Antistin".
(c) Normal guinea pigs injected with control solution. Two guinea pigs (7, 8) were, in addition, given "Antistin".

In Fig. 1 (a) can be seen the sharp rise in circulating eosinophils which took place in 3 of the 4 guinea pigs given Ae.1. In the remaining animal an increase in eosinophils did take place but it was rather late and not very high. The other rises bore a similar pattern - maximal at 12 hours, subsiding somewhat at 24 hours, and initial levels regained at 48 hours.

Fig. 1 (b) shows the effect of "Antistin" on these responses. Little or no change was observed in the 'protected' guinea pigs Nos. 5 and 6. No significant changes took place in guinea pigs Nos. 7, 8 and 9 - the control animals. (Fig. 1 (c)).

In addition to the blood changes reported above, the 'unprotected' animals became dyspnoeic very soon after the injection and had a few convulsions before lying down on the floor of their cages. The dyspnoea lasted about 15 minutes and after an hour the animals were moving about normally again.

Conclusion

In the normal unsensitised guinea pig, a crude extract of Ascaris (Ae.1) can produce on a single injection an increase in the eosinophils circulating in the blood. This effect on the eosinophil level can be prevented completely by the prior administration of an anti-histamine drug.

EXPERIMENT 2

Experimental problem

Having established the eosinophilogenic power of the crude extract (Ae.1) it was decided to find out if this power was possessed by any of the fractions prepared from it.

Procedure

6 normal unsensitised guinea pigs were chosen. 2 of these were given Ae.2 in doses of 0.20 and 0.40 ml.; to a third was given 0.40 ml. control solution. This control solution had been prepared from the control solution of the previous experiment by treating it exactly as in the preparation of Ae.2. To another 2 was given Ae.3 in doses of 10 mgms. and 35 mgms. dissolved in sterile saline. The remaining animal was given 1 ml. sterile saline only.

The changes in eosinophil levels were observed initially and at intervals after the injections for 48 hours.

Results

See Table 2 and Figs. 2 (a) and (b).

It will be seen from Fig. 2 (a) that a distinct rise in the level of eosinophils in the blood took place in the guinea pig (No. 2) receiving the greater dose of Ae.2. This rise reached its peak at 12 hours and although it had fallen a little by 24 hours, the initial level was only slowly regained thereafter. While the guinea pig (No. 1) given the smaller dose of Ae.2 showed only a small rise in eosinophils, the

TABLE 2

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected as follows:

Group 1 No. 1 0.20 ml. Ascaris extract (2)
2 0.40 ml. Ascaris extract (2)
3 0.40 ml. control solution

Group 2 No. 4 10 mgms. Ascaris extract (3) in 1 ml. saline
5 35 mgms. Ascaris extract (3) in 1 ml. saline
6 1 ml. saline

Time after injection	Group 1			Group 2		
	1	2	3	4	5	6
Initial level	20	40	30	80	80	30
3 hrs.	50	640	30	220	290	50
6 hrs.	220	770	20	100	300	20
12 hrs.	150	1200	30	400	850	20
24 hrs.	120	440	90	200	670	20
48 hrs	30	80	80	80	260	40

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

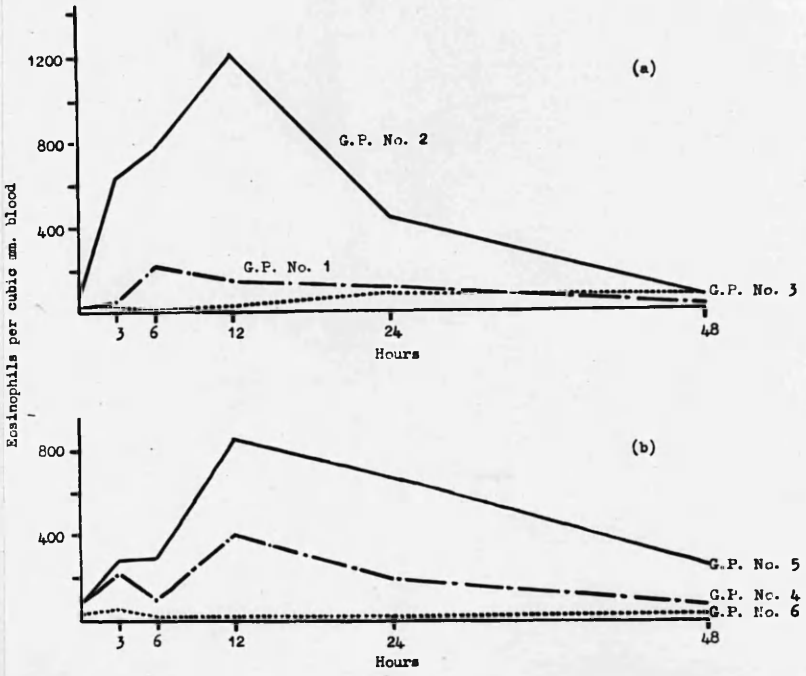


Fig. 2 (a) Two normal guinea pigs (1, 2) injected with Ascaris extract (2). Guinea pig no. 3 injected with control solution.
(b) Two normal guinea pigs (4, 5) injected with Ascaris extract (3). Guinea pig no. 6 injected with saline only.

control animal (No. 3) showed little or no change at all.

In Fig. 2 (b) both animals receiving Ae.3 had quite sharp rises in blood eosinophils, the greater rise being observed in that animal given the larger dose of extract (G.P. No. 5). Maximal at 12 hours, these rises had not subsided much by 24 hours, after which a slow fall to the initial levels took place. No significant changes were recorded in the eosinophil levels of the control animal.

Convulsions occurred in the animals given Ae.2.

Conclusion

In normal unsensitised guinea pigs, an eosinophilia lasting 24 hours or more can be produced by the injection of Ae.2 and Ae.3. Thus the eosinophilogenic power possessed by the crude extract Ae.1 (as demonstrated in Experiment 1) is contained in its two fractions.

EXPERIMENT 3

Experimental problem

A new extract of Ascaris suum being prepared, it was desired to test its potency in producing eosinophilia in guinea pigs and the effect on such changes, if any, of an anti-histamine drug.

Procedure

9 normal guinea pigs were selected, some of which exhibited initial eosinophil levels above the average 500 per cubic mm. 6 pigs were given 30 mgms. Ae.4 in 1 ml. saline and the remaining 3 given the same dose of Ae.4 30 minutes after an injection of 25 mgms. "Antistin". Eosinophils were counted at intervals over 24-48 hours.

Results

See Table 3 and Figs. 3 (a) and (b).

In Fig. 3 (a) it can be seen that with the exception of guinea pig No. 1, all animals receiving an injection of Ae.4 alone showed a sharp rise in the blood eosinophil level. This rise varied a little in pattern. Guinea pig No. 2 showed a steep rise with a very slow subsidence of the eosinophils while guinea pig No. 3 showed a steep rise with a rapid fall. Guinea pig No. 1, after a preliminary fall in eosinophils, eventually showed a steady increase in their levels which at 24 hours was still rising.

In Fig. 3 (b) the blood eosinophil levels in those animals receiving "Antistin" prior to the injection of extract are shown. These

TABLE 3

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected as follows:

Group 1 30 mgms. Ascaris extract (4) in 1 ml. saline

Group 2 30 mgms. Ascaris extract (4) 30 minutes after injection of 25 mgms. "Antistin".

Time after injection	Group 1						Group 2		
	1	2	3	4	5	6	7	8	9
Initial level	730	30	400	330	190	80	690	300	880
6 hrs.	130	1950	970	500	890	1070	50	0	250
12 hrs.	1170	2010	2860	1120	780	880	620	20	320
24 hrs.	1540	1840	630	670	950	1800	450	40	860
36 hrs.	-	1540	-	-	-	-	670	100	-
48 hrs.	-	910	-	-	-	-	530	370	-

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

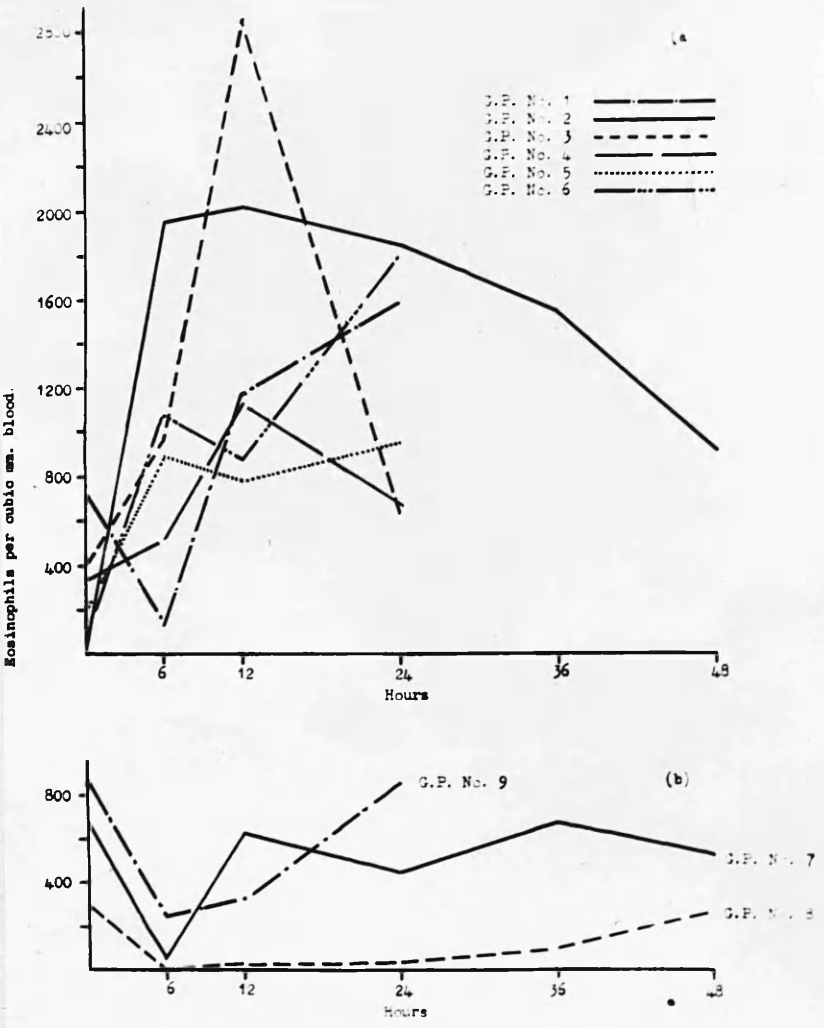


Fig. 5 (a) Normal guinea pigs injected with Ascaris extract (4)
(b) Normal guinea pigs injected with Ascaris extract (4) 30 minutes after being injected with 25 mgms. "Antistin".

3 guinea pigs (Nos. 7, 8 and 9) exhibited no increases in eosinophils but in fact showed an undoubted fall in these cells, and in guinea pig No. 8, the initial level was not regained for 48 hours after the injection.

Ruffling of the coat was noticed in animals given "Antistin".

Conclusion

In normal guinea pigs Ae.4 can produce an increase in the number of circulating eosinophils which lasts 24 to 48 hours. This effect can be prevented by the action of "Antistin". In addition, this drug produces a fall in the level of eosinophils in the blood.

EXPERIMENT 4

Experimental problem

It remained to test the potency of the last extract, Ae.5, in eosinophilogenesis and the effect of anti-histamine drugs on its action.

Procedure

6 normal unsensitised guinea pigs were used, 3 of which showed a high initial level of eosinophils in the blood. The first of these received 1 mgm. Ae.5, the second and third 3 mgms. each, and the fourth 4 mgms. The remaining 2 were given respectively 1 mgm. and 3 mgms. of the extract 30 minutes after an injection of 25 mgms. "Antistin".

Results

See Table 4 and Figs. 4 (a) and (b).

In Fig. 4 (a) it is readily seen that a rise in the blood eosinophil level was produced in guinea pigs Nos. 1, 2, 3 and 4, by an injection of Ae.5. With one exception the eosinophil levels rose slowly and at 24 hours were still rising. In the animal (guinea pig No. 4) which received the greatest dose of the extract, the rise was more rapid, the peak higher, and the fall earlier, than in the animals given the smaller dosage. Guinea pigs Nos. 2 and 3, which were given the same dose, showed a marked similarity of pattern of eosinophil changes. The animal given the smallest dose (guinea pig No. 1) showed no significant changes in eosinophil levels for 12 hours.

The changes observed in the "Antistin" protected guinea pigs

TABLE 4

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected as follows:

Group 1 No. 1 1 mgm. Ascaris extract (5)
2 3 mgms. Ascaris extract (5)
3 3 mgms. Ascaris extract (5)
4 4 mgms. Ascaris extract (5)

Group 2 No. 5 1 mgm. Ascaris extract (5) 30 minutes after injection of 25 mgms. "Antistin"
6 3 mgms. Ascaris extract (5) 30 minutes after injection of 25 mgms. "Antistin"

Time after injection	Group 1				Group 2	
	1	2	3	4	5	6
Initial level	880	190	300	340	640	840
6 hrs.	960	770	720	1300	0	220
12 hrs.	770	1020	1050	2110	580	300
24 hrs.	1410	1100	1310	630	480	860
36 hrs.	1910	-	-	820	620	-
48 hrs.	1160	-	-	400	560	-

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

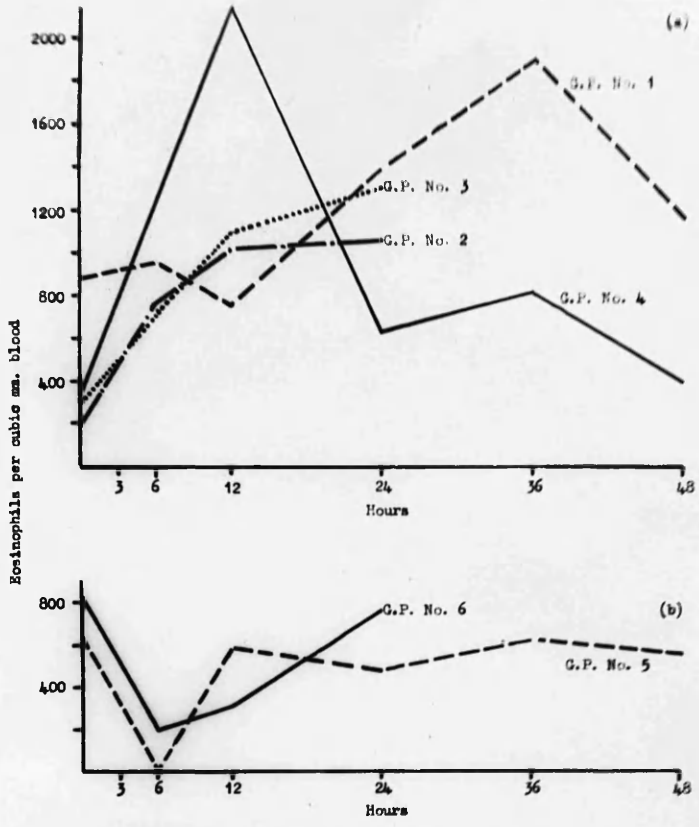


Fig. 4 (a) Normal guinea pigs injected with Ascaris extract (5).
(b) Normal guinea pigs injected with Ascaris extract (5) 30 minutes after being injected with "Antistin".

injected with Ae.5 are shown in Fig. 4 (b). Both animals failed to show any rise in eosinophil levels but instead, a marked depression of these cells in their blood took place, initial levels not being regained for 12 hours or longer.

All the unprotected guinea pigs were dyspnoeic for several minutes after the injection and guinea pigs Nos. 3 and 4, which had received relatively high dosage of the extract, had numerous convulsions. After lying on the floor of their cages for 30 minutes or so, both animals apparently recovered. No changes, apart from ruffled fur, were seen in the animals receiving "Antistin".

Conclusion

Ae.5 can produce a transient eosinophilia in guinea pigs. A greater dose of the extract leads to a more acute rise in the eosinophil level and the initial level is more rapidly regained. Smaller doses seem to produce a more gradual and longer-lasting eosinophilia. It is clear from the dosage of extracts used and the results obtained in Experiments 3 and 4 that Ae.5 is a more powerful eosinophilogenic agent and a more toxic substance than Ae.4.

The effect of Ae.5 can be prevented by the prior administration of an anti-histamine drug. This drug with Ae.5, similarly as with Ae.4 in Experiment 3, produces a temporary but marked depression of the blood eosinophils. This action of "Antistin" resembles that of ephedrine reported by Abelson and Moyes (1950) and may be a sympathetico-mimetic effect.

EXPERIMENT 5

Experimental problem

That the effect of the anti-histamine drug "Antistin" might be sympathetico-mimetic action has been suggested in the conclusion to Experiment 4. It was decided to investigate the undoubted sympathetico-mimetic effect of adrenaline on guinea pigs, recording the changes in the levels of eosinophils which might take place.

Procedure

4 guinea pigs were selected as having a high eosinophil count so that the effect of adrenaline, if any, might be better demonstrated. Adrenaline hydrochloride was used in 1:1000 dilution, of which 0.1 ml. was injected into each animal. The eosinophil count of each guinea pig was recorded hourly for 3 hours and again at 6 hours.

Results

See Table 5 and Fig. 5.

Fig. 5 shows how 3 of the guinea pigs (Nos. 1, 2 and 4) showed a sharp fall in blood eosinophils one hour after the injection of adrenaline. The recovery of these animals took place at a varying rate, initial levels being regained at 2 hours by 2 of the guinea pigs and not even at 6 hours by the other. Guinea pig No. 3 failed to show a fall in eosinophils and in fact showed a small rise.

TABLE 5

Changes observed in the eosinophil level of selected guinea pigs following the injection of 0.10 ml. 1:1000 solution of Adrenaline hydrochloride. These animals were chosen as exhibiting a high level of circulating eosinophils in their blood.

Time after injection	1	2	3	4
Initial level	1950	790	1470	1270
1 hr.	1210	510	1610	360
2 hrs.	2200	770	2220	370
3 hrs.	1870	1320	1820	460
6 hrs.	2280	590	1960	880

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

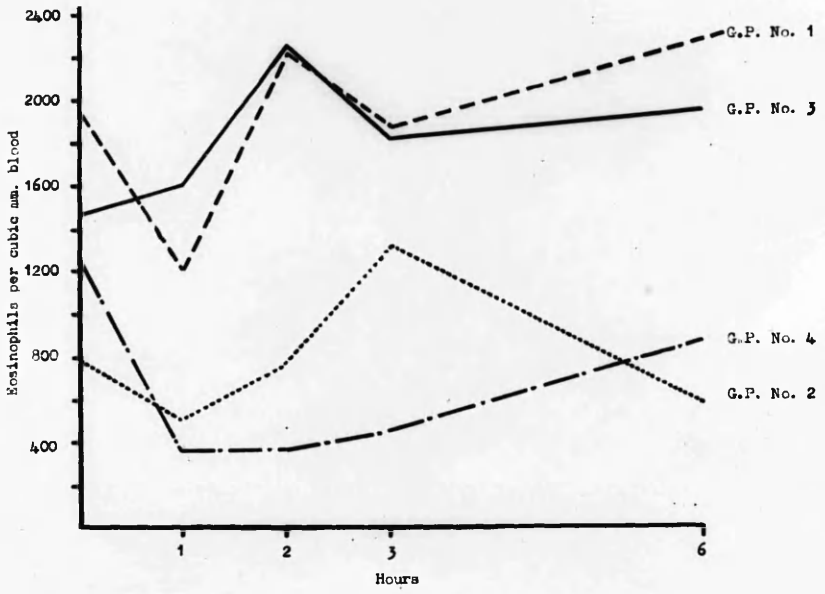


Fig. 5 Guinea pigs injected with Adrenaline hydrochloride.

Conclusion

The production of an eosinopenia by adrenaline suggests that the action of "Antistin" in producing an eosinopenia is a sympathetico-mimetic one. This eosinopenic effect of adrenaline has been amply confirmed and a similar activity has also been reported in the case of other drugs including ephedrine. The guinea pig which did not react to the adrenaline as did the others would, by the standards of Thorn and his co-workers, be considered to have reduced adrenal cortical reserve.

EXPERIMENT 6

Experimental problem

In view of the fact that the eosinophil changes brought about by the Ascaris extracts can be prevented by an anti-histamine drug, the possibility that histamine might have some eosinophilogenic activity engaged our attention. Evidence on the action of histamine has in the past been conflicting (Akerblom and Sjoberg; Giuseppes; Campbell, 1943).

Procedure

Histamine phosphate in a tabloid preparation was used. This was dissolved in sterile saline, prior to injection. 8 normal unsensitised guinea pigs were selected and to 6 of these was given 0.25 mgm. histamine phosphate intra-peritoneally. The remaining 2 were given an injection of the sterile saline only and acted as controls. The level of blood eosinophils was recorded at intervals over a period of 24-48 hours.

Results

See Table 6 and Figs. 6 (a) and (b).

Fig. 6 (a) clearly shows the eosinophilic effect of histamine in guinea pigs. In 4 guinea pigs (Nos. 2, 3, 5 and 6) the response was almost identical and although 2 animals (Nos. 1 and 4) showed rather exaggerated reactions compared to the others, the peak in all cases was reached at 6-12 hours, and by 24 hours the levels were on the way back

TABLE 6

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected with 0.25 mgm. Histamine phosphate with simultaneous observations on two guinea pigs (7, 8) injected with saline only.

Time after injection	Group 1						Group 2	
	1	2	3	4	5	6	7	8
Initial level	0	50	510	170	40	70	30	150
3 hrs.	100	90	470	-	-	-	20	120
6 hrs.	650	280	1030	1950	790	1270	10	180
12 hrs.	2200	850	880	2280	590	880	40	70
24 hrs.	1320	250	610	1450	170	190	100	220
36 hrs.	1100	200	-	-	-	-	-	-
48 hrs.	300	50	-	820	-	-	-	-

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

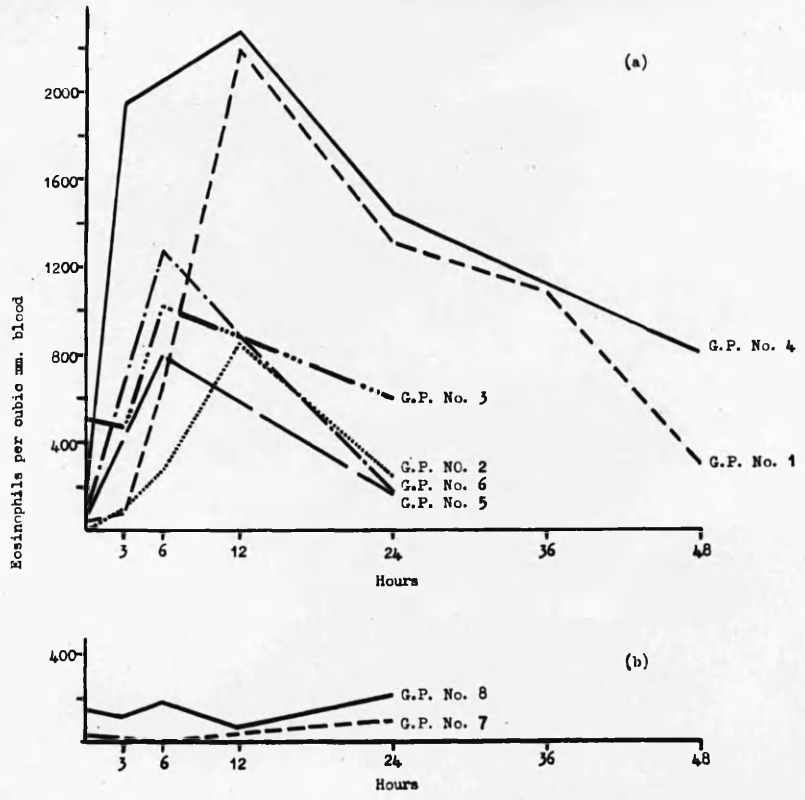


Fig. 6 (a) Normal guinea pigs injected with Histamine phosphate.
(b) Normal guinea pigs injected with saline only.

to the initial values. These were not reached by some, however, by 48 hours.

In fig. 6 (b) it can be seen that no significant changes occurred in the blood eosinophil levels of the control animals.

Conclusion

The ability of histamine phosphate to produce a transient eosinophilia in normal unsensitised guinea pigs has been clearly demonstrated. Individual animals varied in the degree and duration of this response to the drug.

EXPERIMENT 7

Experimental problem

Much has been written on the role of the autonomic nervous system in the stimulus to eosinophil formation and its effect, direct and indirect, has been observed with inconclusive results. It was decided to study the effect of acetylcholine on the blood eosinophils of guinea pigs. This had already been done by Granzner who succeeded in producing an eosinophilia in guinea pigs by the intravenous and intraperitoneal injection of this drug.

Procedure

In view of the toxicity of acetylcholine it was decided to start with small doses of the drug. 4 normal unsensitised guinea pigs were selected, 2 being injected with 2 mgms. acetylcholine per Kg. body wt. and the remainder with 10 mgms. acetylcholine per Kg. body wt.

Results

See Table 7 and Fig. 7.

Although no changes of significance were observed in the blood eosinophil levels of guinea pigs Nos. 1, 2 and 3, the pattern presented in Fig. 7 by guinea pig No. 4 is striking. There is a distinct rise in the eosinophils beginning 6 hours after the injection and rising to a peak at 12 hours. This animal, it may be noted, possessed a relatively high initial eosinophil level.

TABLE 7

Changes observed in the eosinophil level of guinea pigs following the injection of varying doses of Acetylcholine.

Group 1 Normal guinea pigs given 2 mgms. Acetylcholine per Kg. body wt..

Group 2 Normal guinea pigs given 10 mgms. Acetylcholine per Kg. body wt..

Time after injection	Group 1		Group 2	
	1	2	3	4
Initial level	210	590	220	580
3 hrs.	200	220	-	-
6 hrs.	180	360	110	410
12 hrs.	140	520	140	1630
24 hrs.	220	580	200	760

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

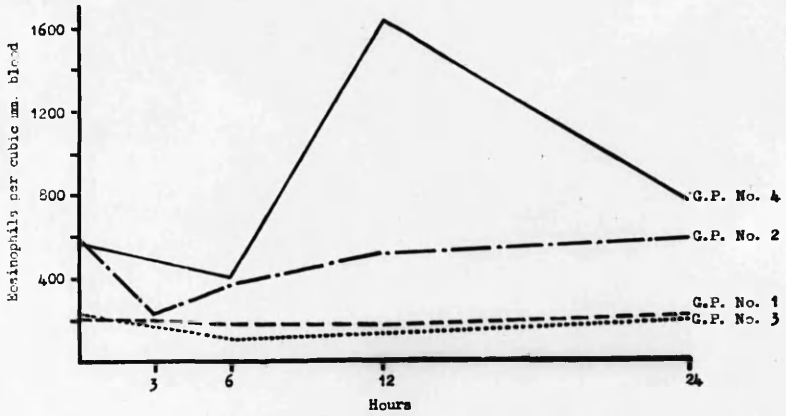


Fig. 7 Normal guinea pigs injected with Acetylcholine in doses of

(a) 2 mgms. per Kg. body wt. Nos. 1 and 2
(b) 10 mgms. per Kg. body wt. Nos. 3 and 4

Conclusion

The fact that the only change observed after the injection of acetylcholine into 4 guinea pigs was an increase in the eosinophil level of a guinea pig with a comparatively high initial eosinophil count may be of significance, but there was another guinea pig with a similar initial level of these cells in the blood which did not so respond. In this case the dose of acetylcholine was smaller. This would suggest that a dosage of the order of 10 mgms. acetylcholine per Kg. body wt. or greater, might produce more conclusive results.

EXPERIMENT 8

Experimental problem

It was decided to administer acetylcholine to guinea pigs in higher dosage than in the previous experiment, observing the effect on blood eosinophil levels.

Procedure

5 normal unsensitised guinea pigs were chosen, some of which showed high initial eosinophil levels. To 3 guinea pigs was given 25 mgms. acetylcholine per Kg. body wt. and the other 2 were given 10 mgms. per Kg. body wt. Eosinophils were counted at intervals for 24 hours.

Results

See Table 8 and Fig. 8.

In guinea pigs Nos. 5 and 7, no changes of note in the blood eosinophil levels followed the administration of 25 mgms. acetylcholine per Kg. body wt. Both these animals had low initial eosinophil counts.

The other guinea pigs, Nos. 6, 8 and 9, all of which had high initial counts, showed an increase in eosinophils after an injection of acetylcholine.

Besides the effects on the blood, the injection of acetylcholine produced in the animals receiving 25 mgms. per Kg. body wt. severe dyspnoea, convulsions and prostration. After 30 minutes or so, they began to move about again.

TABLE 8

Changes observed in the eosinophil level of guinea pigs following the injection of varying doses of Acetylcholine.

Group 1 Normal guinea pigs given 25 mgms. Acetylcholine per Kg. body wt.

Group 2 Guinea pigs with a high level of circulating eosinophils, given 10 mgms. Acetylcholine per Kg. body wt.

Time after injection	Group 1			Group 2	
	5	6	7	8	9
Initial level	100	510	110	1380	920
3 hrs.	110	1200	100	-	-
6 hrs.	110	1250	80	1600	1200
12 hrs.	120	670	70	2410	2100
24 hrs.	140	390	30	2270	890

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

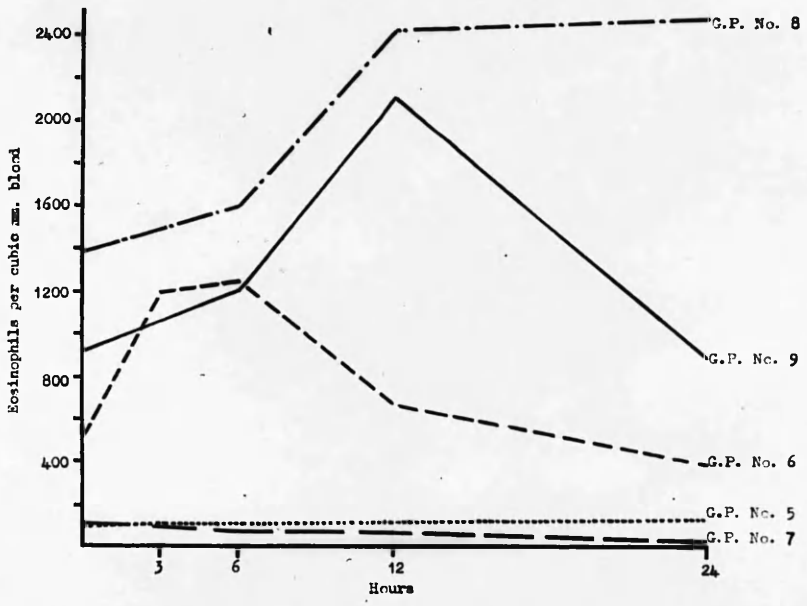


Fig. 8 (a) Normal guinea pigs injected with 25 mgms. Acetylcholine per Kg. body wt. (5,6,7)
(b) Guinea pigs showing a high level of eosinophils in their blood injected with 10 mgms. Acetylcholine per Kg. body wt. (8, 9).

Conclusion

Acetylcholine has some effect on the eosinophils of some guinea pigs. It has no effect on guinea pigs with a low normal eosinophil level but it does further increase the number of eosinophils in animals which already have a high eosinophil count. As it has no effect on the normal animal, it would appear that acetylcholine is not the essential stimulus to eosinophil formation. That it does increase the eosinophil count in some animals is no contradiction since it is possible that this change is brought about by some other mechanism controlled by acetylcholine and hence by the parasympathetic nervous system, e.g., vaso-dilatation.

It is of interest to note that Campbell (1943) found that acetylcholine had no effect on the eosinophils of the normal animal. He could, however, enhance the increase of eosinophils produced by a second dose of antigen in sensitised animals.

EXPERIMENT 9

Experimental problem

The exact nature of the stimulus supplied to the marrow following the injection of Ascaris extracts being unknown, it is possible that it acts through the mediation of the parasympathetic nervous system. In view of the various opinions which have been expressed on this possibility, it was considered worthy of investigation.

Procedure

4 normal unsensitised guinea pigs were given 1 mgm. atropine sulphate per Kg. body wt. 8 hourly for 48 hours. 1 hour after the first injection, when the animals were fully 'atropinised', 3 of them received an injection of 30 mgms. Ae.4, the fourth animal remaining as a control. Eosinophil levels were observed for 48 hours.

Results

See Table 9 and Fig. 9.

The 3 'atropinised' guinea pigs given Ascaris extract developed an eosinophilia thereafter. Although in guinea pig No. 1 the peak of the eosinophilia was not attained until 24 hours, the pattern of these rises is much the same as those produced when Ae.4 was given alone (Experiment 3). The animal receiving atropine alone showed no comparable changes in eosinophil levels, a slight rise only being observed.

TABLE 9

Changes observed in the level of eosinophils in the blood of guinea pigs injected with 30 mgms. Ascaris extract (4) while under the influence of Atropine sulphate (1 mgm. per Kg. body wt. 8 hourly). One guinea pig, which was given Atropine sulphate but not Ascaris extract (4), is included as control.

Time after injection	1	2	3	Control
Initial level	730	400	330	60
6 hrs.	630	970	510	170
12 hrs.	1170	2860	1120	400
24 hrs.	1540	630	670	120
36 hrs.	1410	990	620	170
48 hrs.	1190	1020	690	170

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

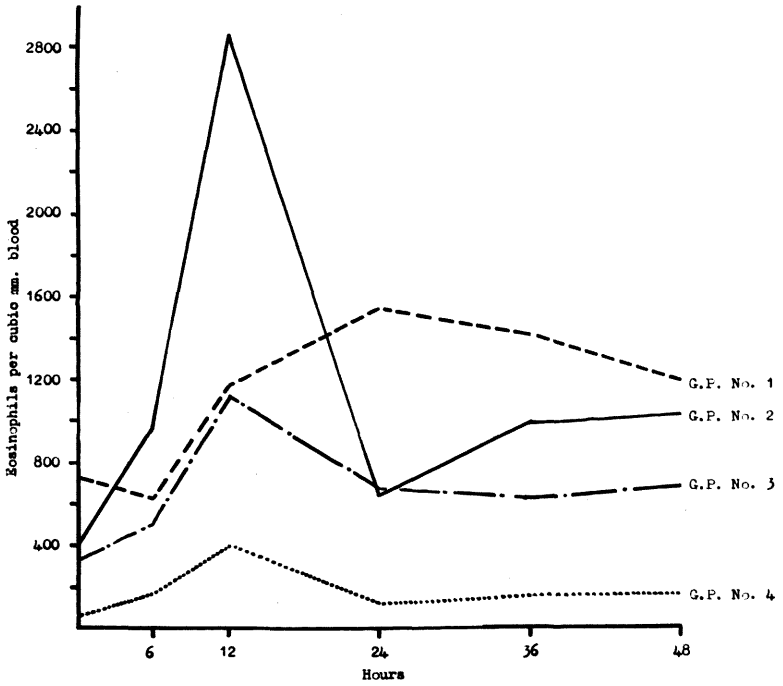


Fig. 9 3 normal guinea pigs injected with Ascaris extract (4) while under the influence of atropine.

1 guinea pig (No. 4) was given atropine alone.

Conclusion

The effect of Ae.4 in stimulating the formation of eosinophils in guinea pigs was not influenced by the temporary inhibition of the parasympathetic nervous system by atropine. It would appear that the mechanism of eosinophil stimulation by Ascaris extracts does not involve the parasympathetic nervous system.

EXPERIMENT 10

Experimental problem

It was decided to observe the effect of another parasympathetico-mimetic drug on the level of eosinophils in the blood of normal and of eosinophilic guinea pigs.

Procedure

4 guinea pigs were selected. 2 of these were chosen as showing a spontaneous eosinophilia; the other 2 were normal animals. The 4 guinea pigs were injected with 0.03 mgm. physostigmine salicylate per Kg. body wt. and the eosinophils observed over 24 hours.

Results

See Table 10 and Fig. 10.

It will be seen that a distinct rise in eosinophils of the blood took place in guinea pig No. 3. This guinea pig had a very high initial eosinophil count. Guinea pig No. 5, which also had a high initial eosinophil level, showed only a very small rise. In the other animals (Nos. 1 and 2) with normal initial levels, no significant changes were observed.

Conclusion

In one guinea pig with a high initial eosinophil count a further rise of eosinophils was produced by an injection of physostigmine. In another eosinophilic animal, a very small increase of doubtful

TABLE 10

Changes observed in the eosinophil levels of guinea pigs injected with Physostigmine sulphate in the dose of 0.03 mgm. per Kg. body wt..

Group 1 Normal guinea pigs.

Group 2 Guinea pigs having a high level of eosinophils in their blood.

Time after injection	Group 1		Group 2	
	1	2	3	4
Initial level	290	100	2200	890
3 hrs.	240	40	2770	870
6 hrs.	300	100	1940	1010
12 hrs.	330	40	2310	990
24 hrs.	240	70	2090	760

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

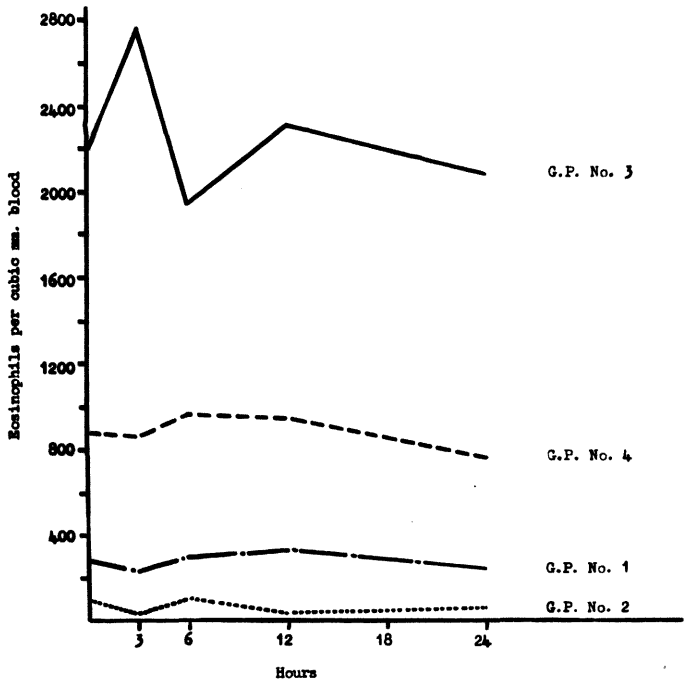


Fig. 10. Guinea pigs injected with Physostigmine sulphate.

significance took place after physostigmine, while no effect was demonstrable in normal animals. In this way physostigmine resembles acetylcholine, the raising of the eosinophil levels of eosinophilic guinea pigs being a parasympathetico-mimetic action. In view of the absence of any effect in normal animals this action cannot be regarded as an essential stimulus, but may be due to some other parasympathetic function.

EXPERIMENT 11

Experimental problem

One of the effects of the parasympathetic nervous system is vaso-dilatation. That the rise in eosinophils produced by parasympathetico-mimetic drugs in Experiments 7, 8 and 10 might be due to that effect, is a possibility meriting further attention. It was decided to produce vaso-dilatation by drugs which operated in a manner different from that of acetylcholine and physostigmine salicylate. For this purpose sodium nitrite, acting on muscle, and hexamethonium iodide, a sympathetic inhibitor (Paton, 1951), were chosen.

Procedure

6 normal unsensitised guinea pigs were selected. To 4 of these were given 30 mgms. sodium nitrite per Kg. body wt. To the other 2, 1 mgm. hexamethonium iodide per Kg. body wt. was injected. Eosinophil levels were observed for 24 hours.

Results

See Table 11 and Figs. 11 (a) and (b).

Fig. 11 (a) shows that no significant changes were observed in the eosinophils of guinea pigs 1, 2, 3 and 4, injected with sodium nitrite.

Of the 2 animals given hexamethonium iodide, No. 5 failed to show any changes but in No. 6 a sharp rise was observed. These animals differed only in the initial levels of the eosinophils in the blood.

TABLE 11

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected with vasodilator drugs as follows:

Group 1 Sodium nitrite 30 mgms. per Kg. body wt.

Group 2 Hexamethonium iodide 1.0 mgm. per Kg. body wt.

Time after injection	Group 1				Group 2	
	1	2	3	4	5	6
Initial level	170	10	0	10	120	390
1 hr.	210	0	20	10	100	480
2 hrs.	120	130	0	10	80	470
3 hrs.	130	20	10	110	90	630
6 hrs.	150	30	20	0	30	370
12 hrs.	120	0	10	20	110	300
24 hrs.	190	20	20	20	90	330

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

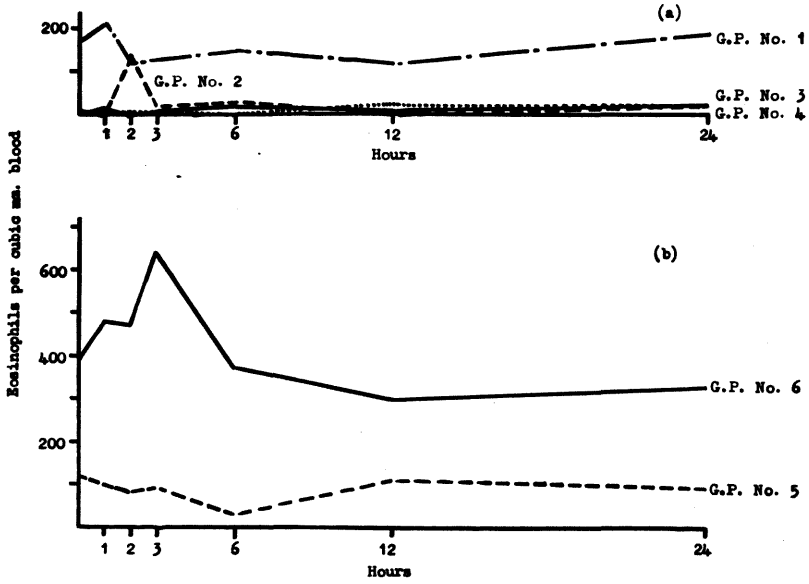


Fig. 11 (a) Normal guinea pigs injected with sodium nitrite.
(b) Normal guinea pigs injected with hexamethonium iodide.

The increase which was observed had subsided completely by 6 hours.

Conclusion

The significance of the fact that only one animal developed a rise in eosinophils following the administration of a vaso-dilator drug may be doubted but all the same, there was only one animal with a high initial level of eosinophils and it was in that animal that the increase in these cells was observed. This suggests that the appearance of a further increase in the circulating eosinophils of a guinea pig which already has a high eosinophil count can be brought about by vaso-dilatation.

Addendum

Since this experiment was carried out it has been noted that the vaso dilating effect of sodium nitrite has been disputed by Weiss, Wilkins and Haynes (1937). As no significance was attached to the results obtained by the use of this drug, the experiment has been allowed to remain as performed.

EXPERIMENT 12

Experimental problem

Since from Experiment 9 it would seem unlikely that the effect of Ascaris extracts are mediated through the autonomic nervous system, the possibility of direct chemical stimulation of the marrow either by the unchanged extract, or by the extract in some altered form must be borne in mind. That such material should gain access to the marrow from the peritoneal sac indicates that at some period it may be found in the blood stream. An attempt was made to find such an eosinophilogenic substance in the blood of guinea pigs after an injection of extract, either in the serum or in the cells.

Procedure

12 normal unsensitised guinea pigs were chosen. 6 of these were given 30 mgms. Ae.4 and were killed at intervals of 3, 4, 8, two at 12 and the last at 24 hours after injection. Blood was taken from the hearts of these guinea pigs and in 4 cases was allowed to clot. In the other two, the heart blood was mixed with a 3.8% solution of sodium citrate to prevent clotting. 2 mls. serum from each of the 4 clotted bloods were injected into 4 guinea pigs respectively. Of the uncoagulated whole bloods, 2 mls. amounts were injected into the remaining 2 animals. All these injections were given intra-peritoneally. Eosinophil levels in these animals were observed for 48 hours.

TABLE 12

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected as follows:

Group 1 No. 1 Serum from guinea pigs killed 4 hours after receiving injection of Ascaris extract (4)
2 Serum from guinea pigs killed 8 hours after receiving injection of Ascaris extract (4)
3 Serum from guinea pigs killed 12 hours after receiving injection of Ascaris extract (4)
4 Serum from guinea pigs killed 24 hours after receiving injection of Ascaris extract (4)

Group 2 No. 5 Whole blood (citrated) from guinea pigs killed 3 hours after injection of Ascaris extract (4)
6 Whole blood (citrated) from guinea pigs killed 12 hours after injection of Ascaris extract (4)

Time after injection	Group 1				Group 2	
	1	2	3	4	5	6
Initial level	130	30	150	80	100	130
3 hrs.	70	-	-	-	70	-
6 hrs.	80	70	200	110	70	110
12 hrs.	220	60	110	90	80	140
18 hrs.	-	20	100	60	80	-
24 hrs.	330	0	70	90	70	140
36 hrs.	320	20	150	150	80	130
48 hrs.	130	30	180	280	110	190

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

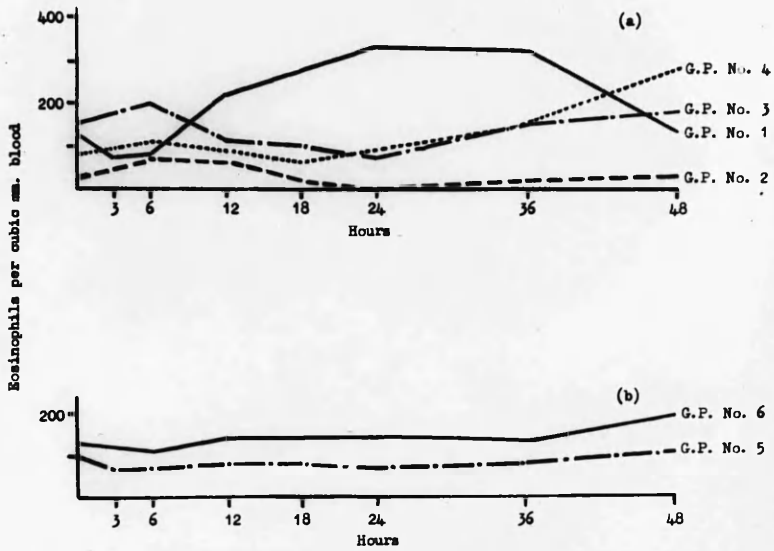


Fig. 12 (a) Normal guinea pigs injected with serum of animals killed at varying intervals after an injection of Ascaris extract (4).

G.P. No. 1 serum from animal killed at 4 hours
2 " " " " " 8 "
3 " " " " " 12 "
4 " " " " " 24 "

(b) Normal guinea pigs injected with whole blood (citrated) from animals killed at varying intervals after an injection of Ascaris extract (4).

G.P. No. 5 blood from animal killed at 3 hours
6 " " " " " 12 "

Results

See Table 12 and Figs. 12 (a) and (b).

The only change observed in the eosinophil levels was a very small increase in the blood of guinea pig No. 1, which received serum from an animal 4 hours after the injection of Ae.4.

No notable changes in eosinophil levels took place in the other animals.

Conclusion

Doubt as to the significance of the rise observed in the circulating eosinophils of guinea pig No. 1 is increased when it is seen that no changes of note took place in guinea pig No. 5 injected with blood from a guinea pig 3 hours after receiving Ae.4. From this it can be concluded that in the above experiment no eosinophilogenic agent has been proved to be present in the blood of guinea pigs 3 hours or more after the injection of Ascaris extract.

EXPERIMENT 13

Experimental problem

In view of the reputation of the eosinophil as a neutralising agent, an attempt was made to test in vitro the effect of eosinophils and other blood elements on Ae.4.

Procedure

A guinea pig was selected which possessed a high level of eosinophils in the blood, the eosinophil level standing at 2,990 per cubic mm. The animal was killed by chloroform and just before it had actually died, blood was withdrawn from the heart in a sterile syringe. Of this blood, about 3 mls. were placed in a dry sterile tube and the remainder, about 8-10 mls., was placed in two sterile tubes containing sterile sodium citrate solution to prevent coagulation.

The citrated blood was gently centrifuged at 1,000 r.p.m. for 10 minutes, separating a turbid supernatant plasma containing the leucocytes. This was removed by a sterile Pasteur pipette. It was estimated that this mixture contained 4-5 million eosinophil leucocytes per ml.

1 ml. of this plasma-eosinophil mixture was placed in a tube and 5 mls. sterile isotonic saline added. This was centrifuged at 2,500 r.p.m. for 5 minutes and the clear supernatant liquid removed. After a further washing with sterile saline, the leucocytes were suspended in 1 ml. sterile saline.

The clotted blood was centrifuged and the serum removed.

30 mgms. Ae.4 was placed in each of 5 sterile tubes to which were added:

Tube 1 : 1 ml. plasma-eosinophil mixture

Tube 2 : 1 ml. plasma-eosinophil mixture

Tube 3 : 1 ml. saline suspension of eosinophils

Tube 4 : 1 ml. serum

Tube 5 : 1 ml. sterile saline

These 5 tubes were agitated so that the extract was dissolved in the various fluids. They were then incubated at 37°C for 1 hour at the end of which they were centrifuged hard. The supernatant fluids were taken off and injected into guinea pigs. Eosinophil counts were then made at intervals for 48 hours.

Results

See Table 13 and Fig. 13.

All the guinea pigs in this experiment showed an increase in the blood eosinophils of a varying degree. The greatest increase took place in guinea pig No. 5 which had received extract incubated with saline only. The eosinophil responses in the other animals were smaller and in one animal (No. 2) so small as to be almost insignificant. All the animals, however, showed a peak level of eosinophils at 12 hours and all the smaller responses (guinea pigs Nos. 1, 2, 3 and 4) had returned to the initial levels by 48 hours. The control animal No. 5

TABLE 13

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected with 30 mgms. Ascaris extract (4) which had been treated as follows:

(a) Incubated with plasma + eosinophils and injected into guinea pigs Nos. 1 and 2

(b) Incubated with eosinophils + saline and injected into guinea pig No. 3

(c) Incubated with serum and injected into guinea pig No. 4

(d) Incubated with normal saline only and injected into guinea pig No. 5

Time after injection	1	2	3	4	5
Initial level	60	90	0	100	70
12 hrs.	750	360	550	510	1230
24 hrs.	680	220	370	510	940
48 hrs.	120	70	20	90	450

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

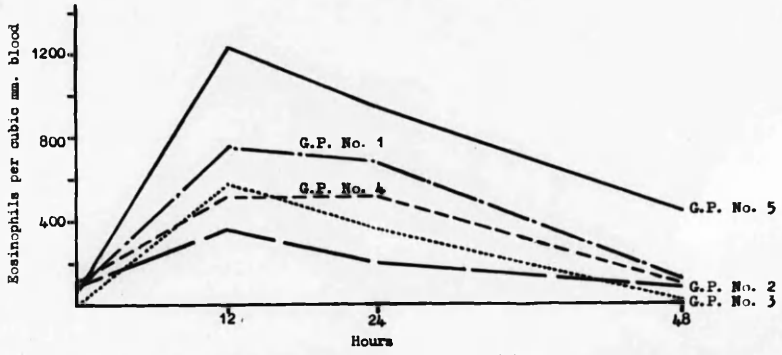


Fig. 13 Normal guinea pigs injected with Ascaris extract (4) which had been incubated with materials as follows:

(a) with eosinophil-rich plasma (G.P. Nos. 1, 2)
(b) with eosinophils in normal saline (G.P. No. 3)
(c) with serum (G.P. No. 4)
(d) with normal saline only (G.P. No. 5).

had not regained its initial level by 48 hours.

Conclusion

It is of interest to compare these results with those obtained in Experiment 3. There is little doubt that in the animals injected with Ae.4 which had been incubated with blood elements, the eosinophil responses are much weaker than in those injected with Ae.4 alone or with the extract after it had been incubated with saline only. In one case, the rise produced was frankly doubtful. From these results it would appear that when Ae.4 is incubated with the materials described there is at least some loss of its eosinophilogenic power. The results do not permit of much more being deduced but perhaps if larger volumes of the blood fractions had been used, some indication of that fraction most active in bringing about this loss might have been gained.

EXPERIMENT 14

Experimental problem

When guinea pigs were injected with Ae.3 which was six months old, they failed to develop an eosinophilia, although the extract had been stored at -10°C. This loss of potency was described by Deschiens but he did not suggest any reason why this should be so. It was decided to test this loss anew by storing at -10°C an extract of undoubted potency and examining its activity after a period of months.

As this loss of potency might be due to the simple exposure of the extract to the oxygen of the atmosphere it was a simple matter to investigate the effect on the extract of exposure to this gas.

The possibility that Ae.4 - "the glycogen fraction" - only possessed its eosinophilogenic effect in virtue of material adsorbed on to it from the more active and more toxic Ae.5 during the separation of these fractions, attracted attention. In this experiment, this also was put to the test.

Procedure

1. A small quantity, about 100 mgms., of Ae.4 was stored at -10°C for 5 months and at the end of this time, a dose of 30 mgms. was injected into a normal guinea pig.

2. About 100 mgms. Ae.4 on a watch-glass was placed in a dessicator containing calcium chloride and the jar filled with pure oxygen. The whole jar was now placed in the refrigerator at -10°C and after 7 days

30 mgms. of the extract was given to a normal guinea pig.

3. To 1.0 gram Ae.4 in a test tube was added 1.0 ml. "Teepol" (a sodium higher alkyl sulphate possessing surface active properties). This was shaken vigorously for a few minutes and then allowed to stand for 1 hour, during which the extract was seen to settle at the bottom of the tube. The tube was centrifuged and the supernatant removed. The extract was then dehydrated by alcohol and acetone and dried by aeration. 30 mgms. of this 'washed' Ae.4 was then given to each of 2 guinea pigs.

Eosinophil levels of these 4 guinea pigs were observed for 24-48 hours.

Results

See Table 14 and Fig. 14.

Guinea pig No. 1, injected with stored extract, showed little change in the eosinophil levels. Guinea pig No. 2 showed a distinct rise in blood eosinophils, maximal at 12 hours and beginning to subside at 24 hours. This animal had been given Ae.4 which had been exposed to an oxygen-rich atmosphere.

The other animals (Nos. 3 and 4) showed little alteration in eosinophil levels following the injection of 'Teepol washed' Ae.4.

Conclusion

Storage at -10°C over months results in a loss of the eosinophilogenic power of Ae.4. As the extract is unaffected by exposure to

TABLE 14

Changes observed in the level of circulating eosinophils in the blood of normal guinea pigs injected with Ascaris extract (4), which had been treated as follows:

(a) Stored at -10°C for 5 months and then injected into guinea pig No. 1

(b) Exposed to an oxygen-rich atmosphere at -10°C for 7 days and then injected into guinea pig No. 2

(c) "Washed" with "Teepol" and injected into guinea pigs Nos. 3 and 4

Time after injection	1	2	3	4
Initial level	10	70	20	70
3 hrs.	40	-	-	-
6 hrs.	50	480	140	70
12 hrs.	50	810	230	120
24 hrs.	20	590	190	20
48 hrs.	-	-	40	-

The figures expressed represent the numbers of eosinophils per cubic mm. blood.

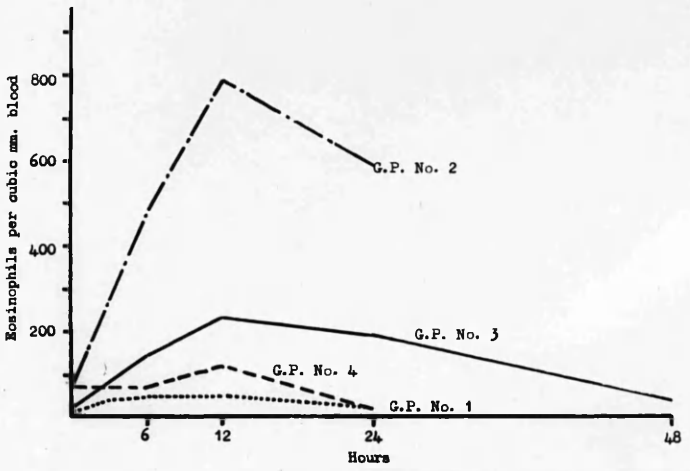


Fig. 14 Guinea pigs injected with Ascaris extract (4) which had been

(a) stored at -10°C for 5 months (G.P. No. 1)
(b) exposed to an oxygen-rich atmosphere (G.P. No. 2)
(c) "washed" with "Teepol" (G.P. Nos. 3, 4).

oxygen for 7 days, it is likely that the manner in which the extract loses its power involves more than just exposure to atmospheric oxygen.

Washing with "Teepol", partially at any rate, deprives Ae.4 of its eosinophilogenic activity. This would suggest that this particular extract possesses the power of stimulating eosinophils only in respect of material adsorbed on to it during the process of separation from Ae.5.

The fact that Ae.5 is a more powerful eosinophilogenic agent and a more toxic substance (Experiment 4) lends support to this conclusion.

EXPERIMENT 15

Experimental problem

It was decided to study the histological changes which took place in the guinea pig following the injection of Ae.4 into the peritoneal cavity.

Procedure

8 normal unsensitised guinea pigs were selected and to each of them were given 30 mgms. Ae.4. These animals were killed by chloroform at 1 hour, 3 hours, 4 hours, 8 hours, two at 12 hours, 24 hours and the last at 48 hours after injection. Autopsies were made and tissues removed for histological study. The specimens removed included samples of bone marrow, sternum, lung, kidney, liver, spleen, peritoneum, intestine and lymph nodes.

Results

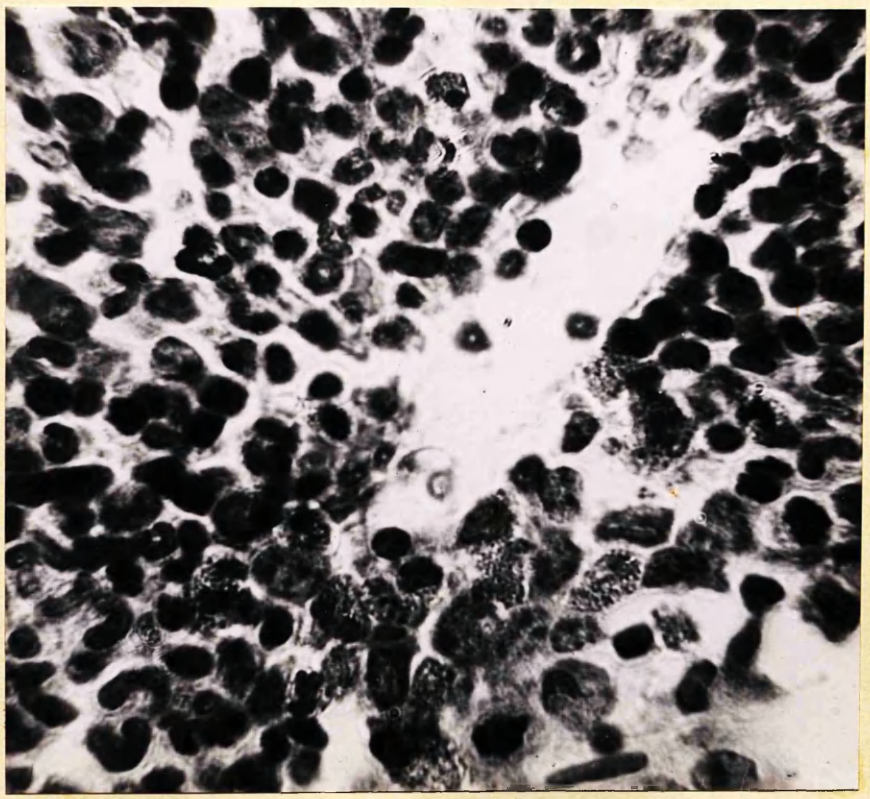
See Plates 1-14.

1 hour. The peritoneum was seen to contain a small number of leucocytes, many of which were eosinophils. These were in the substance of the peritoneum and around the subjacent blood vessels.

The sternal marrow showed an increase in the proportion of eosinophil myelocytes and leucocytes to the other nucleated cell elements and were arranged in foci. (Plate 1)

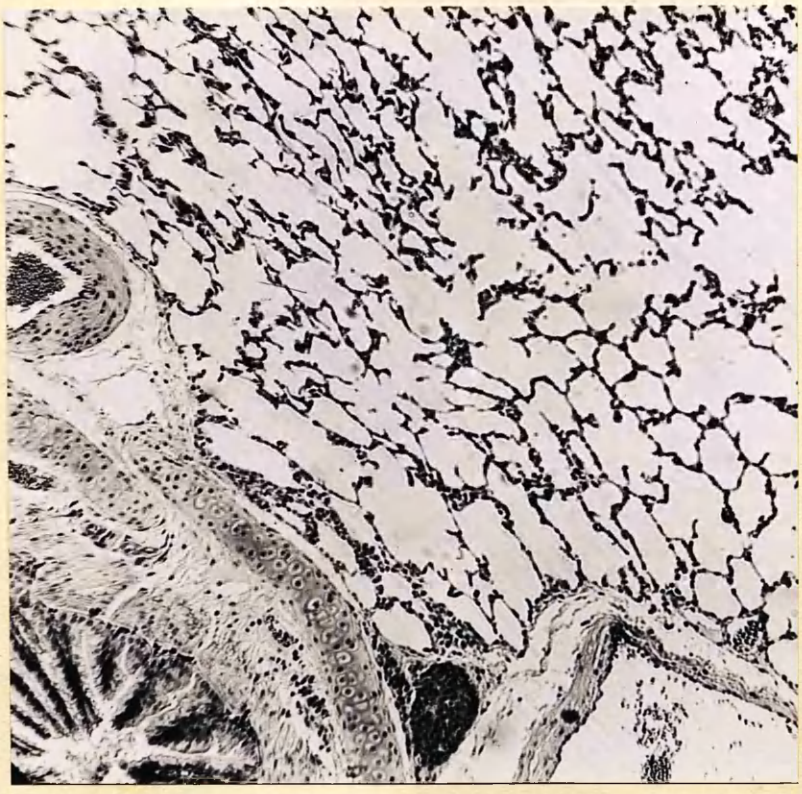
Sections of lung were normal. (Plate 2)

PLATE 1



Eosinophil myelocytes and leucocytes in the sternal marrow of a guinea pig killed 1 hour after being given Ascaris extract.
Stained H. and E. (x 950)

PLATE 2



Section of lung from a guinea pig killed 1 hour after being given Ascaris extract.
Stained H. and E. (x 100)

The level of eosinophils in the blood of this animal at death was 40 per cubic mm.

3 hours. Eosinophils were seen in the wall of the intestine, chiefly around the blood vessels and several were noticed between the vessels and the villi into which a few had penetrated. Comparatively few neutrophils were seen. (Plate 3)

The sternal marrow showed an increase in eosinophils similar to that shown in the specimen taken at 1 hour.

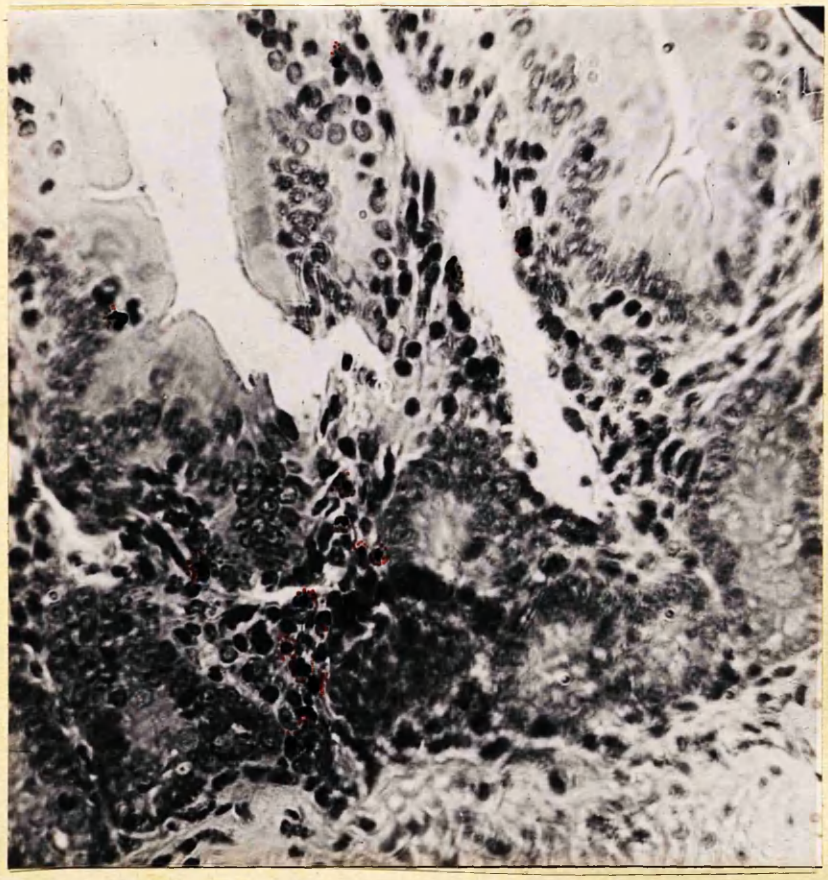
It was in the lung, however, that the most striking changes were seen. There was considerable thickening of the alveolar walls, especially near the vessels and bronchi (Plate 4). On closer examination, this thickening was seen to consist predominantly of eosinophil leucocytes which had infiltrated the alveolar walls. These could also be seen in and around the blood vessels and in the peri-bronchial tissue.

A few eosinophils were seen in the pelvis of the kidney but no actual formation of cells was observed. Spleen and liver were normal.

The level of eosinophils in the blood of this animal at death was 40 cells per cubic mm.

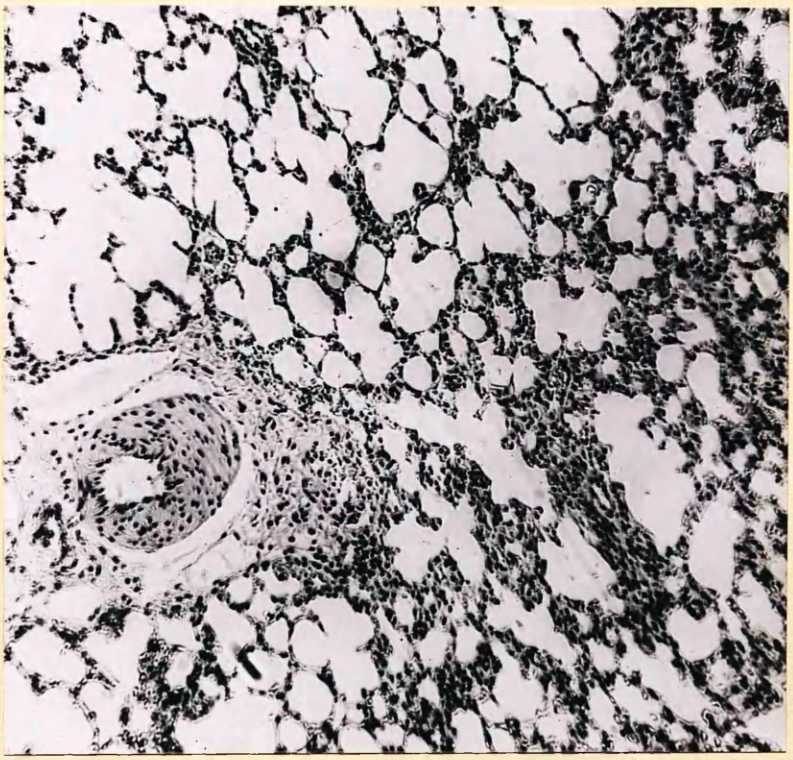
4 hours. The lung changes were now more marked (Plate 5). In the blood vessels of the lung, the eosinophils could be seen arranged in 'pavementing' fashion and could be easily traced into the surrounding tissues. (Plate 6). Around the bronchi, collections of eosinophils were seen some of which had penetrated the wall of the bronchus and a

PLATE 3



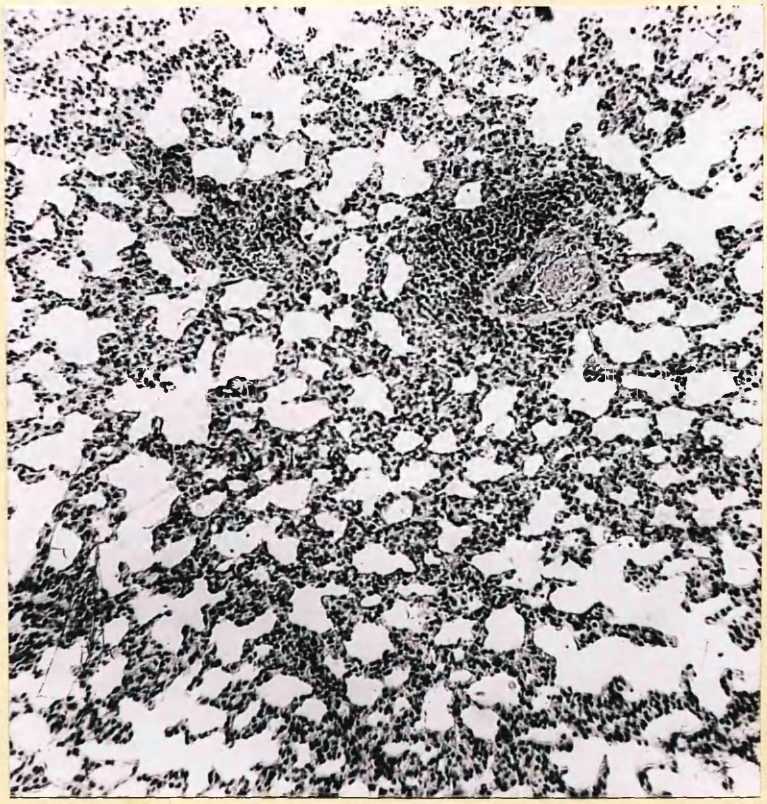
Small intestine of a guinea pig killed 3 hours after being given Ascaris extract. Eosinophils can be seen between the blood vessel in the foreground and the intestinal villi.
Stained H. and E. (x 475)

PLATE 4



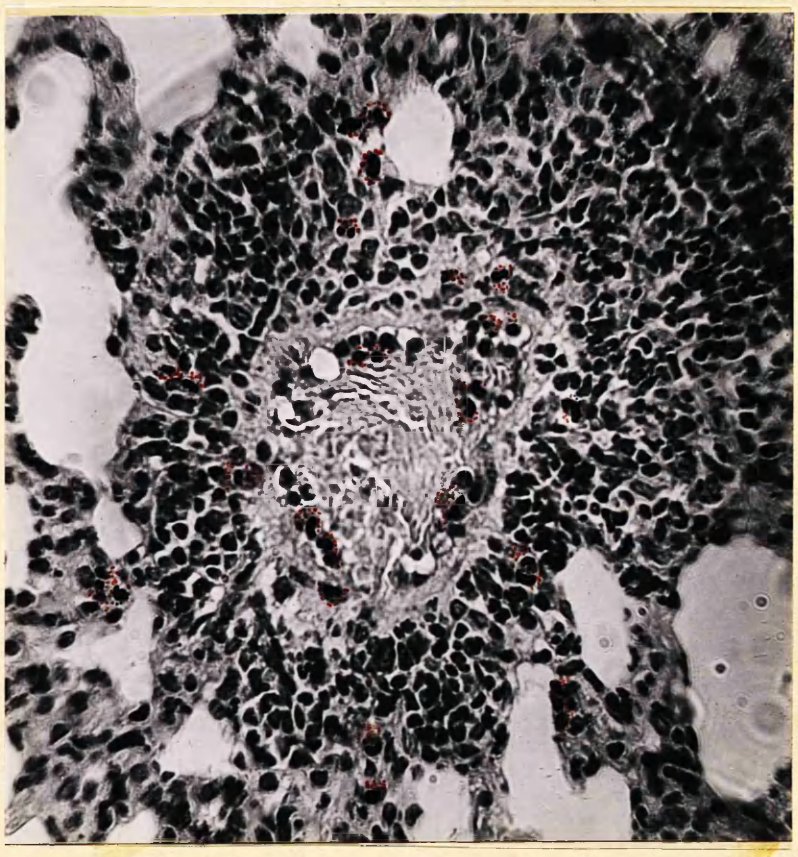
Section of lung from a guinea pig killed 3 hours after being given Ascaris extract. The alveolar walls are thickened and by high power many of the infiltrating cells are seen to be eosinophil leucocytes.
Stained H. and E. (x 100)

PLATE 5



Section of lung from a guinea pig killed 4 hours after being given Ascaris extract. The infiltration proceeds and the eosinophil leucocytes abound.
Stained H. and E. (x 100)

PLATE 6



Blood vessel of lung shown in Plate 5. Eosinophils are seen arranged peripherally in the vessel ('pavementation') prior to emigration. These cells can also be seen in the perivascular tissue. Stained H. and E. (x 475)

few were actually lying free in the lumen (Plates 7 and 8). The alveolar walls were infiltrated as before, and an occasional eosinophil lay free in the alveolar spaces.

Eosinophils were also present in the vessels of the spleen, liver and kidney while the sternal marrow showed increased activity of the eosinophil elements.

The level of eosinophils in the blood of this animal at death was 230 cells per cubic mm.

8 hours. The infiltration of the lung was now dense amounting in places almost to consolidation. The eosinophils were again 'pavemented' in the blood vessels, and were again to be seen in the perivascular and peribronchial tissues, in the bronchial and alveolar walls, and lying free in the bronchial lumens and in the alveolar spaces.

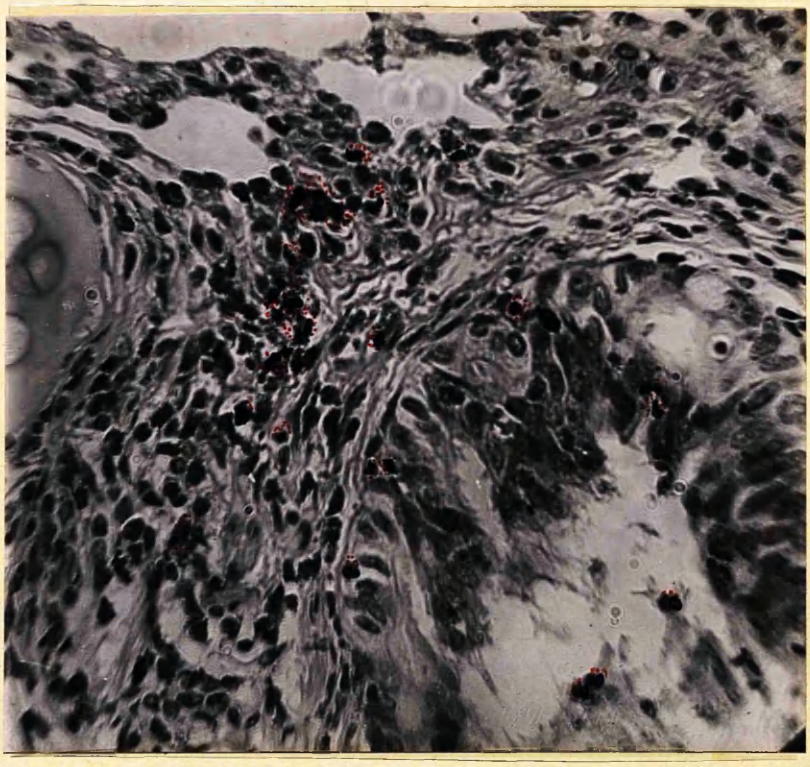
In the spleen several eosinophils were seen in the pulp. None was seen in the peritoneum.

The level of eosinophils in the blood of this animal at death was 1,150 cells per cubic mm.

12 hours. Eosinophilia of the marrow and infiltration of the lungs were observed in the animals killed at 12 hours. Eosinophils were also to be seen in the splenic pulp in rather greater numbers than previously (Plate 9), and also in the submucosa and villi of the intestine. In the liver and kidney the eosinophils were confined to the blood vessels.

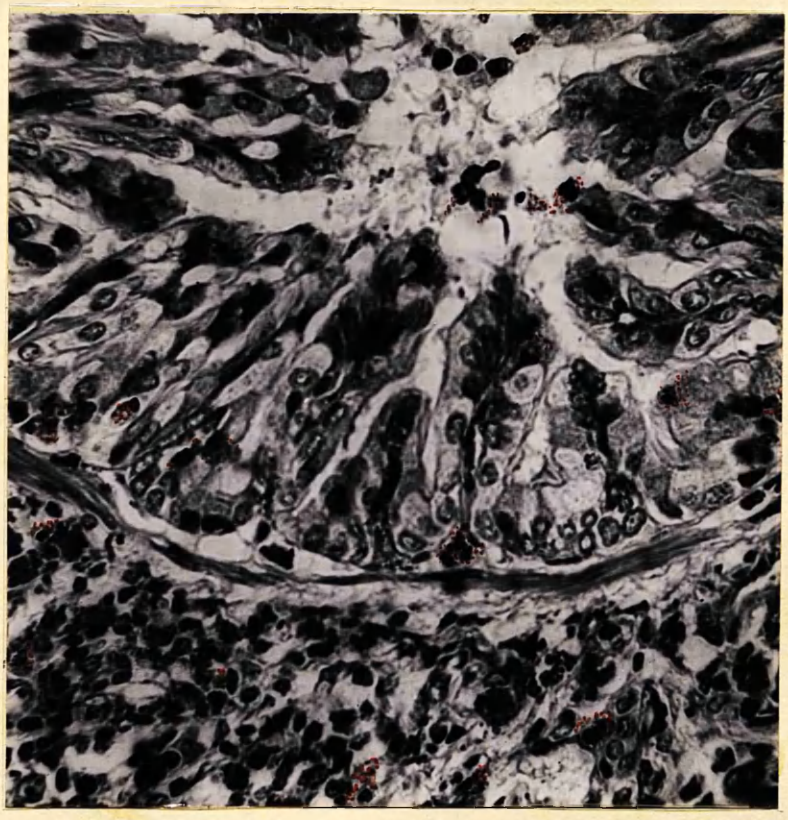
The levels of eosinophils in the blood of these animals at death were 1,020 and 1,130 cells per cubic mm.

PLATE 7



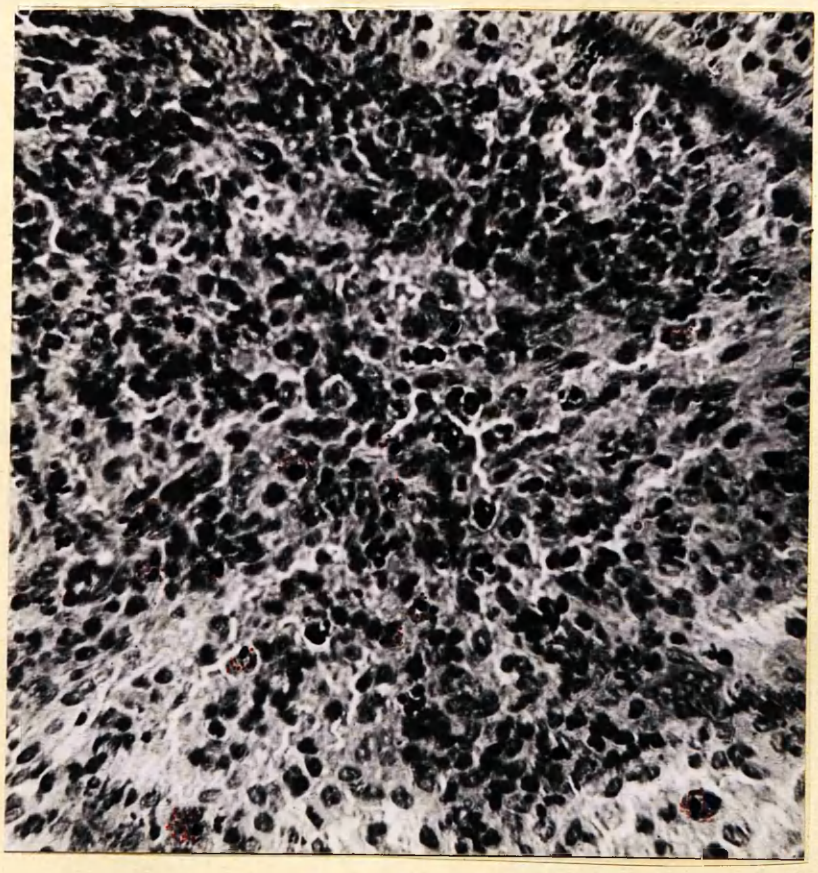
Bronchus from lung shown in Plate 5. Eosinophils are seen in the peribronchial tissue, in the bronchial wall, and lying free in the bronchial lumen.
Stained H. and E. (x 475)

PLATE 8



Bronchus from lung shown in Plate 5. Eosinophils are again seen in the peribronchial tissue and in the mucosa and lumen of the bronchus. Stained H. and E. (x 600)

PLATE 9



Section of spleen from a guinea pig killed 12 hours after being given Ascaris extract. A few eosinophils can be seen in the splenic pulp.
Stained H. and E. (x 475)

24 hours. The changes in marrow and lung already described were again present in this animal. Infiltration of the lungs was still in progress and 'pavementation' with emigration of the eosinophils could still be seen (Plate 10). Eosinophils were also seen in the small peribronchial lymphatics and a few were noticed in the hilar lymph glands (Plate 11).

There was by this time a notable increase in the number of eosinophils caught in the splenic pulp (Plate 12), but in the liver and kidney extravascular eosinophils were not seen.

The level of eosinophils in the blood of this animal at death was 1,120 per cubic mm.

48 hours. Sections of lung taken at this stage showed that the infiltration had cleared a little though emigration of eosinophils still continued (Plate 13). Clumps of cells persisted around the vessels, the bronchi, and at the periphery, but the alveolar thickening was much reduced. (Plate 14) A few cells were observed in the peribronchial lymph nodes.

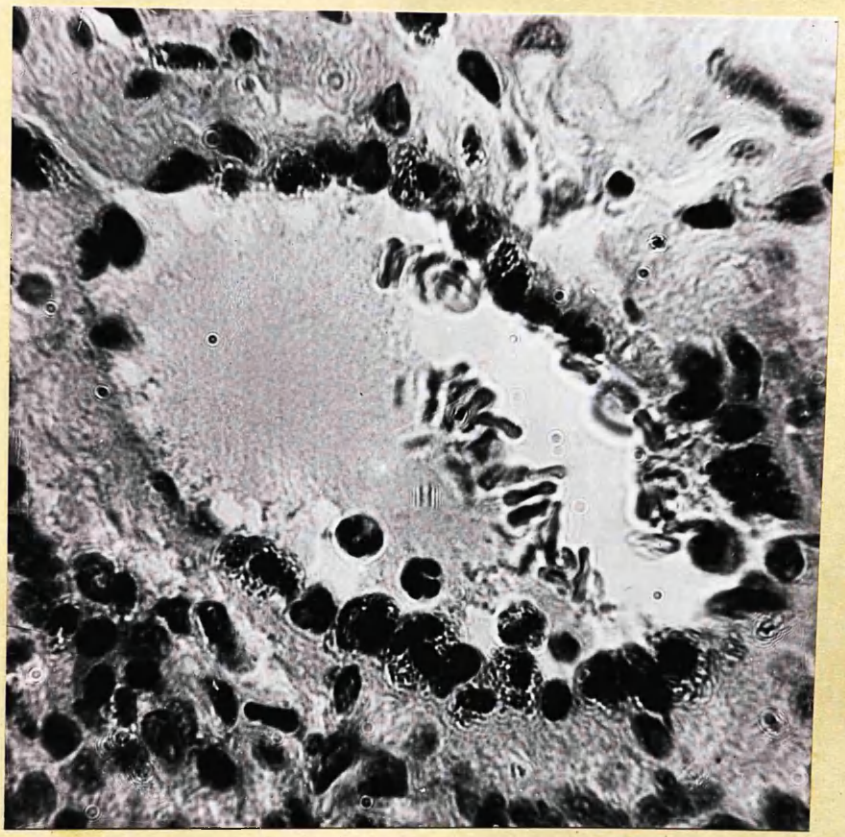
In the intestine a few eosinophils were found in the villi.

The level of eosinophils in the blood of this animal at death was 1,000 cells per cubic mm.

Conclusion

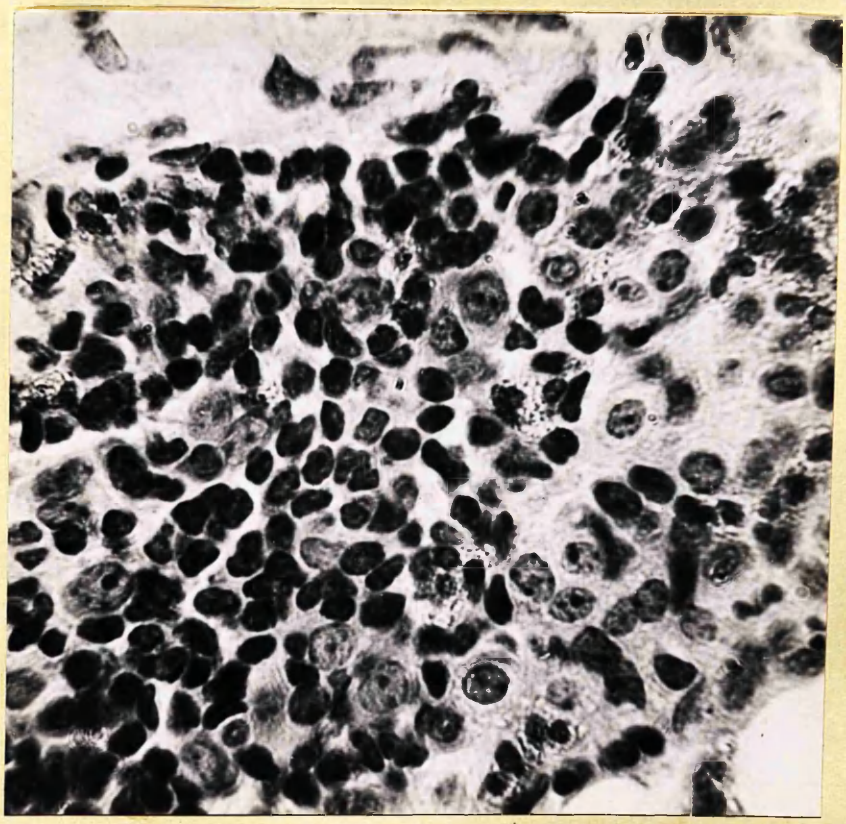
After the injection of an extract of Ascaris into the peritoneal cavity of a normal guinea pig, the bone marrow soon becomes hyperactive

PLATE 10



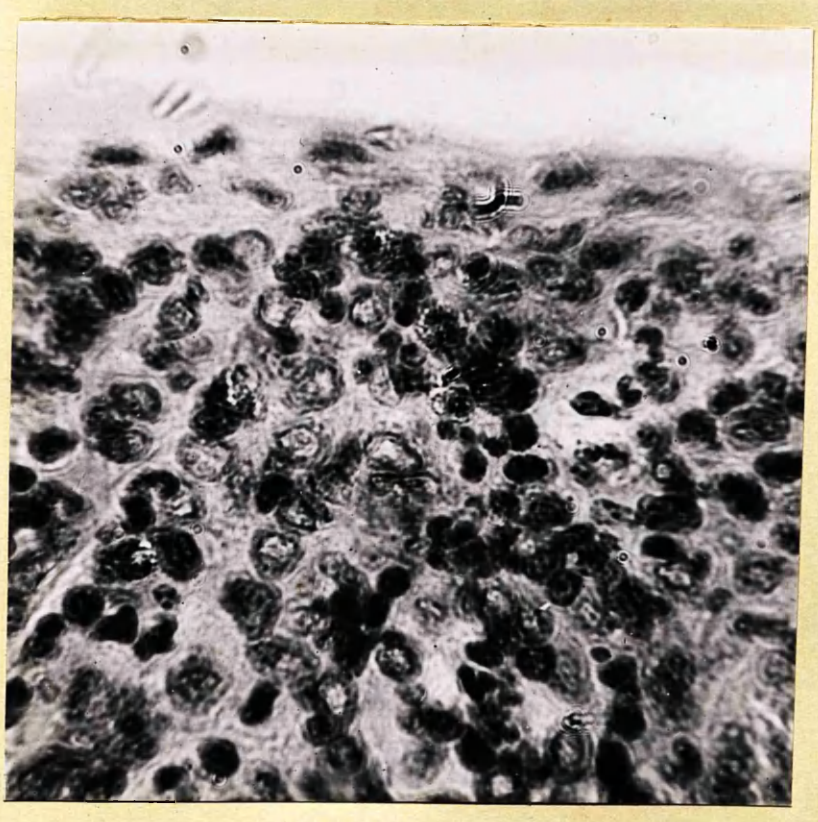
Blood vessel in the lung of a guinea pig killed 24 hours after being given Ascaris extract. Eosinophil leucocytes can be seen in the stages of emigration from the vessel.
Stained H. and E. (x 950)

PLATE 11



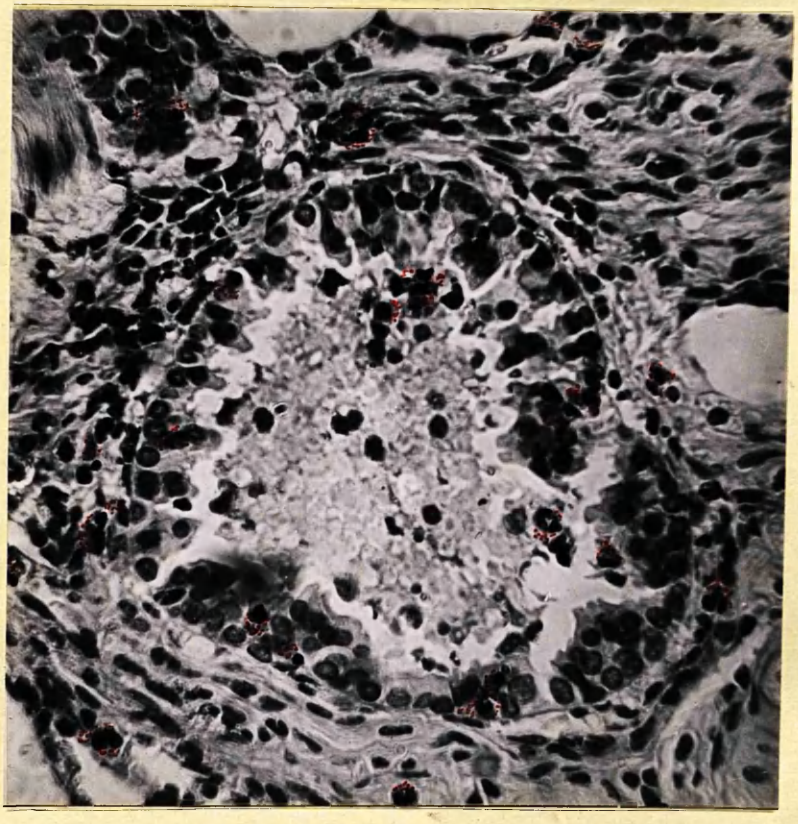
Peribronchial lymph follicle from the lung shown on Plate 10. Eosinophil cells can be seen in the lymphoid tissue.
Stained H. and E. (x 950)

PLATE 12



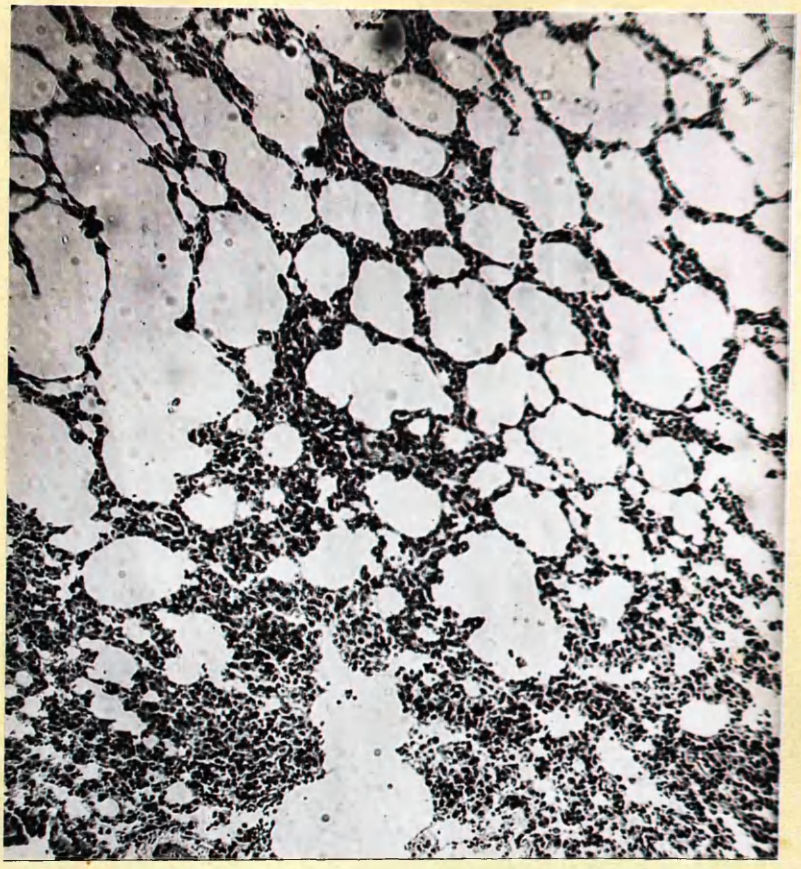
Section of spleen from a guinea pig killed 48 hours after being given Ascaris extract. Numerous eosinophils can be seen.
Stained H. and E. (x 950)

PLATE 13



Blood vessel in the lung of a guinea pig killed 48 hours after being given Ascaris extract. Emigration of eosinophils can still be seen.
Stained H. and E. (x 475)

PLATE 14



Section of lung from a guinea pig killed 48 hours after being given Ascaris extract. Infiltration is not now so dense as in Plate 5, although islands of eosinophilic aggregations still persist. Stained H. and E. (x 100)

the eosinophil elements being conspicuous. Apart from the presence of a few eosinophils in the peritoneum, this was the earliest change seen. Two hours later the lungs are found to be infiltrated with eosinophil leucocytes, these cells having obviously emigrated from the blood stream, the typical preliminary 'pavementation' being observed. Eosinophils are found in the peribronchial tissues and can be seen at various stages in the penetration of the bronchial wall, and eventually lying free in the bronchial lumen. As the peak in the blood eosinophilia is reached eosinophils become more numerous in the pulp of the spleen and later, when the infiltration of the lung and the eosinophilia of the blood subside, these cells are observed in the lymphoid tissue of the lung and the intestinal mucosa, as well as in the spleen. In the liver and kidney few eosinophils were observed outwith the vascular system.

It would seem reasonable to suggest that the path of the eosinophil leucocyte through the body follows the chronological order of the changes recorded above. The eosinophil, formed in the marrow, enters the blood-stream in which, after passing the peritoneum, it is carried to the lungs. Here it leaves the blood and enters the lung, passing from the vessel to the alveolar walls. Some go on to penetrate the bronchus and once in the bronchial lumen, leave the body by that route. Others may be dealt with by the lymphatic system, or on re-entering the blood-stream either directly or via the lymphatics, may meet their fate in the spleen or pass out of the body through the intestinal villi.

The relation between the probable path of the eosinophil leucocyte through the body and its function will be discussed later.

EXPERIMENT 16

Experimental problem

In view of the possibility that changes similar to those described in Experiment 15 might follow the administration of histamine, examination of two animals killed by this drug was undertaken.

Procedure

2 normal guinea pigs which had been injected with 0.25 mgms. histamine phosphate 48 hours earlier were given a fatal dose of 4.0 mgms. of the same drug intra-peritoneally. The animals died in minutes. They were immediately examined and tissues removed for histological examination.

Results

See Plate 15.

The findings in both animals were almost identical. The sternal marrow showed a hypercellularity which was due to an increase of granular cells in which the eosinophil elements were prominent.

The lungs were seen to be infiltrated with leucocytes which on closer examination were seen to be eosinophils (Plate 15). There were signs of the emigration of eosinophils from the blood vessels and these cells were observed in the perivascular and peribronchial tissues as well as in the walls and the lumens of the bronchi. The peritoneum, liver, spleen and kidneys showed no relevant changes.

PLATE 15

Section of lung from a guinea pig killed 48 hours after an injection of histamine. The infiltration is predominantly eosinophilic.
Stained H. and E. (x 100)

Conclusion

When histamine is administered to guinea pigs, an increase in the activity of the bone marrow takes place in which the eosinophil elements are prominent. There occurs too, an infiltration of the lungs by eosinophil leucocytes similar to that which was reported in the previous experiment.

CHEMICAL AND PHYSICAL PROPERTIES OF THE EXTRACTS

The following observations and chemical tests have been carried out on Ascaris extracts (4) and (5). It was considered unnecessary to examine the others since Ae.2 and Ae.3 are respectively identical with Ae.5 and Ae.4, and Ae.1 represents the combination of Ae.2 and Ae.3.

Ascaris Extract (4) - Ae.4

Ae.4 is a white amorphous powder which is very light in weight and is readily soluble in water. The solution, which is neutral to litmus, is white and opalescent like starch. It does not pass through a semi-permeable membrane.

1. Tests for protein

Heat coagulation test :	Negative
Salicyl-sulphonic acid test :	Negative

2. Test for reducing substances

Benedict's test :	Negative
Seliwanoff's test :	Negative

3. Tests for Polysaccharide

Iodine test : With iodine the solution gives a slightly reddish colour.

Hydrolysis : To 1 vol. solution was added 1 vol. 6N hydrochloric acid, and the mixture boiled for 30

minutes, the solution becoming clear. Some of the solution was removed and a Benedict's test carried out. This was negative, so the mixture was boiled for a further 30 minutes and tested again. This time a positive result was obtained. A Seliwanoff's test performed on the hydrolysed solution was negative.

Molisch test :

(a) using a-naphthol : Positive

(b) using thymol : Positive

Conclusion

These tests show that Ae.4 is a polysaccharide of high molecular weight resembling glycogen in its properties.

Ascaris Extract (5) - Ae.5

Ae.5 is a yellow crystalline substance, readily soluble in water giving a yellow solution which is neutral to litmus. It does not pass through a semi-permeable membrane.

1. Heat Test

The effect of heat was observed. The crystals charred, became progressively smaller, finally leaving no residue. An ammoniacal odour which was alkaline to litmus, was given off.

2. Tests for protein

Xanthoproteic reaction :	Negative
Salicyl-sulphonic acid test :	Negative
Biuret reaction :	a faint violet colour

3. Tests for Amino-acids (free and combined)

Millon's test - tyrosine :	Negative
Sakaguchi reaction - arginine :	Negative
Sulphur reaction - S groups	Negative
Nitroprusside test - Cystine, cystein :	Negative
Ninhydrin test - free amino-acids :	very weak positive
Bromine test - free tryptophane :	Negative

Conclusion

Ascaris extract (5) is an organic nitrogenous compound of high molecular weight, containing no protein and very little proteose.

As it has only a trace of free amino acid, it is likely that it represents a stage in protein catabolism before that level, and is probably, therefore, a polypeptide or a mixture of polypeptides.

DISCUSSION

The stimulus to the formation of the eosinophil leucocyte

By injecting drugs into guinea pigs it has been shown that whereas there is no doubt as to the eosinopenic effect of the sympathetico-mimetic drugs, it is not so certain that parasympathetico-mimetic drugs have any eosinophilic effect. Indeed it was observed that in strictly normal animals these drugs did not have any effect whatsoever (Experiments 7, 8 and 10). It is true that a rise in the eosinophil level of already eosinophilic guinea pigs was produced by both acetylcholine and physostigmine, but this point has been met by the demonstration that exactly such a change, namely the raising of an already high level of eosinophils in a guinea pig, is one of the effects of a vasodilator drug (Experiment 11). When one visualises the marrow of such an animal packed with maturing eosinophil myelocytes and fully developed leucocytes, it is easy to understand how a sudden increase in the flow of blood can carry off large numbers of these cells into the general circulation, producing an eosinophilia. This suggests that any activity that acetylcholine and physostigmine may possess in raising the eosinophil count in such animals is solely in respect of the vasodilatation they produce - one of their parasympathetico-mimetic effects.

Perhaps we should bear in mind another property that acetylcholine, physostigmine and hexamethonium have in common and that is relative depression, if not inhibition, of the sympathetic nervous system. In

view of the eosinopenia produced by adrenaline this may be of some significance but the fact that none of these three drugs has brought about an eosinophilia in animals with a low initial eosinophil level, as have the extracts, makes it doubtful. Further, it has been shown (Experiment 9) that complete inhibition of the parasympathetic nervous system did not in the least way have any effect on the eosinophilogenic action of the Ascaris extract, so proving that the stimulation of eosinophil production by the extract does not depend on the parasympathetic nervous system. In the light of these findings it is unlikely that the autonomic nervous system is concerned in the stimulus of formation of the eosinophil.

Some of the earlier evidence which has been submitted on this subject may appear to oppose such a conclusion. In 1910, Bertelli, Falta and Schweeger, and later Wieck, claimed to have produced an increase in eosinophils by injections of pilocarpine. This effect may very well correspond with that demonstrated in parasympathetico-mimetic drugs by which the eosinophil level of an already eosinophilic guinea pig could be increased, because Wieck's "guinea pig" was a 58-year-old hausfrau who had a blood eosinophilia of 20%. This was raised to 28% by pilocarpine. Campbell's (1943) findings that acetylcholine enhanced the eosinophilic effect of a second injection of antigen, in some measure agrees with the results obtained in the present experiments. The effect of direct stimulation of the vagi with electric current as was observed by Hajos, Nemeth and Enyedy produced such profound changes

in the animals that the minor blood variations recorded are extremely difficult to interpret. Moreover, in all these it is important to bear in mind the changes in the blood eosinophils which may be brought about by vasodilatation alone.

The claims of atropine to have an eosinopenic action have been put forward by several authors. In Experiment 9 such a change was not observed in the only animal treated with atropine alone, but in fact a slight rise was recorded. Be that as it may, the same experiment clearly demonstrated that the eosinophilic activity of the extract did not depend on the integrity of the parasympathetic nervous system.

So although at first sight the past evidence appears to conflict with such a conclusion, there is little to gainsay the view expressed above.

The likelihood that the essential stimulus is in the nature of a chemical action appears more probable. The multiplicity of hypotheses, and their individual complexity, e.g., anoxia, acid-base balance, adrenal cortical activity, tissue acidity, Ascaris extracts, Nirvanol therapy, the presence of foreign protein, etc., etc., present a confusing picture. These substances or conditions do not in themselves necessarily constitute the essential stimulus but merely supply the raw materials or provide suitable circumstances for a chemical reaction to take place in which the stimulating agent appears. There is one fairly common factor whose presence in most of these states has long been suspected, and that is the toxic amine, histamine, and it is of interest to find

that histamine, despite previous reports to the contrary, readily produces an eosinophilia in normal guinea pigs. It has been shown (Deschiens) that Ascaris extracts are not rich in histamine, but the obvious relation to their contained polypeptides and other protein end-products cannot be overlooked. This association with proteolysis persists throughout the majority of clinical conditions exhibiting an eosinophilia, while it is commonly accepted that histamine is released in antigen-antibody reactions (Best and Taylor).

In all the examinations of tissues during these experiments, in no instance was eosinophil formation observed other than within the bone marrow.

The function of the eosinophil leucocyte

Whatever substance stimulates the formation of eosinophils, it would not be surprising that a similar if not identical substance was the object of its function. Although the success of Experiment 13 in demonstrating the neutralising power of the eosinophil leucocyte was by no means 'unqualified', the results were encouraging. In any case, most authors to-day are agreed in attributing to this cell some measure of activity in removing or neutralising toxic materials.

Following the injection of extracts of Ascaris there takes place rapidly an eosinophilic reaction in the bone marrow and shortly afterwards the lungs become infiltrated by eosinophil cells. If we relate this sequence of events to the function of the cell as generally

regarded, the aggregation of eosinophils in the lungs must be due to the fact that in the lungs is the greatest concentration of injected material or its derivatives in the body, apart possibly from the site of injection. In this respect it is of interest that Campbell and Nicol (1940) have shown that the lung of a sensitised guinea pig contains a substance or substances which can produce anaphylactic shock in animals. If, then, the lung does contain Ascaris extract or substances derived therefrom, how did they reach that organ from the peritoneal cavity into which they were injected?

It may be that the concentration of toxic material, and hence of eosinophils, in the lung are merely commensurate with their distribution throughout the body. This is unlikely since the degree of infiltration in the lung is striking, by far exceeding anything seen in the other tissues. If, on the other hand, the material is being excreted by the lung as are acetone, urea and other substances, the presence of the eosinophil would seem superfluous and its journey from the bone marrow apparently purposeless, although the mediation of the eosinophil might well be required to accomplish such a task. Another suggestion, and one which is probably nearer the truth, is that the toxic material is conveyed to the lung by the eosinophil cell.

Obviously the solubility of the material injected and the readiness with which, if necessary, it is converted into the active eosinophilogenic substance, will determine response in the tissues and in the blood. In the present case the extract injected was soluble and indeed

was so easily assimilable that within an hour the bone marrow had been stimulated to produce eosinophils. On the other hand, had the material been of a more insoluble nature, or perhaps not releasing the active agent so quickly, then a tissue eosinophilic reaction might have been expected as the cells came to collect the insoluble toxic substance, with a retardation of the appearance of the eosinophilia of the blood as the toxin-laden cells journeyed to the lungs. In this case, however, there was only slight eosinophil activity seen in the peritoneum. This we can readily relate to the solubility of the extract.

The eosinophils did not increase notably in the blood until 4 or more hours after the injection. Nevertheless, in an animal killed 3 hours after the injection in which the eosinophil level stood at 40 per cubic mm. at death, there had already taken place a noticeable infiltration of the lung. This would suggest that the eosinophils were purposefully drawn to the lung rather than deposited there by chance on their passage through the blood vessels of that organ. This view is strengthened by the subsequent observation of active emigration of the eosinophil leucocytes from the vessels into the perivascular tissues. As the eosinophils in the blood rose so the infiltration of the lung became more dense. Inevitably the question comes to mind, why should the eosinophils carry toxic materials to the lungs? In answer to this question perhaps it may be of value at this point to consider the fate of histamine in the body.

Histamine is normally produced in small amounts in the body chiefly

by bacterial action on the amino-acid histidine. These small amounts of histamine are destroyed by an enzyme system - the histamine-histaminase reaction (Best and McHenry, 1930). This takes place in the small intestine as well as at other places, and is sufficient to account for all the histamine normally produced. It is commonly accepted that histamine is released in antigen-antibody reactions and although the exact mechanism is unknown, experimental studies (Best and Taylor) suggest that the antigen reduces the anti-tryptic activity of the cell, thus allowing the intra-cellular trypsins to exert their proteolytic effect with the release of histamine. In the liver of sensitised animals there is an increase in histamine content which, whenever an allergic or anaphylactic reaction takes place is released into the general circulation. This of course, swamps the histaminase of the intestine and kidneys and may very well, owing to the increase of anti-toxic measures necessitated, be the reason for the eosinophilia accompanying such reactions. Eosinophils, which, as Code has observed, can contain histamine, might well carry this toxic substance to the lungs, for it has been shown that eosinophils infiltrate the lungs following an injection of histamine. But why the lungs? The presence of histaminase has not been demonstrated in the lungs but one of the requirements of the histamine-histaminase reaction is present, namely, oxygen. Best and McHenry have shown that this reaction is very sensitive to the lack of oxygen and consider that the destruction of histamine is an oxidative process. The presence of oxygen and the numerous oxygen-binding compounds, if

not actual histaminase, may be the reason that eosinophils are attracted to that organ. Histaminase apart, such compounds could, in virtue of their activity in oxygen exchange reactions, perhaps help in the destruction of toxic material or in the excretion of the wholly or partly neutralised toxin.

If we consider that the other eosinophilogenic materials and reactions give rise to histamine or a histamine-like substance, then the occurrence of eosinophilic infiltration of the lungs can be explained along those lines.

If all this be true one would expect that the eosinophil, having reached the lung and thereby having fulfilled its function, would then either be destroyed or discharged from the body. That this is indeed the case is suggested by the fact that eosinophils have been seen escaping into the bronchi, into the intestine, into the lymph nodes and caught in the pulp of the spleen. The discharge of cells into the natural passages and their presence within the elements of the reticulo-endothelial system are recognised steps in the process by which the body rids itself of effete blood cells.

In allergic tissue reactions the presence of eosinophils locally is probably due to the slow rate of release of histamine, the solubility of the antigen no doubt playing a part - the more soluble agents producing a general rather than a local eosinophilia.

It remains to discover whether any clinical support is obtainable for this hypothesis. Löffler's description of pulmonary infiltration

associated with a blood eosinophilia, and his conclusions that the infiltrations were of an eosinophilic nature, focussed medical attention on this feature. Engel (1935), in China, reported cases of "privet cough", a seasonal allergic condition, which was almost certainly a pulmonary eosinophilic infiltration and such infiltrations have been reported in asthmatic children (Söderling, 1939), in tropical respiratory disease (Frimodt-Möller and Barton, and other workers) and in many parasitic affections including ascariasis, fascioliasis, trichiniasis, ancylostomiasis, strongyloidiasis, schistosomiasis, and amoebiasis (Ball, 1950), as well as in a variety of allergic states including periarteritis nodosa.

The reason that these infiltrations have not been reported more often probably lies in two facts. Many allergic conditions, e.g. eczema, are chronic and in that respect do not give rise to a degree of infiltration likely to capture attention at radiological examination or macroscopically at least, at autopsy. Further, the cases of these diseases which arrive at post-mortem are not numerous. One report by Viswanathan (1947) of a post-mortem examination carried out on a case of tropical eosinophilia is of interest. He noted that in sections of the lungs the eosinophil infiltration seemed to show a peribronchial distribution. This led him to regard the bronchus as being the mode of entry of the toxic material into the body. A similar picture of peribronchial infiltration was seen in the sections of lung from guinea pigs killed 48 hours after Ascaris extract injection.

Here however, the infiltration was clearing, leaving residual clumps of eosinophils many of which were peribronchial in distribution.

The clinical and pathological observations quoted above lend support to the view that some degree of pulmonary infiltration by eosinophil leucocytes is an integral part of an eosinophilic reaction in the body as manifested by either a tissue or a blood eosinophilia or both. The occurrence of an eosinophilia indicates that the body is in the process of dealing with some toxic substance which it has acquired either as a result of an immunological reaction or which has been introduced by such diverse agencies as parasitic helminths and therapeutic injections. Although familial eosinophilia can be explained along these lines, there being a tendency in such cases to use large numbers of eosinophils to compensate for some inherent abnormality of, say, metabolism, the question of eosinophilic leukaemia must be considered in conjunction with the other leukaemic states.

CONCLUSION

It would seem that a direct chemical action on the marrow is the manner in which eosinophil formation is stimulated. Although the nature of this chemical stimulus is unknown there are good reasons for supposing that it is a toxic material, related to protein and occurring in antigen-antibody reactions. Histamine answers such a description and furthermore, has been shown to possess eosinophilogenic properties. The chemical stimulus is probably histamine or a closely related compound.

That the material which stimulates the eosinophil is also the object of its function is not surprising. In the light of previous work and the results of the present experiments it is likely that the eosinophil, formed in the marrow, is attracted to the area of maximum concentration of the toxic material, whence it carries this material to the lung. There the toxic substance is either neutralised or destroyed by enzymatic or other chemical reactions in the presence of oxygen, or is discharged from the body, the neutralisation of the toxin having already occurred within the cell, and excretion of the products effected by the lung. Having thus fulfilled its function the eosinophil meets a fate similar to that of other effete blood cells in that it is either discharged into the body passages, or dealt with by the reticulo-endothelial system.

Thus the presence in the body of a toxic material such as may readily be dealt with by the eosinophil leucocyte results in a local or general eosinophilia, or both, accompanied by a greater or lesser degree of eosinophilic infiltration of the lung.

SUMMARY

1. The early history of the eosinophil leucocyte has been briefly presented with a review of the numerous suggestions made in the past concerning its nature and function.

2. A short account is given of the clinical occurrence and the experimental production of eosinophilia.

3. From Ascaris suum extracts have been prepared and their ability to produce eosinophilia in guinea pigs demonstrated.

4. The mode of action of these extracts has been investigated with reference to the part played by the sympathetic and parasympathetic nervous systems.

5. An unsuccessful search has been made for an eosinophilogenic substance in the blood of guinea pigs after an injection of extract.

6. The effect on the eosinophilogenic property of an extract of incubation with eosinophil leucocytes and other blood elements, and the action of chemical and physical agents has been observed.

7. The blood and tissue changes taking place in the guinea pig following an injection of extract have been closely studied and the probable path of the eosinophil leucocyte through the body traced.

8. The eosinophilogenic effect of histamine in guinea pigs has been

demonstrated and some of the tissue changes following death by this drug recorded.

9. A limited investigation of the chemical properties of the extracts has been carried out.

10. Conclusions as to the nature of the stimulus of eosinophil formation and the function and fate of this cell have been drawn.

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