THE NATURE OF RUSSELL BODIES AND KURLOFF BODIES

OBSERVATIONS ON THE CYTOCHEMISTRY OF PLASMA CELLS AND RETICULUM CELLS

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This paper describes the use of cytochemical methods to demonstrate the presence of a polysaccharide-containing substance which is either mucoprotein or glycoprotein in the cytoplasm of plasma cells and reticulum cells, in Russell bodies, and in the Kurloff bodies of guinea-pig lymphocytes. The work is based on the observation that the cytoplasm of a proportion of plasma cells stains pink by the periodic-acid-Schiff technique of McManus (1946, 1948) and Hotchkiss (1948), which demonstrates polysaccharides in the tissues. From the cytoplasm of plasma cells to Russell bodies in plasma cells is a logical step, while Kurloff bodies and Auer bodies in human myeloblasts were examined on account of a suggestion by Downey (1938) that all three are possible homologues.

Russell Bodies

The development of intracellular acidophil hyaline bodies, now known to take place in plasma cells, has been recognized since 1890 when William Russell, then pathologist to the Edinburgh Royal Infirmary, first described them as the "characteristic organisms of cancer." He regarded them as fungi, and for their demonstration used eosin and logwood or carbol fuchsin and iodine green. Russell examined a large variety of tissues and came to the conclusion that the acidophil bodies were not found in degenerations or in typhoid, tubercle, or inflammatory processes in general. Exceptions to this rule he found in gummata and in a case of "gelatinous degeneration of the knee joint." Simple tumours did not contain the bodies; but he found numerous examples in a wide variety of malignant growths. He noted their distribution especially in the small round-cell infiltration in the margin of cancers

where they occur, he says, in little clusters of two, three, four, or five, up to twenty or more, and "the larger clumps are held together by a delicate cementing substance which stains faintly."

Since then many views have been expressed as to the precise nature of Russell bodies and three main theories have been evolved. First, Downey (1911), Kingsley (1924), and others regard the bodies as pathological secretions or as aggregations of the normal secretions formed as the result of degeneration in the parent plasma cell. Subsequently, it is thought, death of the parent cell liberates the bodies into the tissues. Secondly, Jordan and Speidel (1929) and Dawson and Masur (1929) regard the cells which contain Russell bodies as haemocytoblasts which have failed to transform in the normal manner into erythrocytes or granulocytes. Thirdly, Michels (1935) considers that the bodies are red blood corpuscles which have been taken up by phagocytic plasma cells.

A review of the literature shows that there is some tendency to refer to Russell cells instead of Russell bodies. It is suggested that the latter is the better term, for two reasons. First, Russell himself described bodies and not cells, and, secondly, the parent is now known to be the plasma cell, and whatever the true origin of these there is no need to disinherit them on account of their cytoplasmic inclusions.

Sources of Russell Bodies.—Plasma cells containing Russell bodies are found constantly in a wide variety of chronic infections and in the granulomata (Fig. 3). They are to be found in small numbers in the normal intestinal submucosa of man and in larger numbers in the membrana propria of the abomasum, the fourth or true stomach, of ruminants. In this site they are the

Schollenleukocyten of Weill, described in the abomasum of the sheep. They occur constantly, as described by Russell, in the small round-cell infiltrations at the periphery of malignant tumours (Fig. 1). Russell bodies occur also in the abnormal plasma cells of plasmacytoma, where, according to Willis (1948), abnormal proteins produced in the tumour cells appear as rounded fuchsinophil masses or as crystals.

The main sources of Russell bodies in these investigations have been (1) the periphery of malignant tumours, (2) various chronic inflammatory granulomata, (3) six cases of plasmacytoma.

Kurloff Bodies

Kurloff bodies were first described by Kurloff in 1889, and independently in the same year by Foa and Carbone, as inclusion vacuoles in the cytoplasm of haemic and splenic lymphocytes in the guinea-pig. They occur in from 2 to 20 per cent of the haemic lymphocytes. Bab is quoted by Ledingham (1906) as having found in the bone marrow and spleen of guinea-pigs certain vacuolated cells containing masses of ill-defined granules staining metachromatically with thionin. These Ledingham, but not Bab, regarded as Kurloff cells. Ledingham himself (1940) uses the term "cell," but I suggest, for reasons similar to those adduced for Russell bodies, that the term Kurloff body and not cell is more appropriate.

In air-dried films of guinea-pig blood, fixed in methyl alcohol and stained by Romanówsky methods, the appearance of Kurloff bodies varies considerably. In films wet-fixed in Susa, however, the body appears as a uniformly spherical isotropic globule whose diameter may equal and often exceeds that of the nucleus (Fig. 7). Only where the cells have been crushed by the process of making the film does the material contained in the bodies appear granular. I consider that the appearances described by Ledingham (1940) based on air-dried methyl alcohol fixed films stained by Giemsa are largely artifact.

There are four theories as to the nature of Kurloff bodies.

Secretory Theory.—Kurloff himself originally suggested that they were some kind of secretion (*Sekretstoff*), and it has also been considered possible that they may represent aggregations of the azurophil granules normally present in lymphocytes.

Parasitic Theory.—According to Ledingham, Mochkovski in 1937 suggested that the bodies were rickettsial and proposed the name *Ehrlichia*

kurlovi in honour of the discoverer, who worked in Ehrlich's laboratory.

Phagocytic Theory.—The bodies have been considered to represent phagocytosed red cells or nuclear remnants derived from other cells (Lazzeroni, 1935).

Nuclear Theory.—This theory derives from Leinati (1932), who regards the body as representing half the nucleus of the lymphocyte which has undergone pycnosis. Lymphocytes with double nuclei connected by a thin band of chromatin are not uncommon in the guinea-pig.

In this investigation, the sole source of Kurloff bodies has been films of adult guinea-pig's buffy coat, wet-fixed in Heidenhain's Susa fixative or in saturated aqueous basic lead acetate.

Method

The use of the periodic acid Schiff reagent (P.A.S.) method in histology was first described by McManus (1946) for the demonstration of mucin and by Hotchkiss (1948) for a wide variety of polysaccharide structures.

By this method the monosaccharide component of polysaccharide structures remaining in the tissues after the use of ordinary aqueous fixatives are oxidized by periodic acid (HIO_4) to polyaldehydes, and the latter are combined *in situ* with Schiff's reagent (leucofuchsin) to form a red substituted dye. If the periodic acid method is used as described in the appendix on paraffin sections after aqueous fixatives, then five groups of substances may be expected to give positive results and these will not be visible in control sections unexposed to periodic acid. The five groups are given below.

I. Polysaccharides.—Of this group glycogen is the only member remaining after aqueous fixation and paraffin embedding.

II. Mucopolysaccharides (simple and complex).—These are defined by Meyer (1938) as polysaccharides containing hexosamine as one component occurring either free (simple) or in combination with protein substances of higher molecular weight and as esters of sulphuric acid (complex). Both simple and complex acid mucopolysaccharides contain uronic, usually glycuronic, acid as their second carbohydrate component. They include hyaluronic acid, intestinal mucins, chondroitin sulphuric acid, and heparin. The smaller fraction of gastric mucus is included here as neutral mucopolysaccharide.

III. Mucoproteins (Mucoids).—These are defined by Meyer as substances containing mucopolysac-

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charide in firm chemical union with a peptide where the hexosamine content is greater than 4 per cent. The mucoproteins contain hexosamine and hexose as their carbohydrate component as a rule, though uronic-acid-containing mucoids do occur. In this group are included the pituitary and chorionic gonadotropic hormones, submaxillary gland mucoids, and the serum mucoproteins seromucoid and seroglycoid. The larger fraction of gastric mucus is a mucoprotein.

IV. Glycoproteins.—These are divided from the mucoproteins on the basis of their hexosamine content of less than 4 per cent. They include fractions of serum albumin and serum globulin. According to Meyer (1945) simple proteins containing hexoses without hexosamine have not been found in nature.

V. Glycolipids.—These substances, on hydrolysis, yield one molecule each of fatty acid, sphingosine, and galactose or glucose. They stain positively with the periodic acid method by virtue of the sugar. The cerebroside, which are the main members of the group, occur in a variety of tissues outside as well as within the nervous system. After fixation, even in formalin alone, their solubility in fat solvents is altered so that they may still be present in paraffin sections. Hotchkiss suggests that the alcoholic solutions used in his periodic acid method complete the removal of those traces of cerebroside and other polysaccharide-containing lipids which may remain in paraffin sections. Unfortunately, I have found evidence, at least in the case of the anterior hypophysis, that this is untrue.

The three substances hexuronic acid, hexosamine, and hexose are responsible for the development of colour when the periodic acid method is employed. *In vitro*, equimolecular portions of hexose and hexosamine give the same intensity of colour when oxidized by periodic acid and allowed to react with leucofuchsin.

Cytochemical Distinctions.—*Group 1.*—Glycogen can be removed from sections by hydrolysis with diastase or ptyalin. During this work control sections, where necessary, have been exposed to the action of saliva for ten minutes at room temperature before applying the periodic acid or other techniques.

Group 2.—The only simple mucopolysaccharide with which we are concerned is hyaluronic acid. Experiments suggest that the amount of this substance present can be reduced by exposing sections to the action of hyaluronidase. Complex muco-

polysaccharides contain higher esters of sulphuric acid or phosphoric acid groups and are distinguished by their property of staining metachromatically in dilute aqueous solutions of thionin or toluidine blue. Lison (1936) regards metachromasia as a specific histochemical indication of the presence of higher esters of sulphuric acid, but it is apparent that the phosphoryl groups of ribonucleic acid, in some circumstances, induce metachromatic staining with dilute aqueous thionin. Hyaluronic acid is metachromatic where it occurs in the form of its sulphuric acid ester as, for instance, in the cornea. In paraffin sections, therefore, metachromasia may signify sulphuric or phosphoric acid groupings.

Further assistance may be obtained by a study of the dye-binding capacity for methylene blue measured at various pH levels (Dempsey and Singer, 1946). These authors found that mast cell granules and the ground substance of cartilage still bound methylene blue at pH 2 whereas the binding capacity of tracheal mucus was extinguished at pH 3 and that of ribonucleic acid at pH 4. The ribonucleic acid they studied was that of thyroid colloid, but I have found that the ribonucleic acid of plasma cell cytoplasm still binds methylene blue at pH 2.6. After ribonuclease, plasma cell cytoplasm is only faintly blue at pH 6. If the nucleic acids can be excluded the capacity to bind methylene blue below pH 4 almost certainly indicates sulphate ions and thus mucopolysaccharides.

Groups 3 and 4.—These two groups have been subdivided according to Meyer's arbitrary though convenient classification, and no cytochemical distinction is possible between them. Theoretically, substances in Group 3 might be expected to give more colour than those in Group 4 if their hexose components are equal. Factors such as concentration and availability apparently modify the development of colour to such an extent, however, that no conclusion can be derived from its intensity. The most important point is that substances in Groups 3 and 4 do not show metachromasia with thionin and will not bind methylene blue below pH 6.

Group 5.—The possibility that periodic acid material in paraffin sections may still belong to this group must always be recognized. Control frozen sections stained for lipid by Sudan IV or Sudan black B and compared with paraffin and frozen periodic acid sections will usually allow the lipoidal nature of the substance to be established or excluded.

Results

The results of the present series of experiments are shown in the Table. In the accounts of these findings and in the description which follows, substances in Groups 3 and 4 are referred to, jointly, as mucoprotein.

Russell Bodies.—If a tissue is chosen in which plasma cells containing Russell bodies are present in addition to the ordinary types, then intermediate states can be found between the plasma cell with faintly periodic-acid-positive cytoplasm and one

containing the fully developed Russell bodies. These states are best seen in sections stained by periodic acid after ribonuclease (Figs. 2 and 4).

The stages of development appear to be as follows. The faintly positive plasma cell, which is usually normal in size, first enlarges and becomes more strongly positive. The contained material then becomes finely granular with a decrease in the total intensity of staining. The granules enlarge, increasing until they become globules the size of adult red cells or larger, and the intensity of staining rises simultaneously. Finally, the cell

TABLE
RESULTS OF THE EXPERIMENTS

Structure	Eosino- philia	Basi- philia	P.A.S.*	P.A.S. after ribo- nuclease	Pyronin	Pyronin after ribo- nuclease	P.A.S. after hyal- uronidase	P.A.S. after diastase	Meta- chrom- asia and M.B.E.†	Constitution
Plasma cell cytoplasm	+ Degree varies	+	+ Degree varies	+ Degree varies	+	Basiphilia reduced	+ No reduction	+	— About pH 6.0‡	(1) Ribonucleic acid (2) mucoprotein
Russell bodies (various sources)	+	+ Around globules	+	+	+ Around globules	Surround- ing sub- stance removed	+ No reduction	+	— About pH 6.0‡	(1) Mucoprotein surrounded by (2) ribonucleic acid
Kurloff bodies	+	—	+	+	+ Around globules	Surround- ing sub- stance removed	+ No reduction	+	— pH 6.9	(1) Mucoprotein surrounded by (2) ribonucleic acid
Tissue mast cell granules	+	Slight (H & E)	+	+	+	+	+ No reduction	+	+ Nil at pH 2.6	(1) mucopoly- saccharide
Pituitary basophil cells	+	+	+	+	+	Basiphilia removed	+ No reduction	+	— pH 4.9‡	(1) Ribonucleic acid (2) mucoprotein
Auer bodies	Azuro- phil	—	+—							Inconclusive
Azurophil granules (lympho- cytes)	Azuro- phil	—	—							Not mucopro- tein
Blood and tissue eosinophil polym'phs	+	—	—						—	Not mucopro- tein
Paneth cell granules	+	—	— To faint +						—	Not mucopro- tein
Red blood cells	+	Reticu- locyte forms +	—	—	—	Reticulo- cytes not basiphil			—	Not mucopro- tein

* P.A.S. = Periodic acid Schiff.

† Methylene blue extinction.

‡ After ribonuclease.

may be so full of globules and their colour so deep that they appear to coalesce. In some cases the process appears to stop at the stage of a cell scarcely larger than the normal plasma cell, with bright red non-granular cytoplasm. These cells appear in the majority of chronic infective conditions, and with haematoxylin and eosin staining look like eosinophils with unlobed nuclei. Similar cells containing fine eosinophil granules which are P.A.S.-positive and metachromatic with thionin occur in various small round-cell infiltrations. These are the plasma-mast cells of Krompecher described by Michels and Globus (1929) in cerebral syphilis.

Comparison with sections stained by the Unna-Pappenheim method shows that the amount of pyronin-positive material in the plasma cell (ribonucleic acid) decreases as the amount of P.A.S.-positive substance (mucoprotein) rises. Furthermore each Russell body is surrounded by, to use Russell's own words, "a delicate cementing substance which stains faintly." It is almost certain that this is the basiphilic and pyronin-positive material which is removable by hydrolysis with ribonuclease and is, most probably, ribonucleic acid itself (Fig. 5).

The substance of which the bodies are composed is unaffected by ribonuclease, diastase, or hyaluronidase, fails to bind methylene blue at pH 6, and is devoid of metachromatic properties. It is, therefore, mucoprotein or at least contains mucoprotein as a major constituent.

Kurloff Bodies.—Guinea-pig lymphocytes have basiphilic cytoplasm which is pyronin-positive; as in the case of human lymphocytes this is due to ribonucleic acid and is removable by ribonuclease. A thin layer of this pyronin-positive ribonucleic acid surrounds each individual Kurloff body (Fig. 7a), and it is this layer which, irreversibly precipitated as a network either by the vital stains or by air drying of blood films and subsequently fixed and counterstained, has been described by various authors as the inclusion body of the so-called Kurloff cell. In fact the body within this reticulum is ribonuclease-fast and brilliantly P.A.S.-positive (Figs. 7b and 7c). It is fast also to diastase and hyaluronidase and does not show metachromasia. In preparations wet-fixed in Susa, however, and mounted in a watery medium the cytoplasm of the lymphocytes and the thin layer covering the Kurloff body show reddish metachromasia with thionin. This finding probably explains the results obtained by Bab, mentioned above.

It is concluded that the Kurloff body consists wholly or partly of mucoprotein.

Other Structures in the Table.—The remaining structures in the Table have been included for various reasons. Tissue mast cells conveniently provide themselves as controls for metachromasia in many sections containing plasma cells and Russell bodies (Fig. 6). It has been suggested by Holmgren and Wilander (1937) that mast cell granules contain the acid mucopolysaccharide heparin, and the results listed in the Table are in agreement with this view.

The pituitary basiphil cells present a problem similar to that posed by plasma cells. It must be emphasized that their granules are primarily only weakly basiphil and that their cytoplasm is secondarily basiphil by virtue of its ribonucleic acid content. As Desclin (1940) has shown, and as Dempsey and Wislocki (1945) have confirmed, this secondary basiphilia is removable by hydrolysis with ribonuclease. After such hydrolysis with ribonuclease, the true β granules, which now contain no ribonucleic acid, remain in the basiphils and are weakly stained by 5×10^{-4} M methylene blue at pH 6.4 whereas the α granules are stained strongly blue at this pH. The α granules are thus more basiphil than the true β granules at this pH. The basiphil (β) granules are strongly P.A.S.-positive both before and after ribonuclease and they resist the action of diastase and hyaluronidase. Although Bienwald (1939) was able to demonstrate in thionin-stained formol-fixed frozen sections the presence of a metachromatic substance in the basiphils, the β granules have not been found to exhibit metachromasia in either frozen or paraffin sections. The substance demonstrated by Bienwald was probably fatty material (*Fettstoffe*), as he suggested.

Pearse (1948) considers that the true β granules represent the gonadotropic hormone (F.S.H. or F.S.H. + L.H.) which has been shown by Evans and co-workers (1939), and by various other workers, to be a mucoprotein.

The results obtained with Auer bodies have been unsatisfactory for the following reasons. Cells containing Auer bodies are seldom numerous in either blood or bone marrow, and the bodies cannot be demonstrated except in air-dried films fixed in methyl alcohol and stained by one or other of the Romanowsky methods. Auer bodies in myeloblasts, located by this method, marked, decolorized, and subsequently treated with periodic acid, have been found to show a faint red colour demonstrable only by the use of green filters but not shown by the decolorized control. The azurophil granules of lymphocytes are periodic-acid-negative.

Discussion

In the light of the present work, only one of the three theories of origin of Russell bodies can be maintained. This is the secretory theory of Downey (1911) and Kingsley (1924).

Although it is clear that Russell bodies are formed in large numbers in response to abnormal stimuli, such as may be supposed to occur in the vicinity of malignant tumours or in long-continued chronic inflammation, their presence is clearly not invariably associated with obvious pathological processes. If one of the functions of the plasma cell is to produce mucoproteins, then the formation of Russell bodies may represent a disorder due to over-stimulation and over-production. The evidence presented in this paper suggests that plasma cells normally can produce mucoproteins and that the quantity is usually insufficient or too rapidly secreted for granular and globular retention stages to occur.

Most supporters of the secretory theory believe that the formation of Russell bodies is a degenerative phenomenon, and the cytological appearances certainly support such a view. The large cart-wheel nucleus of the plasma cell becomes distorted by the pressure of accumulated secretion, shrinks, and becomes pycnotic in the majority of cases, so that death of the cell and liberation of its content appear to be the usual end-result.

In his work on the genesis of red blood corpuscles from eosinophil leucocytes Duran-Jorda (1943a and b, 1948) describes one of the stages of erythropoiesis as a cell, originally called by him the "stem cell" and the "plasma cell with eosinophilic granules." The situation and appearance of this cell parallel so exactly that of the plasma-cell-containing Russell bodies as to make it almost certain that they are identical. Since Russell bodies are of mucoprotein nature and since red blood cells and the granules of tissue and blood eosinophils are uniformly P.A.S.-negative, I consider that he is mistaken in including the Russell-body-containing plasma cell in his erythropoietic series.

As far as Kurloff bodies are concerned, the conclusion arrived at in these studies is that they are produced in guinea-pig lymphocytes by a process of secretion similar to that involved in the production of Russell bodies by human plasma cells. Freed from the surrounding ribonucleic acid reticulum, which has misled so many investigators, the structure of Kurloff bodies is seen to be very simple indeed. Of the four theories of origin presented, only Kurloff's original one, which regards them as a secretion, fits the evidence.

Ledingham (1940) found that the number of circulating lymphocytes containing Kurloff bodies was normally greater in female than in male guinea-pigs, and greater still in pregnant females, and that the number could be greatly increased by injection of oestrogenic substances (oestradiol). Here is evidence of a controlled stimulus to secretion of mucoprotein affecting guinea-pig lymphocytes.

The present experiments show that, apart from the Russell bodies, the cytoplasm of a proportion of normal plasma cells contains mucoprotein. This substance may have been phagocytosed by the cells or synthesized, or its precursors may have been absorbed and subsequently converted into mucoprotein. It is important to know which of these hypotheses is the true one, because if the mucoprotein is synthesized the fact may have some bearing on the possible production of antibody by plasma cells and on the possible production of abnormal globulins by plasmacytoma.

Rohr (1936) states that in the bone marrow plasma cells are specialized reticulum cells which phagocytose fat, erythrocytes, and nuclear debris, and Waldenström (1944) goes further and states that in plasmacytoma they take up and store abnormal globulins formed elsewhere in the body. The majority of workers, however, hold the opposite view. Bing and Plum (1937) noted that hyperglobulinaemia and plasma-cell increase tended to be associated, and considered that plasma cells produced the globulins. Björneboe and Gormsen (1943) demonstrated in the rabbit immunized against pneumococci that the formation of plasma cells in various tissues accompanied the rise of antibody titre. They were unable to increase the number of plasma cells by injection of globulins, and concluded that antibody globulins are formed by plasma cells but that storage does not take place. Ranström (1946) noted the discrepancy between the views of Waldenström and the observations of Björneboe and Gormsen, and agreed with the latter, though it is fair to note that the findings of their second experiment can only be applied to the particular globulins employed and not to globulins in general. Teilum (1948) also holds that plasma cells and other reticulo-endothelial cells produce the various antibodies and also hyaline and amyloid.

It is evident that the weight of opinion upon plasma cells is against storage and in favour of secretion. It is considered that the inverse relationship between ribonucleic acid and mucoprotein, noted especially in plasma cells which are forming Russell bodies, supports synthesis and not

absorption. In order to explore the possibility that connective tissue mucin (hyaluronic acid) might be absorbed to form Russell bodies in connective tissue plasma cells, the action of hyaluronidase upon these was tested and found to be absent. This evidence is against the absorption of hyaluronic acid and in this respect favours secretion.

In cases of plasmacytoma the abnormal circulating globulins may belong to the α , β , or γ fractions, and Blix and others (1941) have shown that normal β and γ fractions contain carbohydrate. Normal globulins may therefore be glycoproteins. If the abnormal globulins of plasmacytoma contain sufficient carbohydrate they should be P.A.S.-positive. In the present work I have examined six cases of plasmacytoma and found a positive reaction in a small proportion of the plasma cells in four of them. In the fifth and sixth cases, the only ones in which undecalcified tissues were examined, a large proportion of the plasma cells and reticulum cells showed a positive reaction (Figs. 8a and 8b). It seems probable that the acid used for decalcification hydrolyses the mucoprotein and renders the P.A.S. reaction negative. It is hoped that a study of further cases will confirm these findings.

Summary

1. The periodic acid Schiff method of McManus and Hotchkiss has been used together with other techniques to investigate the cytochemistry of plasma cells, Russell bodies, and Kurloff bodies. A number of other structures whose chemistry throws light on the problem have been investigated simultaneously.

2. Evidence is presented that Russell bodies in human plasma cells and Kurloff bodies in guinea-pig lymphocytes consist of mucoprotein probably secreted by the parent cell. The implication of these findings on the various theories of origin of the two types of body are briefly discussed.

3. The presence of mucoproteins in the cytoplasm of human plasma cells is demonstrated, and it is suggested that these mucoproteins are polysaccharide-containing globulins (glycoglobulins, mucoglobulins). The question whether the mucoglobulin is secreted or absorbed by plasma cells is considered and evidence adduced in favour of the former.

Appendix

Periodic Acid Schiff (McManus, Hotchkiss, modified).—For paraffin sections mercurial fixatives are used for choice, but formalin is quite adequate. For blood films wet fixation in Susa for 10 minutes

(White, 1947) is employed. The films are preferably stained at once.

After removal of mercury salts by means of iodine and thiosulphate, (1) bring to 70 per cent alcohol; (2) leave for 5 minutes in periodic acid solution (A) at room temperature; (3) flood with 70 per cent alcohol and transfer to reducing rinse (B) for 1 minute; (4) flood with 70 per cent alcohol and leave for 15 to 45 minutes in fuchsin-sulphite solution (C); (5) wash in running water for 10 to 30 minutes; (6) stain the nuclei in 0.5 per cent celestine blue in 5 per cent aqueous iron alum (Lendrum and McFarlane, 1940) for 1 minute, followed by Meyer's haemalum for 1 minute. The object of the short staining period is to avoid emphasis of cytoplasmic basophilia to a degree sufficient to obscure the red colour of Schiff-positive substances. The times in stage 6 may be increased without limits after ribonuclease. (7) Differentiate strongly in 2 per cent acid alcohol, wash, dehydrate, clear in xylol, and mount in D.P.X.

(A) HIO ₄	...	...	...	400 mg.
Aq. dest.	...	...	...	10 ml.
M/5 sodium acetate buffer	...	...	...	5 ml.
Pure ethyl alcohol	...	...	...	35 ml.
(Keeps in the dark at 4° C. for at least 14 days.)				
(B) KI	...	...	...	1 g.
Na ₂ S ₂ O ₃ .5H ₂ O	...	...	...	1 g.
Aq. dest.	...	...	...	20 ml.
Pure ethyl alcohol	...	...	...	30 ml.
2N HCl	...	...	...	0.5 ml.

(The precipitate of sulphur which forms is ignored. This solution must be reacidified if necessary from time to time.)

(C) The Schiff's solution of Feulgen (see Lison, 1936, p. 178) or any of its modifications may be employed.

Ribonuclease.—This was used as a 0.5 per cent solution in isotonic veronal acetate buffer at pH 6.75. To destroy any remaining proteolytic action the enzyme is heated for 3 minutes at 100° C. in aqueous solution, and this is added to double-strength acetate buffer to make the solution for use (White, 1947). A crystal of thymol is added to the solution in a coplin jar to prevent bacterial contamination.

Sections are incubated at 37° C. for 1 hour.

Hyaluronidase.—500 mg. of dried bull's testis is added to 50 ml. of isotonic veronal acetate buffer at pH 6.99 and stirred gently with a glass rod. The mixture is left for 30 minutes for undissolved solid matter to settle; it is then decanted, and sufficient proflavine (2.8 diamino-acridine hydrochloride) is added to give a faint yellow tinge. Hyaluronidase is quickly destroyed by shaking, but its action is unimpaired by bacteriostatics of the acridine series.

Sections are incubated at 37° C. for 24 hours. A 30 to 40 per cent reduction of P.A.S.-positive colour is obtained in frozen sections of umbilical cord and cornea, and this reduction is not shown in control sections incubated for 24 hours in buffer at pH 6.99.

Unna-Pappenheim.—Sections, preferably fixed in Helly or other mercurial fixatives, are stained for 30 minutes in the following solution:

Methyl green (C.I. No. 684) ...	0.15 g.
Pyronin G. (C.I. No. 739) ...	0.25 g.
90% ethyl alcohol ...	2.5 ml.
Glycerine ...	20.0 ml.
0.5% aqueous phenol to 100 ml.	

The solution is boiled for 2 minutes, and filtered before use.

Metachromasia.—Sections fixed in mercurial fixatives are stained for 1 hour in 0.25 per cent aqueous thionin (C.I. No. 920), or in 0.5 per cent aqueous toluidine blue (C.I. No. 925), and are mounted in water for examination or in glycerine jelly for permanency.

For thionin staining, films of guinea-pig's buffy coat were fixed in saturated aqueous basic lead acetate, which is said to facilitate the demonstration of metachromasia.

Methylene blue extinction.—Sections fixed in formalin or mercurial fixatives were immersed for 24 hours at 25° C. in $5 \times 10^{-4} M$ methylene blue dissolved in veronal acetate buffer at the various pH levels. The sections were examined in water and subsequently mounted in glycerine jelly.

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FIG. 1.—Plasma cells and Russell bodies (arrows) in the lamina propria of the gastric mucosa. Haematoxylin and eosin, $\times 580$.

FIG. 2.—The same tissue as Fig. 1. Lower right, positively-stained mucus in gland cells. P.A.S. celestin blue-haemalum, $\times 580$.

FIG. 3.—Russell bodies in a granuloma (breaking-down sebaceous cyst). P.A.S. celestin blue-haemalum, $\times 580$.

FIG. 4.—Part of Fig. 2 to show detail of Russell body formation. The P.A.S.-positive cytoplasm of some of the plasma cells is well shown. P.A.S. celestin blue-haemalum, $\times 1,150$.

FIG. 5.—Shows the “delicate cementing substance” between the Russell bodies, stained by pyronin. On hydrolysis with ribonuclease this disappears. Pyronin-methyl green, $\times 1,150$.

FIG. 6.—The section is mounted in glycerine jelly, which accounts for the poor optical qualities of the photograph. Below, red metachromasia shown by tissue mast cell granules. Above, blue stained Russell bodies in a plasma cell. 0.25 per cent aqueous thionin, $\times 1,150$.

FIG. 7a.—Lymphocyte with semilunar nucleus below and above, pyronin-positive cytoplasm surrounding an unstained Kurloff body. Pyronin-methyl green, $\times 1,150$.

FIG. 7b.—Single lymphocyte showing, lower left, blue staining nucleus; upper right, red P.A.S.-stained

Kurloff body. There is an unstained cytoplasmic halo around the body in which three greyish dots can be seen. The darker dots overlying the body are thought to be precipitated ribonucleic acid. P.A.S. celestin blue-haemalum, $\times 1,150$.

FIG. 7c.—Below, the nucleus of the lymphocyte. Above the Kurloff body. Note the absence of dots seen in Fig. 7b. P.A.S. celestin blue-haemalum, $\times 1,150$ (after ribonuclease).

FIG. 8a.—Plasma cells from a case of plasmacytoma showing P.A.S.-positive material in the cytoplasm. P.A.S. celestin blue-haemalum, $\times 1,150$.

FIG. 8b.—Above, a plasma cell, and, below, a reticulum cell, from another case of plasmacytoma. Both cells contain P.A.S.-positive material. P.A.S. celestin blue-haemalum, $\times 1,150$.