# OBSERVATIONS ON THE FORMATION AND NATURE OF RUSSELL BODIES.

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THE term Russell body is used in this paper to denote certain intracellular acidophil hyaline bodies, which have been produced experimentally in animals by the injection of bacteria, and which have similar appearances and staining reactions to the structures first recognized by William Russell of Edinburgh and described by him as " the characteristic organism of cancer."

Russell (1890) found examples in a large number of malignant growths of human origin as a feature of the small round-cell infiltration at the margin. Russell concluded that these bodies were an essential component of the neoplastic process, unrelated to degeneration or inflammation of tissue secondary to such neoplastic change. He recorded two apparent exceptions to this rule, a granulomatous ulcer of the leg and an ulcer of the nasal cavity, but he interpreted his sections as showing early signs of the secondary development of a neoplastic change and concluded that the acidophil bodies (fungus particles) were the agent causing this change.

However, the literature contains ample references to the regular presence of Russell bodies in granulomas from a wide variety of chronic infections. An early group of reports described their presence in rhinoscleroma; Pellizzari (1883), Cornil and Alvarez (1885), Konstantinowitsch (1902) and Marschalko (1900). The literature contains numerous accounts of their presence in chronic inflammatory lesions, brain abscesses, syphilis of the central nervous system, skin diseases, syphilitic condylomata, and inflammatory reactions surrounding gastric ulcer.

Russell bodies are also found in small numbers in the " normal " intestinal mucosa of man. Lisco (1942) demonstrated their striking localisation within lymph follicles of the intestinal tract of pigs. Remarkably large numbers occur in the abomasum or fourth stomach of ruminants (Weill, 1919) where they are referred to by this author as *Schollenleukocyten*. Russell bodies are also not uncommon in the abnormal plasma cells of plasmacytoma (Apitz, 1937).

In contrast with the very extensive literature relating to the occurrence of these structures in human and veterinary material is the paucity of reference to their occurrence in the experimental animal. Miller (1931) injected rabbits repeatedly by the intraperitoneal route with a variety of protein fractions derived from the tubercle bacillus. Plasma cells resulted in the omentum and in reticular tissues, and at some of these sites contained eosinophilic bodies (Russell bodies).

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They were thought to represent a degenerative change in the cytoplasm of the mature plasma cells. It was noticed that Russell bodies were numerous where plasma cells were crowded and crowding was suggested as a possible cause of the cytoplasmic degeneration.

The first part of this paper concerns the cellular reactions in reticular tissues following the intravenous injection of a variety of antigens, in relation to the production of Russell bodies. The second part deals with attempts to characterise these structures by histochemical and immunological methods.

## MATERIALS AND METHODS.

*Vaccines and antigen solutions.*

Proteus vulgaris *and* Klebsiella pneumoniae.—The organisms were grown in a tryptic digest broth for 18 hr. at 37°. It was found that this constantly gave opacities approximately equal to that of tube 1 " Wellcome " opacity tubes or $2 \times 10^8$ organisms per ml. (Wright method).

Mycobacterium tuberculosis.—A culture of avirulent human bacilli derived from the strain H37Rv was provided by Dr. F. C. O. Valentine. This was grown for 10 days in Dubos medium with added human serum albumin and Tween 80 (Sorbitan mono-oleate). The organisms were heat-killed, thrice washed in physiological saline and reconstituted to a volume half that of the original culture.

*Bovine γ-globulin.*—Supplied by Armour & Co. Ltd., Lindsey Street, London, E.C.1.

*Staphylococcal toxoid (strength B).*—Kindly provided by Dr. C. L. Oakley, The Wellcome Research Laboratories, Beckenham, Kent.

Streptococcus pneumoniae.—Type III organisms were provided by the National Collection of Type Cultures. Cocci from the peritoneal cavity of a moribund mouse were grown overnight (14 hr.) on blood agar and washed off into sterile saline. The suspension was standardized to an opacity equal to that of tube 2 " Wellcome " opacity tubes or $6 \times 10^8$ organisms per ml. (Wright method). The intravenous dose in the rabbit was 1 ml.

*Preparation of frozen sections.*

Small blocks of tissue, up to 5 mm., were obtained immediately after death and placed in thin-walled test-tubes, closed with rubber stoppers and quickly frozen by rapid immersion in alcohol-$CO_2$ mixtures at − 70°. Frozen sections at 4μ were prepared from these tissues by the modification of the Linderstrøm-Lang and Mogensen technique (1938) described by Coons, Leduc and Kaplan (1951). Subsequent handling of sections followed the methods of these authors except that gelatinized slides were not used. The sections were fixed in 95 per cent ethanol for 15 min. at 37° for the demonstration of antigen and antibody, and in Bouin's fixative for staining by Giemsa's method.

*Demonstration of antigen and antibody in tissues.*

*P. vulgaris* antigens in the frozen tissue sections were demonstrated by the technique of Coons and Kaplan (1950). Briefly, the procedure (A) for staining of antigen involves a precipitation of fluorescein-tagged *Proteus* antibody, when a solution of the latter is layered over the tissue section and allowed to react for 30 min. The ability of *P. vulgaris* antibody conjugate to reveal homologous antigens in tissues was demonstrated by the use of sections from a mouse injected 10 min. before death with living organisms by the intravenous route (Fig. 8). Staining for antibody (B) was by the method described by Coons, Leduc and Connolly (1953), appropriately modified for *P. vulgaris*. The procedure involved treatment of the frozen sections with a dilute solution of *P. vulgaris* antigen for 30 min. to allow precipitation between antigen and specific antibody in the section. The antigen which was thus precipitated was then itself localised with fluorescein-tagged antibody as above (A), after washing in saline for 5 min. to remove excess of unfixed antigen. Staining according to (B) will reveal both antigen and antibody, and thus sections stained by both methods are necessary to determine that part of the staining in (B) which is due to specific antibody.

In all cases therefore sections were stained by procedures (A) and (B). Further, procedure (A) will also indicate any non-specific staining associated with the use of the antibody con-

jugate solution. Another control for the staining of antibody consisted in the use of heterologous systems, *e.g.*, ovalbumin and fluorescein conjugated homologous antibody, to determine that the first stage of procedure (B) is not a non-specific fixation of antigenic protein.

*Antigenic fractions of* P. vulgaris.

The organisms were grown for 5 days in 12 l. of digest broth (a modification of Hartley's digest broth as prepared in bulk at the Department of Bacteriology, Harvard Medical School) and separated in a Sharples continuous flow centrifuge. They were next disintegrated in a Booth-Green ball mill (Cambridge Instrument Company Ltd.) and the saline washings from this yielded on centrifugation an easily delimited thick layer of disintegrated organisms between the pearly supernatant fluid and the intact bacilli below. This intermediate layer was carefully removed and lyophilized. The dried, light-brown powder was stored in a desiccator over anhydrous $CaCl_2$ at 4° until required for use.

The antigen solution used in the staining technique above was prepared by extracting 50 mg. of the dried disintegrated bacilli with 5 ml. of phosphate saline buffer (0·15 M-NaCl and 0·01 M-phosphate pH 7·0) at 4° for 2 hr. The suspension was centrifuged at 20,000 r.p.m. for 2 hr. in a refrigerated centrifuge at 4°. The supernatant was taken off carefully with a pipette, heated at 56° for 20 min. and merthiolate (sodium ethyl mercuri-thiosalicylate 1/100 solution, Eli Lilly & Co. Ltd.) added to a final concentration of 1/10,000. The antigen was used at a 1 in 100 dilution in the staining procedures.

*Preparation of* P. vulgaris *antisera and fluorescein conjugates.*

*P. vulgaris* antisera were prepared by repeated intravenous injections of rabbits with increasing amounts of 18-hr. cultures of living organisms grown in digest broth. The titres determined by agglutination with a formolised suspension of *P. vulgaris* diluted to correspond with opacity of 5 (Macfarland scale) were 1 : 20,280 and 1 : 40,560.

The sera from two rabbits were pooled, a crude globulin fraction prepared by precipitation with half-saturated ammonium sulphate, conjugated with fluorescein isocyanate and purified by dialysis (Coons and Kaplan, 1950).

Before use the conjugate was absorbed twice with lyophilized mouse liver powder. This is necessary in order to eliminate certain non-specific staining of mouse and rabbit tissues (Kaplan, Coons and Deane, 1949).

## RESULTS.

### *Production of Russell Bodies in Rabbits.*

*Injections of a* P. vulgaris *vaccine.*

During the course of investigations in which rabbits were injected repeatedly intravenously with a variety of antigenic substances it was noticed that numerous cells containing Russell bodies were present in spleen sections. The first antigen found to have this effect was *P. vulgaris*. A suspension of living organisms from an 18-hr. culture in nutrient broth was employed. The course of intravenous injections was started with 0·1 ml., 0·25 ml., 0·5 ml., 1·0 ml., given every third day and continued with the same time interval at the 1·0 ml. dose level for 1–3 months.

Sections of such an animal autopsied 6 days after the last injection showed great hyperplasia of the red pulp, with broadening of the pulp strands by the presence of closely packed mature and immature plasma cells. There were also prominent groups of macrophages, some of which were multinuclear and of great size, often containing phagocytosed polymorphonuclear leucocytes, nuclear remnants and haemosiderin pigment granules. The perifollicular zone was broadened but the plasma cell elements here were not quite as numerous as in the pulp strands.

Russell bodies were found in the mature plasma cells of such cellular proliferations of the splenic medulla (Fig. 1 and 2). It was characteristic that certain

areas showed large numbers of cells containing Russell bodies, while other areas were free from them, although containing numerous plasma cells. The Russell bodies were present as eosinophilic spherical masses in the cytoplasm of plasma cells. No other cell was found to show such changes in this material. First, tiny round " vacuoles " appeared throughout the basophilic cytoplasm. At this stage the bodies are not eosinophilic. Apparently by a process of aggregation these spherical bodies become larger until the cytoplasm is occupied by five to ten or more rounded eosinophilic spherical masses, flattened where they make contact with each other and separated by thin strands of cytoplasm, which are still strongly basophilic. The nucleus, densely stained with haematoxylin and pyknotic, is displaced to the periphery of the cell and distorted by the eosinophil masses. The cells are at this stage very similar to the cells described and illustrated by Russell (1890) for human tissues.

In some of the animals there were also present lozenge-shaped eosinophilic structures within the cytoplasm of neighbouring plasma cells. These structures appeared to be crystals of the material composing the Russell bodies (Fig. 3). In animals receiving *P. vulgaris*, the characteristic form of these appeared to be an equal-sided lozenge with the acute angle of approximately 60°, although bundles of long, narrow crystals were also found.

Russell bodies were consistently absent from those reticular tissues of the normal rabbits which were examined (liver, spleen and bone marrow). This is contrary to statements in the literature (*e.g.*, Downey, 1938) that Russell bodies appear in the reticular tissues of normal rabbits. If this is so, they must be rare, particularly if a thorough autopsy is done to exclude a natural infection.

Russell bodies have never been seen in the reticular tissues of the rabbit after a single injection of *P. vulgaris* even with a 1 ml. dose of 18-hr. culture.

In the course of investigations planned for a different purpose the tissues were examined of 14 rabbits which had been injected with a course of 4 intravenous

EXPLANATION OF PLATES.

FIG. 1.—Russell bodies in plasma cells of the medulla of rabbit spleen after repeated intravenous injections of *P. vulgaris*. Paraffin section. Haematoxylin and eosin. × 880.

FIG. 2.—Rabbit spleen showing a greatly broadened medullary strand with numerous Russell bodies and a few crystals within plasma cells. Repeated intravenous injections of *P. vulgaris*. Paraffin section. Periodic-acid-Schiff technique. × 430.

FIG. 3.—Rabbit spleen medulla. Crystal formation in plasma cells after repeated intravenous injections of *P. vulgaris*. Paraffin section. Phloxin tartrazine. × 880.

FIG. 4.—Rabbit spleen showing crystals within plasma cells of the medulla after repeated intravenous injections of *Kl. pneumoniae*. Paraffin section. Haematoxylin and eosin. × 1280.

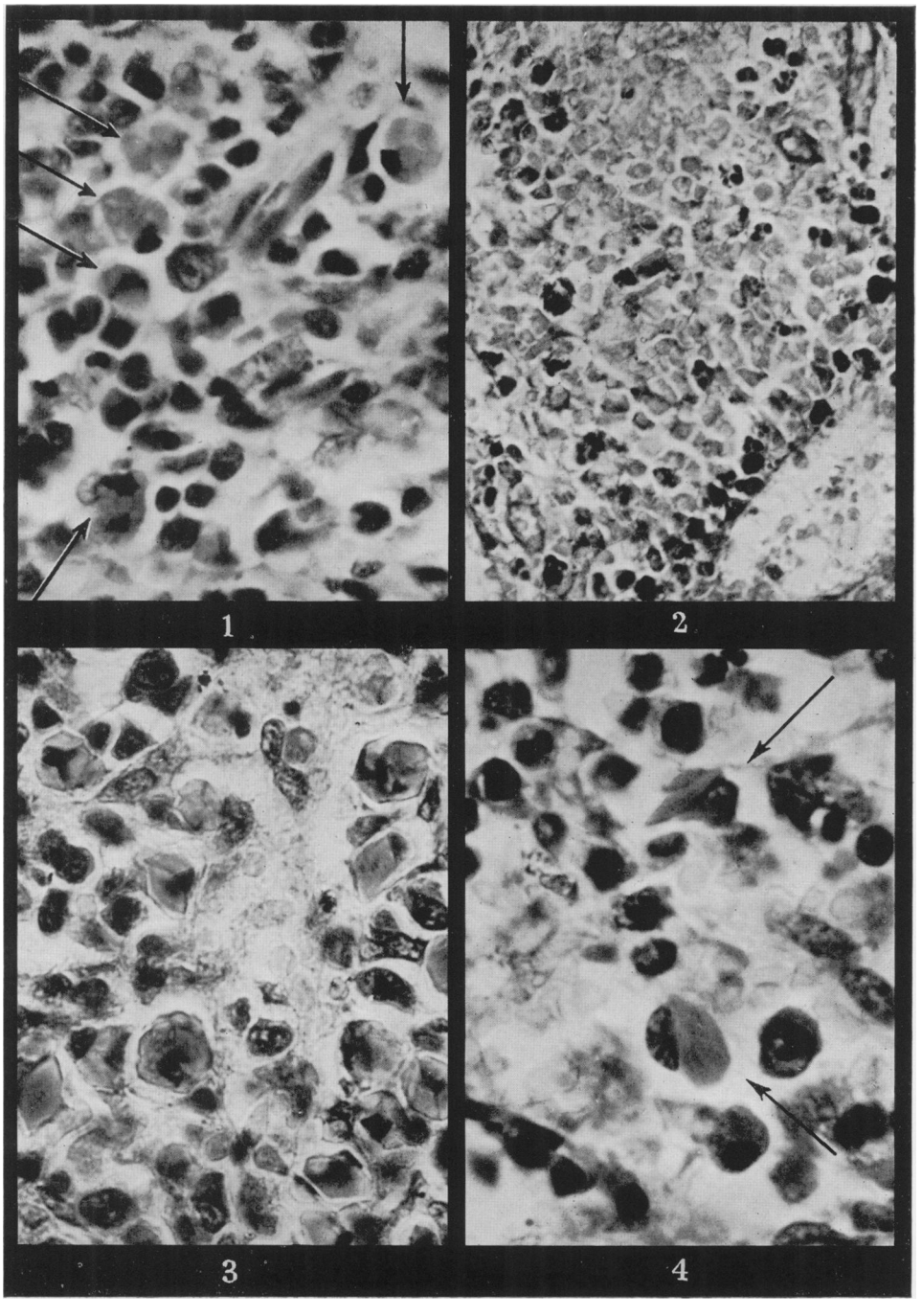
FIG. 5.—Rabbit subcutaneous tissue. Group of mature plasma cells at the edge of a granuloma produced by the injection of diphtheria toxoid adsorbed to aluminium phosphate. Note the stages of development of Russell bodies in the cytoplasm of a group of mature plasma cells (Pc). Eo = Eosinophil. Frozen section. Giemsa. × 900.

FIG. 6.—Fluorescence photomicrograph. Mouse spleen showing numerous cytoplasmic clusters of Russell bodies. The individual Russell bodies are outlined by bright yellow-green surface fluorescence. Frozen section stained for *P. vulgaris* antibody. × 600.

FIG. 7.—Fluorescence photomicrograph. Mouse spleen showing several cytoplasmic clusters of Russell bodies very intensely stained in comparison with the usual antibody-containing plasma cells. Frozen section stained for *P. vulgaris* antibody. × 600.

FIG. 8.—Fluorescence photomicrograph. The light areas represent the bright yellow-green, specifically stained *P. vulgaris* and their disintegration products within Kupffer cells of mouse liver. The liver parenchyma is faintly outlined by its natural blue fluorescence. Frozen section stained for *P. vulgaris* antigen. × 840.

FIG. 9.—Fluorescence photomicrograph. Mouse spleen. Cytoplasmic cluster of Russell bodies. N = cell nucleus. Frozen section stained for *P. vulgaris* antibody. × 1200.

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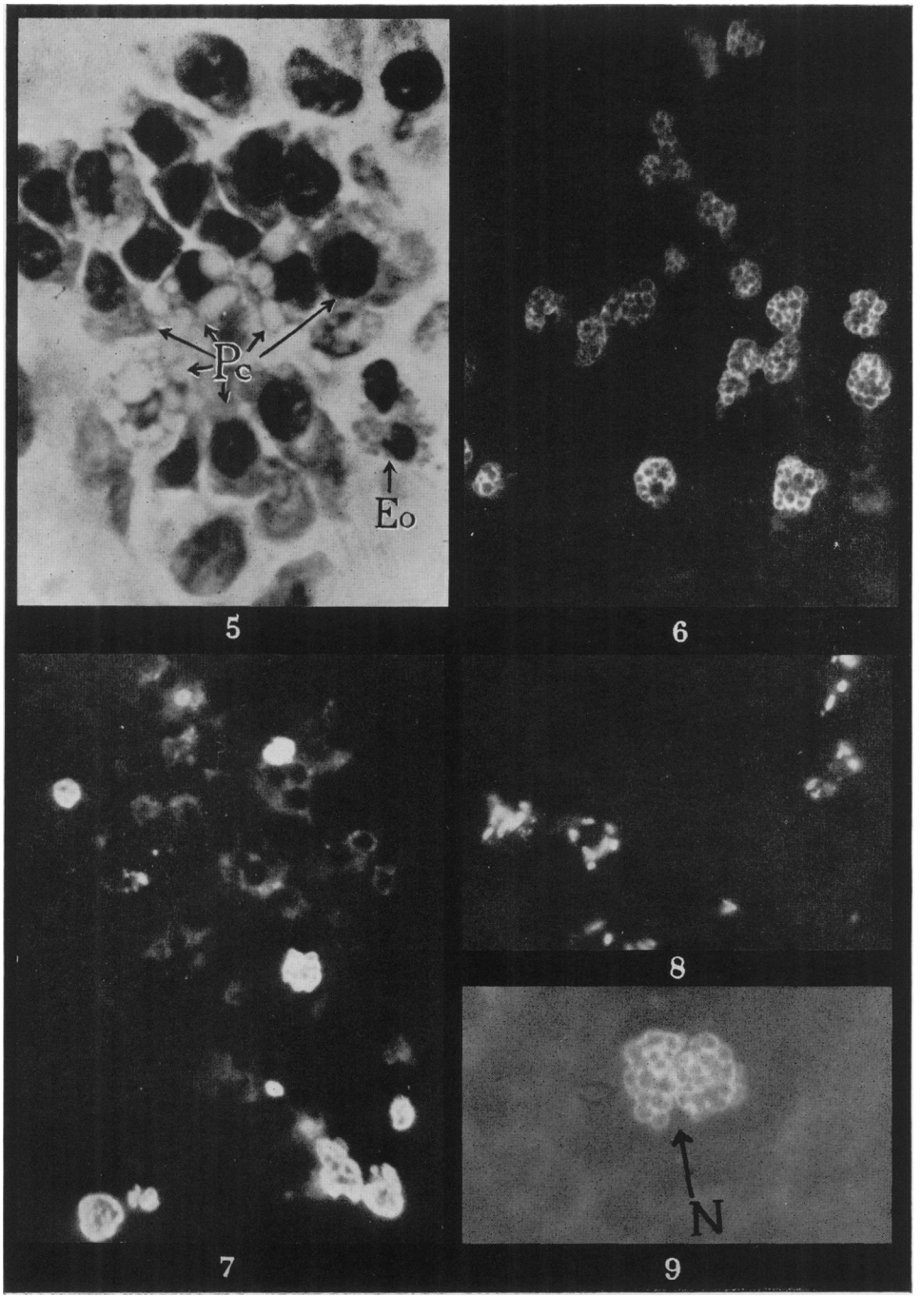


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injections of *P. vulgaris* (an 18-hr. culture in nutrient broth), starting with 0·1 ml. and continuing with 0·25 ml., 0·5 ml., 1·0 ml. at 3-day intervals. With this restricted course of injections, the sections of the reticular tissues do not commonly contain Russell bodies. Occasionally a solitary plasma cell is seen to contain them and in 1 out of the 14 animals a local area of the splenic pulp contained a large number of such cells.

Since, in these experiments, nutrient broth was used, the possible influence of the constituents of this in the production of Russell bodies was checked by the use of organisms which were thrice washed in saline. Two rabbits injected bi-weekly for two months with identical numbers of organisms to those used in broth cultures showed large numbers of Russell bodies in sections of the spleen. Thus it appears unlikely that broth constituents had any influence in the production of Russell bodies.

*Experience with organisms other than* P. vulgaris.

Russell bodies appeared in the reticular tissues of rabbits when repeated injections of *Kl. pneumoniae* were given intravenously. An 18-hr. culture was used and starting with a dose of 0·1 ml., 0·25 ml., 0·5 ml., and 1 ml. were given with an interval of 3 days, and injections were continued at the 1 ml. level for 5 weeks. Such a course of injections resulted in intense plasma cell proliferation in the reticular tissues with very numerous bodies and crystalline inclusions (Fig. 4).

A similar course of injections of *Str. pneumoniae*, Type III rabbit avirulent, living organisms, was found to produce scattered Russell bodies in the rabbit spleen, although these were far fewer than after courses of *P. vulgaris* and *Kl. pneumoniae*.

The bacillary vaccines used for the production of Russell bodies are unsatisfactory since they consist of complex mixtures of antigenic substances. Accordingly attempts were made with simpler antigens. Intravenous doses of 20 mg. bovine $\gamma$-globulin repeated every 3 days for 4 weeks were unsuccessful in two rabbits. Intravenous staphylococcus toxoid 1 ml. dose, repeated every third day for 4 weeks was also unsuccessful (2 rabbits). It should be remarked that both these antigens caused far less impressive plasma cellular accumulations than the bacillary vaccines.

In experiments in which subcutaneous injections of aluminium phosphate precipitated diphtheria toxoid were made into previously immunized animals, Russell bodies were seen in plasma cells in the outer zone of granulation tissue. Fig. 5 shows Russell bodies at varying stages of development in the cytoplasm of a group of mature plasma cells at the edge of such an alum granuloma.

*Production of Russell Bodies in Mice.*

Experiments were also made in mice. Injections of a suspension of living *P. vulgaris* were given into a tail vein. A course of 0·1 ml., 0·25 ml., 0·5 ml., 0·5 ml. of living organisms from an 18-hr. culture with 3-day intervals between injections and with sacrifice of the animals 6 days after the last, constantly produces in the mouse spleen an intense proliferation of plasma cells. Large numbers of the mature forms of the latter were found to contain Russell bodies. Uninoculated mice from the same source as the experimental animals constantly had small collections of mature and immature plasma cells around arterioles of the pulp, and an occasional one of these contained Russell bodies.

In view of the experiences of Miller (1931) in which fractions from *Myco. tuberculosis* were injected intraperitoneally in rabbits with the production of plasma cells containing Russell bodies, two mice were injected with repeated doses of an avirulent human strain derived from H37Rv. Bi-weekly injections of a suspension of heat-killed organisms were given. The spleen of one mouse autopsied 6 days after the last injection showed very numerous plasma cells with Russell bodies ; but in the other animal they were scanty.

*Staining and Histochemical Reactions.*

The description given below refers to Russell bodies produced in the rabbit by injections of *P. vulgaris*. Paraffin sections were prepared from tissues fixed in formol alcohol. Exceptions to these procedures are stated.

*Haematoxylin and eosin.*—A noteworthy observation was that the depth of staining with eosin varied. Thus the Russell bodies in the spleen of mice injected intravenously with *Myco. tuberculosis* were intensely eosinophilic. The intracytoplasmic crystalline structures were also stained to a variable degree with eosin.

*Haematoxylin-pyronin* (Marshall and White, 1950).—The Russell bodies appeared as colourless spherical masses in a network of delicate, intensely pyroninophilic, cytoplasmic strands.

*Gram-Weigert stain* (Mallory, 1938).—Russell bodies and intracellular crystals were purple when decolorization was such that traces of positive staining remained in collagen.

*Phloxin-tartrazine method* (Lendrum, 1947).—The larger Russell bodies were strongly phoxinophil but the smaller forms were not stained. The delicate strands of cytoplasm between the bodies were a deep olive-green. The crystals were also phloxinophil.

*Giemsa stain.*—Frozen sections with subsequent fixation in Bouin's solution. Russell bodies in rabbits were usually a pale pink colour, but those produced in mice by *P. vulgaris* were uncoloured, or pale pink.

*Periodic-acid-Schiff* (Hotchkiss, 1948).—The details of the technique were taken from Pearse (1949), employing reagents in 70 per cent ethanol. Russell bodies and intracytoplasmic crystals were an intense red-purple. The extended technique as suggested by McManus and Cason (1950) was also used. Acetylation abolished the positive periodic-acid-Schiff reaction of these structures, but treatment with alkali following such acetylation resulted in its restoration. This was regarded as evidence that a chemical structure bearing the 1–2 glycol linkage is a component of such bodies. In some cases control sections were exposed to the action of diastase (saliva) and hyaluronidase (hyalase, Benger) following the technique of Pearse (1953). The P.A.S. reaction was still positive. No evidence was thereby provided that the P.A.S. positive material was either glycogen or hyaluronic acid.

*Method for acid polysaccharides* (Hale, 1946).—Both the Russell bodies and intracellular crystals gave negative reactions.

*Method for arginine-containing proteins* (Thomas, 1946).—A weakly positive reaction was given by both the Russell bodies and the intracellular crystals.

*Millon's reaction for tyrosine.*—A positive reaction (brown-orange coloration) resulted.

*Sudan black B.*—Staining was by the modification described by Ackerman (1952). No sudanophilia of the Russell bodies could be demonstrated.

In summary Russell bodies produced experimentally as described gave a uniformly positive reaction with periodic-acid-Schiff reagent. This finding taken together with the evidence for the absence of glycolipids, glycogen and acid polysaccharides, suggests that the Russell bodies investigated in this material consisted wholly or partly of mucoprotein or glycoprotein.

The uniformly similar histochemical and staining reactions of Russell bodies and the intracytoplasmic crystals suggested that both are composed of similar material.

### *Production of Russell Bodies by Injection of Previously Sensitized Rabbits.*

In two rabbits the intravenous injection of *P. vulgaris* was continued with two doses of 1 ml. of an 18-hr. broth culture per week for 4 months. The animals were then rested for 6 weeks and at the end of this period a biopsy of the spleen was taken under nembutal and ether anaesthesia and examined histologically. Immediately after the biopsy the animal was injected intravenously with a further 1 ml. and 6 days afterwards was sacrificed. Two controls were injected with a 1 ml. dose of the same culture and sacrificed 6 days later and another two were subjected to biopsy of the spleen before receiving the same intravenous dose of culture. They were also sacrificed 6 days later. These controls were used to show that a single injection with or without the operation on the spleen failed to produce Russell bodies.

The biopsies taken before the recall injection showed a complete absence of the plasma cell reaction, and no Russell bodies were seen. Spleens from the rabbits 6 days after the recall injection showed greatly broadened pulp strands packed with immature plasma cells and lesser numbers of haemocytoblasts and activated reticular cells. The sinuses also contained many immature plasma cells. Also present within the pulp strands were groups of eosinophils and eosinophil myelocytes, as well as scattered pseudo-eosinophils. Russell bodies were very numerous within the plasma cells, of which approximately 10 per cent contained them. Some of these plasma cells contained eosinophil crystals.

To summarize, in rabbits previously sensitized by repeated injections of *P. vulgaris* and rested to allow reversion to a normal splenic histology, the injection of a booster dose of organisms results, 6 days afterwards, in the production of striking numbers of Russell bodies within the plasma cells in the medulla of the spleen.

### *Time Relations in the Formation of Russell Bodies in Response to a Recall Injection of* P. vulgaris.

For economy in animals and material mice were used, and 30 were given a course of intravenous injections of an 18-hr. culture of living *P. vulgaris*. Starting with doses of 0·1 ml., 0·25 ml., 0·25 ml., and 0·5 ml., given at 3-day intervals injections were continued with two further doses of 0·5 ml. (a total of 6 injections). All injections were made into the tail vein. The animals were then rested for 5 weeks and at the end of this period 2 animals were sacrificed as controls, while the remainder received 0·5 ml. as a recall injection. Pairs of these animals were sacrificed at 24 hr., 3, 5, 7, 9 and 11 days following this recall injection.

As a further control group, mice were given a single intravenous injection of 0·5 ml. of the culture used for the recall injection above. Pairs of animals

were sacrificed at the same intervals following this injection. Thus the histological findings in these animals represented the primary response to the organism.

In a rough attempt to quantitate the response in the spleens, the number of Russell bodies in 40 fields using a 43 × objective and a 10 × ocular was counted (an area 30 mm.$^2$). Frozen sections were cut at 4$\mu$ and stained by Giemsa and by the method for revealing *P. vulgaris* antibody. The count of cells which contained Russell bodies was made with the latter sections using a fluorescence microscope, since it was very simple to pick out the brightly green-fluorescent cells.

The intravenous injection of *P. vulgaris* in the mouse caused a great proliferation of plasma cells in the red pulp of the spleen. Since spleens from control uninjected mice contained numerous plasma cells in Giemsa-stained sections, it could not be inferred that all the plasma cells were formed in response to the antigenic stimulation of the injected organisms. The plasma cell proliferations were more intense in the animals which were previously sensitized and examined after a recall injection. These differences in cellular content of the two groups were dramatically shown in sections treated by the method for revealing *P. vulgaris* antibody. The primary injection resulted in small numbers of antibody-containing cells, appearing as isolated single cells at the third day and increasing thereafter. The secondary injection resulted in the appearance on the third day of scattered groups of 6–12 antibody-containing cells as well as isolated cells. The animals sacrificed on the fifth day following the recall injection showed large " colonies " of a hundred or so antibody-containing cells producing a generalised mottling of the red pulp. No antibody-containing cells were shown in the white pulp. In animals which were sacrificed at 7 days and after, the proliferations in

TABLE.—*Comparison between the Production of Russell Bodies by Injection of* P. vulgaris *into Normal and Previously Sensitized Animals.*

| | Primary response (A). | | Recall response (B). | |
|---|---|---|---|---|
| Days after injection of *P. vulgaris* | Mouse no. | No. of Russell body-containing plasma cells in 30 mm.$^2$ in spleen section. | Mouse no. | No. of Russell body-containing plasma cells in 30 mm.$^2$ of spleen section. |
| 1 | — | — | 798 | 0 |
| | — | — | 799 | 0 |
| 3 | 802 | 0 | 800 | 0 |
| | 803 | 0 | 801 | 10 |
| 5 | 809 | 0 | 807 | 2 |
| | 810 | 0 | 808 | 6 |
| 7 | 813 | 1 | 811 | 35 |
| | 814 | 203 | 812 | 64 |
| 9 | 890 | 0 | 815 | 100 |
| | 883 | 0 | 816 | 8 |
| 11 | 889 | 0 | 819 | 20 |
| | 893 | 0 | 820 | 40 |

*Controls :* Animals 796 and 797 which had received the same course of sensitizing injections of *P. vulgaris* organisms as other animals in group B were sacrificed on the day of the recall injection. Both animals showed absence of Russell bodies from spleen sections.

discrete groups of cells were less obvious and a confluent mass of antibody-containing cells now outlined the whole of the red pulp of the spleen.

The Table summarises the findings in regard to the numbers of Russell body-containing plasma cells in the spleens of these animals. It will be seen that the primary response is not accompanied by Russell body formation except for one animal (814) of the group which responded not only with a marked plasma cell reaction, but also showed large numbers of Russell bodies in its spleen. Since the number of antibody-containing cells which were found in the sections from this animal was far in excess of that expected in a primary response it was considered possible that natural sensitization to the antigenic components of the test organism had been previously acquired. The results with the group of animals which were stimulated by a recall injection suggested that while Russell bodies can be formed as early as the third day their numbers increase to a peak at a later stage of the reaction, *i.e.*, 7–9 days.

*Attempts to Characterize Russell Bodies by Immunological Methods for Revealing Specific Antigen and Antibody.*

Since Russell bodies appear in the spleen in response to a variety of antigens it was considered possible that their composition might include specific antibody. As a result of the investigations of Bing (1940), Bjørneboe and Gormsen (1943), Fagraeus (1948) and others, the plasma cell has been thought to be active in the secretion of antibody globulin. Thus it was considered appropriate to investigate the antibody content of such structures by the technique for the localization of antibody in frozen tissue sections described by Coons *et al.* (1953), adapted so as to be appropriate for *P. vulgaris*. Since it was also a possibility that the precipitated antibody of such bodies could result from the presence of specific antigen within such plasma cells, the antigen content was also studied by the technique of Coons *et al.* (1951).

Frozen sections at 4$\mu$ thickness were obtained from the spleens of the mice used in the previous section. Areas of antigen or antibody in appropriately treated sections (see " Materials and Methods ") were revealed by the deposition at these sites of fluorescein-conjugated antibody and appeared as bright apple-green areas in the fluorescence microscope.

The sections of animals 801, 807, 808, 811, 812, 814, 815, 816, 819, 820, all showed the presence of varying numbers of Russell bodies in Giemsa-stained sections. In corresponding areas of adjacent sections, treated by the method for antibody, Russell body-containing cells appeared as shown in Fig. 6. It is seen that each Russell body is outlined by a brilliant surface reaction characteristic for *P. vulgaris* antibody. The control sections treated to show the presence of *Proteus* antigen failed to demonstrate any in the Russell bodies. In Fig. 7 the striking brilliance of the fluorescence of the Russell-body surfaces is seen and contrasts with the faint cytoplasmic fluorescence of antibody-containing plasma cells. This appeared similar to the appearance described by Coons, Leduc and Connolly (1953), and White, Coons and Connolly (1953) for plasma cells showing a cytoplasmic content of antibody to diphtheria toxoid and ovalbumin antigens, except that the cytoplasmic fluorescence given by *Proteus* antibody was usually more granular.

In interpreting the results of this technique it may be questioned whether a distinction can be drawn between the presence of antibody within the cytoplasmic

strands surrounding the Russell bodies and the presence of antibody on the surface of the latter. Repeated observations strongly favoured the latter interpretation. As can be appreciated from Fig. 6 and 9, the appearances are those of a reaction at the surface of spherical bodies rather than of a stained network with " holes " for the non-fluorescent bodies. Also the gap shown at N in Fig. 9 represented the compressed and distorted nucleus and it is to be observed that fluorescence does not extend along the peripheral side of this as would be expected if the distribution of the fluorescence coincided with the basophilic cytoplasm.

## DISCUSSION.

The histological observations which have been described support the idea that Russell bodies occur within the cytoplasm of plasma cells formed in response to a specific antigenic stimulus. It has been shown that Russell bodies may occur in mice and rabbits in response to a variety of antigenic stimuli. This concept would appear to accord well with the reports of the occurrence and distribution of Russell bodies in regions of inflammatory cellular reaction to chronic infections. The evidence is not sufficiently extensive to allow any generalisations concerning the classes of antigenic material which have this effect. The failure with repeated heavy doses of certain antigens which nevertheless are active in stimulating antibody production and proliferation of plasma cells suggests that Russell body production may be limited to certain types of antigen. Physical properties of antibodies vary and it is to be expected that certain among these would be more easily precipitated within the cytoplasm of plasma cells than others.

Interpretation of the results of the technique for revealing the presence of specific antibody in Russell bodies is not without difficulties, due to the limitations involved in the use of a microscopic precipitin reaction between antigen and antibody. Unlike most tissue stains, the reagents consist of macromolecules with limited penetrating power. A mass of antibody might only give a precipitation of homologous antigen at its surface. It must therefore be realized that the results of the technique as employed here do not allow a distinction between a Russell body structure consisting of a solid mass of antibody and one in which antibody is restricted to a surface layer.

The negative results of the technique for revealing *P. vulgaris* antigens are not, of course, regarded as conclusive evidence that these are absent from Russell bodies occurring after injections of this organism. Antigens could be present and undetectable owing to their valencies being completely occupied in combination with antibody. It was in order to explore this possibility that the technique for revealing antigen was modified; first, by omitting any previous fixation of the section with ethanol, and secondly, by allowing interaction with fluorescein conjugated antibody for prolonged periods up to 2 hr. It was thought that substitution of the antibody molecules of the conjugate might thereby occur with antibody within the Russell body. These manoeuvres also failed to reveal the presence of specific antigens as constituents of the Russell bodies.

The reason for the very strongly positive reaction with the periodic acid-Schiff technique is not very obvious on the interpretation of the structure of the Russell bodies as precipitated antibody globulin. In this connection the work of Blix, Tiselius and Svensson (1941) that normal $\beta$- and $\gamma$-globulins contain carbohydrate may be relevant. In the experience of the author the cytoplasm of

some normal plasma cells also shows a diffuse reaction with the P.A.S. technique. Pearse (1949) described a similar finding in the case of the cells of plasmacytomata.

There can be little doubt that the " crystals " occurring within the cytoplasm of plasma cells are similar in composition to Russell bodies for they give identical staining reactions and occur in the same conditions and within the same groups of plasma cells. In this connection it may be relevant to note that certain antibodies have been obtained *in vitro* in a crystalline state although this is apparently no evidence that they are pure (Pope and Stevens (1953) in regard to diphtheria antitoxin).

Intracellular crystals associated with plasma cells have also been reported from a variety of human pathological sources. Glaus (1917) and Steinmann (1919) described their presence within the abnormal cells of a plasmacytoma. They have also been described in the cells of granulomas. Thus, the structures " *Zigarrenbunde ähnlich* " described by Mibelli (1889) appear to be examples of crystals within plasma cells of a rhinoscleroma. Zettergren (1949) described protein crystals within the plasma cells of a polyp of the ear, and Freifeld (1913) also described similar crystals in the small round cell infiltration at the margin of an adenocarcinoma.

This account of their occurrence in the experimental animal has not included irrevocable evidence that these structures are truly crystalline, and the opinion is based solely on their morphology. Repeated attempts to demonstrate anisotropism of the " crystalline bodies " or Russell bodies have failed. Zettergren (1949) in describing the crystals present within the plasma cells of an aural polyp concluded that since he could not demonstrate them to be anisotropic and since " inclusions sectioned in a plane parallel with one of the surfaces of the inclusions are always quadrate " the inclusions were members of the cubic crystalline system. If this observation were correct it would be extremely unusual for a protein substance to crystallize within this system. Possibly the fixative (formol alcohol) or the dehydration in absolute alcohol is responsible for a disorientating action upon the protein molecules within the crystal lattice. In the present study frozen sections were restricted to mouse tissues. Crystals were never observed in this material and it was thus not possible to study them in sections uninfluenced by fixatives.

## SUMMARY.

Cytoplasmic bodies have been observed in the mature plasma cells which occur in the spleen of rabbits and mice after intravenous injection of repeated doses of various bacillary vaccines.

These structures have a close morphological resemblance and common staining reactions with the " fuchsin bodies " described in human anatomical material by William Russell (1890) and commonly referred to as Russell bodies. This term has been adopted for the bodies with this appearance produced experimentally in animals as described in this paper.

Russell bodies can be produced by a single antigenic stimulus (*P. vulgaris* vaccine) providing the animal has been previously sensitized to this antigen. In mice, it was shown that Russell bodies may appear as early as the third day following such a recall stimulus but occur in maximum numbers at the seventh to the ninth day.

Intracytoplasmic crystals may also develop in mature plasma cells in the same experimental conditions as were found to produce Russell bodies. The material composing such intracytoplasmic crystals has identical staining reactions with the material of Russell bodies.

The surface of the Russell bodies produced in the mouse spleen by the intravenous injection of *P. vulgaris* yielded an intense reaction characteristic for antibody to this organism. The findings with this technique are compatible with the view that antibody is a major component of such Russell bodies.

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## REFERENCES.

ACKERMAN, G. A.—(1952) *J. nat. Cancer Inst.*, **13**, 219.
APITZ, K.—(1937) *Virchows Arch.*, **300**, 113.
BING, J.—(1940) *Acta med. scand.*, **103**, 565.
BJØRNEBOE, M. AND GORMSEN, H.—(1943) *Acta path. microbiol. scand.*, **20**, 649.
BLIX, G., TISELIUS, A. AND SVENSSON, H.—(1941) *J. biol. Chem.*, **137**, 485.
COONS, A. H. AND KAPLAN, M. H.—(1950) *J. exp. Med.*, **91**, 1.
*Idem*, LEDUC, ELIZABETH H. AND KAPLAN, M. H.—(1951) *Ibid.*, **93**, 173.
*Idem*, LEDUC, ELIZABETH H. AND CONNOLLY, JEANNE M.—(1953) *Fed. Proc.*, **12**, 439.
CORNIL, V. AND ALVAREZ, E.—(1885) *Arch. Physiol. norm. path.*, **6**, 11.
DOWNEY, H.—(1938) 'Handbook of Haematology.' London (Hamish Hamilton).
FAGRAEUS, ASTRID—(1948) *Acta med. scand.*, Suppl., 204.
FREIFELD, H.—(1913) *Beitr. path. Anat.*, **55**, 168.
GLAUS, A.—(1917) *Virchows Arch.*, **223**, 301.
HALE, C. W.—(1946) *Nature, Lond.*, **157**, 802.
HOTCHKISS, R. D.—(1948) *Arch. Biochem.*, **16**, 131.
KAPLAN, M. H., COONS, A. H. AND DEANE, HELEN W.—(1949) *J. exp. Med.*, **91**, 15.
KONSTANTINOWITSCH, W. N.—(1902) *Virchows Arch.*, **167**, 443.
LENDRUM, A. C.—(1947) *J. Path. Bact.*, **54**, 399.
LINDERSTRØM-LANG, K. AND MOGENSEN, K. R.—(1938) *C.R. Lab. Carlsberg*, Série chim., **23**, 27.
LISCO, H.—(1942) *Anat. Rec.*, **82**, 59.
MALLORY, F. B.—(1938) 'Pathological Technique.' Philadelphia (Saunders).
MARSCHALKO, T.—(1900) *Arch. Derm. Syph., Wien.*, **54**, 235.
MARSHALL, A. H. E. AND WHITE, R. G.—(1950) *Brit. J. exp. Path.*, **31**, 157.
MCMANUS, J. F. A. AND CASON, JANE E.—(1950) *J. exp. Med.*, **91**, 651.
MIBELLI, V.—(1889) *Mh. prakt. Derm.*, **8**, 531.
MILLER, F. R.—(1931) *J. exp. Med.*, **54**, 333.
PEARSE, A. G. E.—(1949) *J. clin. Path.*, **2**, 477.—(1953) 'Histochemistry Theoretical and Applied.' London (Churchill).
PELLIZZARI, C.—(1883) *Arch. Sc. Anat. Patol., Firenze*, ii, 123.
POPE, C. G. AND STEVENS, MURIEL F.—(1953) *Brit. J. exp. Path.*, **34**, 56.
RUSSELL, W.—(1890) *Brit. med. J.*, **2**, 1356.
STEINMANN, B.—(1919) *Dtsch. Arch. klin. Med.*, **185**, 49.
THOMAS, L. E.—(1946) *J. cell. comp. Physiol.*, **28**, 145.
WEILL, P.—(1919) *Arch. mikr. Anat.*, **93**, 1.
WHITE, R. G., COONS, A. H. AND CONNOLLY, JEANNE M.—(1953) *Fed. Proc.*, **12**, 465.
ZETTERGREN, L.—(1949) *Acta path. microbiol. scand.*, **26**, 696.