THE INTERPRETATION AND SIGNIFICANCE OF GORDON'S TEST

IN THE DIAGNOSIS OF HODGKIN'S DISEASE

A Study of 92 Cases

by

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Submitted for the Lewis Cameron Post-Graduate Prize for an Essay on the Diagnosis of Disease.
The aim of this article is an endeavour to present to the reader a concise account of recent experimental work on lymphadenoma. Gordon (1932) demonstrated that the intracerebral inoculation of a rabbit with an emulsion of lymphadenomatous lymphatic tissue was followed by paralysis, ataxia, musculo-incoordination and sometimes death of the animal. His discovery has been utilised to form the basis of a biological test for the diagnosis of Hodgkin’s disease by van Rooyen (1933) and Ogilvie and van Rooyen (1933-4), who have drawn attention to the value of this procedure to the clinician and pathologist alike. Their findings have received the confirmation of several independent workers, such as van der Hoeden and Hulst (1934), Davidson (1934), Paterni, Maroncelli & Corsi (1935), Davis (1935) & Smith (1935), to whose publications reference should be made for further details concerning the practical utility of Gordon’s test. Whereas the test is useful as an aid to the routine histological examination of gland tissue derived from suspected cases of Hodgkin’s disease, much dubiety has existed with regard to the exact nature of the pathogenic agent present in this condition and responsible for the encephalitic syndrome in the rabbit. Considerable light has been shed on this problem by the work of Friedemann (1934), who showed that normal/
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normal leucocytes, bone-marrow and splenic tissue may also exhibit an encephalitogenic property towards the rabbit similar to that produced by lymphadenomatous lymphatic glands.

Friedemann (1934) further suggested that this agent is Jochmann's proteolytic enzyme, and demonstrated its ability to withstand the prolonged action of alcohol, ether and glycerol.

Mackenzie and van Rooyen (1935) have confirmed Friedemann's findings as regards the distribution of the pathogenic agent in human bone-marrow, spleen and leucocytes and have drawn the following conclusions from their studies - (1) the encephalitogenic principle present in normal tissues (vide supra) is indistinguishable from Gordon's agent observed in lymphatic glands affected with lymphadenoma. (2) Gordon's agent cannot be identified with Jochmann's proteolytic enzyme since no relationship can be found to exist between the proteolytic activity of a specimen of tissue examined on the one hand and its encephalitogenic properties on the other.

Both investigators have agreed, however, that the pathogenic agent represents a product associated with certain normal and pathological cells.

On account of the resemblance existing between the encephalitic syndrome in the rabbit produced by lymphadenomatous tissue and that produced by certain neurotropic viruses, the possible virus nature of the pathogenic agent has been investigated (van Rooyen 1934).
Efforts to transmit the encephalitic condition from one rabbit to another have failed, and those animals which recovered from it were found to be again susceptible on re-inoculation. No definite immunity phenomena have been demonstrable and a search for inclusion bodies in the central nervous system of affected rabbits has failed to reveal them.

On the other hand Gordon (1932) and Coles (1934) have reported the presence of minute particles resembling the elementary bodies of vaccinia in glands affected with Hodgkin's disease.

It is difficult, however, to accept their presence alone, as conclusive evidence for the assumption that lymphadenoma is a virus disease, especially when considered along with certain noteworthy negative findings such as those already referred to above.

There is no alternative therefore, but to conclude that Gordon's agent represents a tissue derivative of certain normal and pathological cells which exhibits a toxic effect towards the central nervous system of the rabbit.

With regard to the distribution of the encephalitogenic agent in animal tissues other than those of the human subject, Friedemann (1934) has drawn attention to its occurrence in the Bone-marrow of monkeys. This question is under investigation, and will be dealt with elsewhere.

The Limitations of Histological Evidence as a means for the Diagnosis of Hodgkin's disease.
When the histopathological appearance presented by biopsy or necropsy material are typical of Hodgkin's disease in a suspected case of this condition, the diagnosis may be readily established.

In an attempt to elicit the maximum information from a given case and obviate the chance of securing an enlarged gland which is inadequately representative of the nature of the pathological process as a whole, both Cunningham (1915), and Barron (1926), have advocated the simultaneous resection and examination of several glands at one time.

Unfortunately, however, there remains a proportion of instances in which no definite conclusions can be drawn from the results of microscopical examination. This is in accordance with the experience of numerous morbid histologists and the existence of a group of conditions closely resembling but not identical with classical Hodgkin's disease has long been recognised. Thus, after an extensive review of the subject dealing with the histological diagnosis of Hodgkin's disease, Wallhauser (1933) states "the criteria necessary to establish a diagnosis of Hodgkin's disease are hardly standardised or uniform, and the apparently increasing inclination of some writers to submerge all or many neoplastic or neoplasmoid structures of lymphoid origin under the enveloping and unqualified term of lymphoblastoma has further complicated the problem."

He continues to quote Sternberg, who records that/
that "The diagnosis of a typical lymphogranuloma has come to mean about as much as pseudoleukaemia, into which everything was placed that did not fit elsewhere. This is a pitiable back-step."

Likewise Kettle (see Pullinger 1934) has suggested the term "Hodgkin group" as a suitable designation in order to include those cases which presented atypical characteristics.

In a paper dealing with the diagnosis of lymphadenoma, Ritchie (1935) advocated the routine performance of Gordon's test in all suspected cases of Hodgkin's disease. He further remarks that "the histological findings are often difficult of interpretation and depart very widely from the classical description of Reed and others, whilst blood examinations are often of more value in excluding the diagnosis than in confirming it. Nevertheless amongst the ill-defined group of progressive enlargements of the lymph glands there are certain cases concerning which the clinician, the pathologist and haematologist can reach ultimate unanimity, even if this happy issue is reached only over the autopsy table."

Calvert and Saiguinetti (1933) also draw attention to the extraordinary difficulties which may be sometimes met with in the diagnosis of Hodgkin's disease.

Remarks such as these indicate the unsatisfactory state of our knowledge concerning the nature of hyperplastic conditions affecting the lymphoid and the reticulo-endothelial systems generally. They furthermore/
furthermore illustrate that notwithstanding the multiplicity of available dyes employed for staining lymphoid structures, the total data gleaned from microscopic examination of such tissue, still leaves much to be desired.

It must be appreciated that the morbid histologist is only able to observe the staining properties, the shape, size, contents and configuration of particular cells, or group of cells, from which deductions have to be made as to the possible nature of the pathological process at work. In lymphadenoma owing to the fact that a multiplicity of cell types are probably derived from the reticulum of the gland, it is only to be anticipated that intermediate and ill-defined groups of cells are to be encountered in a certain percentage of cases, thereby complicating the histological picture.

The property of lymphadenomatous tissue to produce an encephalitic syndrome in the rabbit must accordingly be looked upon as an important characteristic. It will be shown later in this paper that Gordon's test is closely associated with the reticulum cell itself, and as such indicates a particular biological property of this type of cell, apart from its appearance in stained sections.
The Interpretation of the Biological Test.

Merely because a similar syndrome to that produced by lymphadenomatous glands may also be elicited by the intracerebral inoculation of bone-marrow and splenic tissue, it must not be concluded that the value of the test is lessened.

It should be clearly understood that Gordon's Biological test for Hodgkin's disease only refers to the results following the intracerebral inoculation of rabbits with enlarged lymphatic glands and is not applicable to the effects of any other tissues when injected in the same way.

During the course of this work, extending over a period of 4 years, biopsies have been performed on 92 cases of suspected Hodgkin's disease and the tissue examined both histologically as well as subjected to biological test. Comparison of findings has revealed that the test is negative in Tuberculosis, lymphosarcoma, lymphatic leukaemia, syphilis and pseudoleukaemia (or a-leukaemic leukaemia), but positive in a high percentage of cases of lymphadenoma.

It may therefore be presumed that Hodgkin's disease is the commonest cause of lymphadenohyper trophy that yields a positive result in this test.

Exceptions to this statement have been few in number: in one case, a positive result was obtained in a case of Hodgkin's disease complicated by glandular tuberculosis. In another, a similar finding was observed/
observed in a boy age 11, who clinically presented
the text book description of lymphadenoma but, on
histological examination displayed a marked eosino-
philia without any other features sufficiently
characteristic to warrant a diagnosis being pronounced.

Exceptional instances such as these are only to
be expected when dealing with a laboratory method of
diagnosis, and it may therefore be safely said that
the percentage of error associated with Gordon's test
compares favourably with those of other procedures
involving the use of biological reagents in routine
diagnosis.

The test applied to lymphatic glands yields
information that is of assistance to the morbid-
histologist in the interpretation of histological
results in suspected cases of lymphadenoma, especially
when doubt exists with regard to the appearances seen
in sections.

A positive result in circumstances such as these
should be regarded as evidence in favour of Hodgkin's
disease. See Ogilvie and van Rooyen (1935).

A negative result, however, is of limited
significance and may sometimes be encountered in
typical cases of lymphadenoma. The test should be
repeated in such a case, for it has been noticed that
individual specimens of lymph nodes have been found
to vary in their encephalitogenic activity. For
example, in an earlier case of lymph adenoma it was
found that whilst a small cervical gland removed at
biopsy gave a negative test, after death of the
patient/
patient, glands removed from the mediastinum and groin gave a positive reaction.

Gordon's biological test is positive in 85% cases of lymphadenoma (Gordon 1934), and positive in 75% and 69% cases according to van Rooyen (1934), and Davis (1935), respectively.

It will thus be observed that the test has been found to be negative in a certain percentage of cases which were both clinically and histologically characteristic of Hodgkin's disease.

Furthermore, it should be pointed out that great variability exists with regard to the degree of encephalitogenic action displayed by material derived from different cases of Hodgkin's disease. For example, whereas one specimen may cause marked ataxia, paralysis, and death within 3 days, another, may produce milder lesions from which the animal may recover completely or be entirely unaffected by it.

Attention has been paid to this aspect of the problem and accordingly glandular tissue has been examined microscopically with a view to determining whether the variability in encephalitogenic behaviour may be explained on a basis of cellular composition.

It was previously reported (van Rooyen 1933) that those lymph glands which were tough and fibrous in consistency frequently exhibited little or no encephalitogenic activity towards the rabbit and thus gave a negative result. Further work has showed that, to some extent, the result of the test may be influenced by the amount of fibrosis present in any particular specimen examined. This feature was illustrated/
illustrated in the case of two specimens removed from a single case of lymphadenoma, the one obtained at biopsy which gave a negative result, and the other at necropsy that yielded a positive.

Comparison of sections in such a case revealed that whereas the former contained excessive fibrous tissue in its composition, the latter displayed a greater degree of cellularity in structure. There has thus been some evidence to indicate that the presence or absence of the test is related to the degree of cellularity manifested by various specimens of glands and therefore explanation has been sought to interpret the reaction on a basis of such findings.

The Relationship of Gordon's Reaction to Histological Structure in Lymphadenoma.

The microscopic anatomy of typical Hodgkin's disease has been fully described by several observers such as Greenfield (1878), Andrewes (1902), and others, no further account is therefore necessary. The author would remind the reader, however, that the usual sequence of events occurring in lymphatic glands are essentially, initial hyperplasia of lymphoid tissue, followed by the appearance of numerous endothelial cells, with a corresponding diminution in the numbers of existing lymphocytes.

These reticulo-endothelial cells are large in size, oval or fusiform in shape, contain a pale staining nucleus of vesicular type, showing chromatin granules within it and surrounded by a distinct nuclear membrane. Considerably larger cells called Hodgkin
giant cells, are also observed at this stage, and these are recognisable by virtue of their comparatively large size and characteristic multiple or convoluted nuclei. Eosinophilic polymorphonuclear leucocytes are frequently present and neutrophilic leucocytes are sometimes seen in addition. As the condition progresses the normal glandular architecture tends to be obliterated and replaced by the elements described above. The gland capsule is usually unaffected by these changes. Connective tissue fibres eventually make their appearance, and wide areas of fibrosis may be seen throughout the section.

Hitherto, attempts to correlate the occurrence of a positive reaction in the rabbit with the existence of a particular type of cell in the tissue used for inoculation, have been unsuccessful.

Subsequent work, however, has proved that in a number of instances it has been possible to associate closely a positive result with the presence of the reticulum cell in lymphadenoma. This conclusion has been reached after systematic microscopical examination of Hodgkin lymph nodes that varied in their encephalitogenic activity towards the rabbit and by observing the incidence of various cell elements contained within them.

Attention was therefore focussed upon those tissues which showed the greatest cellularity in their composition. All sections were thus divided into two classes according to whether they had been derived/
derived from positive or strongly positive encephalitogenic material. An endeavour was then made to detect any significant histological differences between individual specimens with the object of discovering if any particular cells or group of cells were to be linked with the reaction in the rabbit.

Sections were stained by Haematoxylin eosin and eosin Methylene-blue as well as by Foot’s method when available.

The results indicated that gland tissue which contained numerous Hodgkin giant cells was found to be no more active than those which did not.

Likewise the relative incidence of neutrophilic and Eosinophilic polymorphonuclear leucocytes did not appear to affect the results. The number of lymphocytes was also found to be unconnected with the reaction and this was proved not only in certain lymphadenomatous glands in which numerous lymphocytes were noticed but also from the negative results recorded in lymphosarcoma, lymphatic and a leukaemic leukaemia respectively.

On the other hand it was repeatedly observed that large numbers of reticulum cells were encountered in those glands which gave a positive reaction. For example, in five specimens in which positive results were recorded it was observed that the tissue was composed principally of numerous large reticulo-endothelial cells with only occasional eosinophil and polymorphonuclear leucocytes intermingled among them.

The/
H.15.
Several reticulo-endothelial cells and Hodgkin giant cells present. No eosinophil leucocytes could be detected.

Biological test — STRONGLY POSITIVE.

From a case under care of Professor Murray Lyon.
Tissue is highly cellular in composition. Numerous reticulo-endothelial cells, Hodgkin Giant cells and lymphocytes are present. A few eosinophil polymorphonuclear leucocytes were also found. Biological test - STRONGLY POSITIVE. Section supplied by courtesy of Professor Shaw Dunn.
H.40.
Tissue shows active reticulo endothelial proliferation. Numerous reticulum cells of various shapes and sizes are present throughout the field and form the predominant type of cell in this specimen. An occasional Hodgkin giant cell can be seen and eosinophil leucocytes are absent.

Biological test - STRONGLY POSITIVE.
BT.1.
Highly cellular tissue. There are large numbers of reticulum cells with lymphocytes present amongst them. Giant cells are scanty in distribution and eosinophils absent. Neutrophil polymorphs were seen in a few fields only.
Biological test - STRONGLY POSITIVE.
Section supplied by courtesy of Professor Stanley Davidson from a case of lymphadenoma.
H.23.
Specimen is less cellular in composition than H.33, H.40, H.15 and BT.1. respectively.
Numerous cells are to be found in this section. They are principally actively proliferating reticulo-endothelial cells with several Hodgkin giant cells. A few eosinophil polymorphs are also present and some small areas of fibrous tissue formation exist.
Biological test - POSITIVE.
Section supplied by courtesy of Professor Shaw Dunn.
Specimen has been obtained from an advanced case of lymphadenoma. There are considerable areas of fibrosis, several giant cells and numerous lymphocytes. Biological test - WEAKLY POSITIVE.
H. 16.
Tissue shows extensive fibrosis. Cells present are chiefly lymphocytes, large numbers of eosinophil leucocytes and occasional Hodgkin giant cells. Biological test - NEGATIVE. Specimen supplied from a case of chronic Hodgkin's disease by courtesy of Professor Wilkie.
HR.
Section from a case of chronic Hodgkin's disease of four years duration showing much fibrosis.
Biological test - NEGATIVE.
Section supplied by courtesy of Professor Utz, Sydney, Australia.
Case of Dr. Gow, Barts. Hospital, London.
This specimen illustrates the fact that although tissue may be markedly cellular in composition and contain numerous reticulum cells, giant cells and eosinophil leucocytes it may sometimes give a negative result in the rabbit. Four animals were injected intracerebrally with tissue derived from this case but with negative results throughout. Compare this with the next specimen H.37.

Biological test - NEGATIVE.
Section supplied by courtesy of Professor Shaw Dunn.
This section is almost identical in general appearance with that evident in the previous specimen which gave a negative reaction, but it produced a positive reaction in the rabbit after intracerebral inoculation.

Biological test — POSITIVE.

Section supplied by courtesy of Professor Shaw Dunn.
The former cells when stained by Haematoxylin and eosin appear as large oval or fusiform structures, with a pale blue cytoplasm and a vesiculated nucleus, surrounded by a definite nuclear membrane. For a detailed description of their morphological characters the reader is referred to the careful description devoted to them by Pullinger (1932).

Whilst it is only reasonable to conclude that these cells (in an analogous manner to normal leucocytes) contained the encephalitogenic agent to the rabbit, it should be emphasised that in certain cases, although they were present in sections, the tissue failed to produce a strongly positive reaction in the rabbit.

It would thus appear that the encephalitogenic agent is not always demonstrable in the reticulo-endothelial cells found in lymphadenoma, and its inconstancy in this tissue is similar to its occurrence or absence in various specimens of pus or leucocytes, see Gordon (1934) and Mackenzie and van Rooyen (1935).

It is of particular interest, however, that both the normal polymorphonuclear leucocytes as well as the reticulum cells observed in lymphadenoma should be found to contain a similar encephalitogenic agent towards the rabbit. It remains to be seen whether this finding would help to strengthen the views of Kidd and Turnbull (1908) and MacNalty (1928) and Pullinger (1932) who have postulated the local origin of the granular leucocytes in lymphadenoma, in/
in contradistinction to the general opinion that they are the result of colonization and deposition via the blood stream. Whichever of these possibilities ultimately proves to be correct, the current work indicates the presence of a common characteristic existing between reticulum cell and the polymorphonuclear leucocyte. Pullinger (1932) quotes a case in which direct heteroplastic transformation of reticulum cells into myelocytes and promyelocytes occurred without passage through a common blood cell stage. This author also states earlier that "No further discussion on the exact nature of these changing cells can be pursued on account of lack of data."

Under the circumstances the encephalitogenic property manifested by leucocytes, bone-marrow and the reticulum cells in Hodgkin's disease would appear to supply the lacking information regarding the hitherto suspected relationship existing between these group of cells. Medlar (1931) has suggested that Hodgkin's disease and myelogenous leukaemia are genetically allied, and Pullinger has likewise concluded that the disease may be described as an extramedullary fibro-myeloid reticulosis.

There would appear to be no reason for the onset of these changes and the failure to find a specific-micro-organism, strengthens the belief that the disease is of neoplastic origin. It must be remembered that minute particles resembling elementary bodies/
bodies found in virus diseases have also been seen in lymphadenomatous tissue by certain workers. Apart from their occurrence, numerous attempts to seek additional evidence to support the virus hypothesis have failed. After due consideration of the findings of earlier investigators and from the results of continuous research work into the aetiology of lymphadenoma extending over a period of 4½ years, carried out by the author, he has concluded his studies on this subject.

At the present time there is the strongest possible evidence to support the opinion that lymphadenoma is essentially a neoplastic process affecting lymphatic gland tissue.
AETIOLOGY OF HODGKIN'S DISEASE
WITH SPECIAL REFERENCE TO
B. TUBERCULOSIS AVIS

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The relation of Hodgkin's disease to tuberculosis has been the subject of extensive inquiry, and many workers, such as Sternberg (1898), Ewing (1927), and others, have repeatedly drawn attention to the similarity of tissue changes produced in both conditions. Opinion, however, is divided on the incidence of tuberculosis in patients suffering from lymphadenoma. Ewing says that "tuberculosis follows Hodgkin's disease like a shadow"; Lemon (1924) reports, to the contrary, that out of 191 cases of Hodgkin's disease under observation in the Mayo Clinic only eight showed evidence of tuberculosis. The latter worker further states that out of twenty-six cases of the disease in which the mediastinal glands were involved there was definite evidence of tuberculosis in only one.

Infective agents other than the tubercle bacillus, such as the diphtheroid organism (Bunting and Yates, 1913), certain moniliae (Dias, 1918) and amoebae (Kofoid, Swezy, and Boyers, 1922), have been described in Hodgkin's disease, but such work has lacked confirmation. Stewart and Dobson (1924) attempted to reproduce the disease in monkeys (rhesus and bonnet), but without results. Twort in 1930 published the results of six years' research into the causation of the condition, and arrived at the conclusion that the disease was probably of neoplastic origin, since he was unable to isolate any one organism with constancy or to reproduce the disease in laboratory animals with pathological material.

More recently American workers have advanced two fresh views on the aetiology of the condition. Medlar (1931) maintains that the disease is one primarily affecting the bone marrow, for which he proposes the name "megakaryoblastoma" in view of the particular type of cell involved. L'Esperance (1929-31) claims to have reproduced...
Hodgkin's disease in birds and "treated" guinea-pigs, from whose tissues she recovered the avian tubercle bacillus. This investigator therefore states that Hodgkin's disease is produced by infection with B. tuberculosis avis. This view has been further elaborated by Utz and Keatinge (1932), who claim to have treated successfully several cases of lymphadenoma by the use of serums obtained from chickens previously immunized with lymphadenomatous tissue. I have investigated the claims of L'Esperance by the inoculation of chickens and other laboratory animals with material obtained at biopsy from three cases clinically and histologically characteristic of Hodgkin's disease, and also with material removed at necropsy from three other cases of the disease, two of which showed mediastinal involvement.

The following brief extracts of case and post-mortem notes describe the subjects from which material was obtained.

**CASE HISTORIES**

1. **Case of Professor Fraser's.**—A male, aged 52, had swelling in left axilla of eleven months' duration, with later ulceration and secondary infection lasting three months. There was slight enlargement of the inguinal and infraclavicular glands, with no evidence of mediastinal involvement. Ten per cent. eosinophils were present. Histological diagnosis: Hodgkin's disease.

2. **Case of Dr. Comrie's.**—A female, aged 28, had a swelling of the glands of the neck for eight months, with later involvement of abdominal glands and spleen. These changes were accompanied by extensive bronzing of the skin and intolerable itching. Throughout her illness a fever conforming to the Pel-Ebstein type was present, and the blood picture showed an eosinophilia. At post-mortem the cervical, mediastinal, bronchial, and abdominal para-aortic glands were grossly enlarged, together with slight enlargement of the lymph nodes of the lesser omentum. The spleen was enlarged to about three times its normal size, and contained numerous nodules throughout. The heart, liver, and lungs also showed involvement with lymphadenomatous tissue.

3. **Case of Professor D. Murray Lyon's.**—A male, aged 46, complained of vague symptoms of general illness lasting three months, with loss of appetite and weight, diarrhoea, colic, and dyspnoea. Temperature reaction of the Pel-Ebstein type was present, accompanied by enlargement of liver and spleen. At post-mortem there was considerable enlargement of para-aortic, abdominal, and mediastinal lymph glands, with commencing enlargement of the cervical and inguinal groups. The bone marrow revealed red patches suggestive of erythroblastic reaction, and microscopically proliferation of bone marrow cells affecting the red cell series was found to be present.

4. **Case of Professor Bramwell's.**—A male, aged 27, gave a
three months’ history of loss of weight and appetite, accompanied by distension of the abdomen and bronzing of the skin. On admission the patient was found to have an enlarged spleen, dullness over the apex of the right lobe, with crepitations and vesicular breathing. Pel-Ebstein type of fever was present. Red blood cells were 4,200,000 per c.m.m., and white cells 3,800; differential count was normal, and haemoglobin 70 per cent. Post-mortem revealed typical lymphadenoma of spleen, liver, and para-aortic and cervical glands. Brown atrophy of the heart. No evidence of tuberculosis demonstrable.

5. Case of Professor Fraser’s.—A female, aged 31, first noticed lumps on her neck two years before examination, initially confined to the left side, but later involving the right. These painless swellings rapidly increased in size, and later gave rise to pressure symptoms in that region. There was no palpable enlargement of spleen, liver, or lymph glands in inguinal or axillary regions. An intermittent fever accompanied her illness. Red blood cells were 4,540,000 per c.m.m., and white cells 9,200; differential count was normal, and haemoglobin 70 per cent.; colour index, 0.08. Histological diagnosis: Hodgkin’s disease. Deep x-ray therapy and subcutaneous injections of Coley’s fluid over the affected area were employed, which resulted in marked diminution in the size of the glands and considerable general improvement.

6. Case of Dr. Chalmers Watson’s.—A schoolboy, aged 8, gave a five years’ history of progressive enlargement of submental, supraclavicular, axillary, and inguinal glands, and of the spleen. Increasing weakness and cachexia, severe secondary anaemia, accompanied by cardiac haemic murmurs, commencing integumentary dryness and pigmentation were present. During the last two years patient exhibited a typical Pel-Ebstein pyrexia. The histological diagnosis, clinical manifestations, course of the disease, and reaction to deep x-ray therapy have shown this case to be almost a textbook picture of the disease.

EXPERIMENTAL WORK

Enlarged lymph glands removed at biopsy, together with liver, spleen, and bone marrow obtained in necropsy, were examined for the presence of avian tubercle bacilli by direct attempts at cultivation on Dorset’s egg media, but no such organisms were isolated. It is of interest, however, to state that in both Cases 1 and 6 a small, typical, Gram-positive, aerobic diphtheroid organism was isolated after about ten days’ incubation. Both strains subsequently proved to be non-pathogenic to laboratory animals, and so further investigations were discontinued. About 300 histological sections of affected glands and viscera were stained for acid-fast organisms, but none were found.

Animal Inoculation

Four bantam chickens and four street pigeons were inoculated both intravenously (through the wing vein) and intra-
peritoneally with 2 to 3 c.c.m. of a dense emulsion of affected material, comprising enlarged lymph glands, spleen, and bone marrow. Animals were kept under observation for a period of seven to ten months, but no signs of illness were noticed. Two chickens which had been inoculated with emulsions of spleen and lymph gland by the two routes were examined post mortem ten months later, but found to be perfectly healthy.

**Grafting Experiments**

Five healthy chickens (three buff orpingtons and two white leghorns) were used for these experiments. With strict aseptic precautions small fragments of an enlarged lymph gland (removed at biopsy by Professor Fraser, from Case 5) were implanted into the birds as follows. (1) *Extraperitoneally*. (2) *Intraperitoneally*. (3) *Intrahepatically*: This was carried out by making a V-shaped incision into the free margin of the left lobe of the liver, and inserting into this a wedge-shaped portion of gland, which was then secured by means of catgut sutures. (4) *Into shaft of femur*: This was done after the manner of an intramedullary graft, and performed by exposing the femur, removing a U-shaped fragment of bone with the aid of bone-nibbling forceps, and then inserting small fragments of lymphadenomatous tissue into the proximal and distal ends of the medullary canal. (5) *Intramuscularly*: Implantation was in the pectoral muscles. All five birds made a good recovery from the operation, and at the end of a week appeared to be in good health.

Animal No. 1 died after three months, succumbing to accidental injury. Necropsy revealed that the attempted graft had undergone necrosis and subsequent extrusion into the superficial tissues, while the surrounding tissues showed extensive fibrosis and cicatricial contracture, presumably in the nature of a reaction towards a foreign body. No evidence of tuberculous ulceration or caseation was to be found in the underlying tissues, peritoneum, or viscera. Animal No. 3 was killed after six months and the site of the attempted graft examined both macroscopically and microscopically, but no changes indicative of tuberculosis were evident and no bacterial growth was obtained on culture. The remaining animals are still under observation, and appear to be healthy after seven months.

**Discussion**

In the experiments described above no evidence of infection with *B. tuberculosis avis* was found in material taken from six accurately diagnosed cases of Hodgkin's disease.

Cases of human infection due to the avian tubercle bacillus have already been described by several European workers. Löwenstein (1913) obtained the organism from cases of pulmonary, renal, and dermal tuberculosis. Rabinowitsch (1907) succeeded in isolating the organism
from the spleen of a case of generalized miliary tuberculosis, and Janscó and Elfer (1910) also demonstrated organisms of a similar type in the mesenteric glands of a child. It would thus appear that the occurrence of human infection by the avian tubercle bacillus has been proved, and isolation of the causative organisms seems to have been achieved by the usual well-recognized methods. With reference to the claims made by L'Esperance, I have not only failed to produce tuberculosis in chickens by inoculation with lymphadenomatous material, but I have also been unable to demonstrate the presence of avian tuberculosis in affected material, either by direct cultural methods or by examination of a large number of stained histological preparations.

It is noteworthy that in the literature of the cases of human tuberculosis due to avian tubercle bacillus there is no mention that the clinical manifestations of these cases in any way differed from the syndrome produced by infection with the ordinary human or bovine types. Indeed, it is questionable whether the true nature of the causative agent would have been determined had a special search not been made for it.

The feeble pathogenicity of the avian tubercle bacillus to the guinea-pig and its more pronounced effect on the rabbit are well-recognized characteristics (Medlar, 1931) that cannot be lost sight of when one also remembers that the non-pathogenicity of lymphadenomatous material to rabbits and guinea-pigs is a fact of wide acceptance. A notable exception to this is found in the results obtained by M. H. Gordon and co-workers of the Rose Research on Lymphadenoma (1932), who have demonstrated that the intracerebral injection of lymphadenomatous tissue into rabbits is followed by ataxia, muscular spasms, and paralysis. Such effects are not produced, however, by the injection of leukaemic, sarcomatous, and carcinomatous tissue. L'Esperance nevertheless claims to have reproduced avian tuberculosis in birds by the injection of lymphadenomatous material into them, without having simultaneously done so in rabbits. The same worker's claim to have reproduced Hodgkin's disease in guinea-pigs which had previously been inoculated with killed cultures of B. tuberculosis hominis is also unconvincing.

It should also be pointed out that, according to Griffith (1930), under domestic conditions poultry exposed to infection readily develop tuberculosis, frequently following the ingestion of contaminated food. Organs most commonly involved are the intestine, liver, and bone marrow, but the writer has been unable to reproduce avian tuberculosis
by the introduction of lymphadenomatous tissue directly into these areas.

SUMMARY AND CONCLUSION

Pathological material obtained from six accurately diagnosed cases of Hodgkin's disease have been examined by direct cultural methods for the presence of the avian tubercle bacillus, and histological sections examined for acid-fast organisms, but both with negative results. Tissue emulsions have proved to be non-infective to rabbits, guinea-pigs, pigeons, and chickens on intravenous, intraperitoneal, and intramuscular administration. Attempts to graft lymphadenomatous material obtained from an acute case of Hodgkin's disease into the bone marrow, peritoneum, liver, and muscles of chickens have likewise failed.

No evidence of *B. tuberculosis avis* can be found in cases of Hodgkin's disease.

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Recent literature has shed fresh light on many hitherto obscure factors concerning the nature of Hodgkin's disease. Thus the workers of the Rose Research on lymphadenoma have shown, after a careful search, that neither spirochaetes, yeasts, diphtheroids, nor acid-fast bacteria have any relation to the condition. Similarly, the finding of L'Esperance (1931) that the avian tubercle bacillus may be causally associated with the disease has not been confirmed (van Rooyen, 1933).

Perhaps the most interesting contribution towards the study of the problem has been the work of M. H. Gordon (1932). He showed that the intracerebral inoculation of rabbits and guinea-pigs with suitable suspensions of lymphadenomatous tissue was followed in a few days by spastic paralysis of the hind limbs, rigidity, ataxia, and muscular weakness. Such effects, however, were not produced by the injection of similarly prepared suspensions of normal, leukaemic, sarcomatous, and carcinomatous lymphatic tissue. It thus appeared that lymphoid tissue affected by Hodgkin's disease acquires properties which this tissue does not exhibit when affected by certain other pathological conditions. Work bearing on the precise nature of the agent responsible for the syndrome produced experimentally in rabbits is without the scope of the present article, and demands further investigation. It is proposed, however, to draw attention to the possibilities which this phenomenon offers as a means of identifying true lymphadenomatous tissue, and also to its clinical value as an aid in the diagnosis of certain doubtful cases. The following brief abstracts from case and post-mortem reports of patients treated in the Royal Infirmary of Edinburgh describe subjects from which material was obtained. The first five were cases of Hodgkin's disease.

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the sixth was one of pseudo-leukaemia, and the seventh one of lymphosarcoma.

CASE I
A miner, aged 43, under the care of Dr. Goodall, had a swelling on the side of his neck for three to four years, and complained of cough for six months. Enlarged glands were palpable in the neck and axillae, whilst x-ray examination revealed enlargement of the superior mediastinal and bronchial groups as well. The spleen was not palpable. The blood count was as follows: red cells, 4,700,000 per c.mm.; white cells, 6,800 per c.mm.—neutrophils 82 per cent., eosinophils 3 per cent., lymphocytes (large and small) 15 per cent.—haemoglobin 68 per cent.; Wassermann reaction was negative. Histological diagnosis: Hodgkin’s disease.

CASE II
A forester, aged 53, under the care of Dr. Matthew, had noticed progressive enlargement of his right axillary glands for ten weeks before admission. Temperature varying between 102° and 103° F., accompanied the illness. X-ray treatment produced no benefit, and death followed shortly. Post-mortem Appearances.—Glands about three inches in diameter were present in both axillae, together with bilateral enlargement of those occupying the anterior triangles of the neck; the retrosternal, tracheal, para-aortic, and lumbar lymphatic glands were also enlarged. The spleen was about twice its normal size and typical in appearance. The liver was slightly enlarged. The pleural and pericardial sacs contained effusions; the heart showed fatty degeneration, and the bone marrow slight haemopoietic activity.

Histology.—The lymphatic glands exhibited extensive replacement of normal glandular architecture with Hodgkin’s tissue, numerous giant cells being noticeable. The liver and spleen revealed similar changes. The bone marrow showed some erythroblastic and leucoblastic reaction.

CASE III
A miner, aged 52, under the care of Professor Murray Lyon, for five weeks had had pain in the abdomen extending into his right leg. Enlarged glands were present in inguinal, axillary, and posterior cervical areas. There were considerable bilateral muscular atrophy and loss of motor power, lateral nystagmus, and weakness of facial movements. Tenderness was elicited over third lumbar vertebra. Fever accompanied his illness. The blood count was: red cells, 4,800,000 per c.mm.; white cells, 3,200 per c.mm.; haemoglobin, 70 per cent.; colour index, 0.7; a film showed slight anisocytosis. Wassermann reaction was negative.

Post-mortem Appearances.—There was considerable enlargement of the para-aortic glands, with some increase in size of those in the right groin; the left inguinal group was slightly enlarged. The spleen was twice its normal size, and
contained numerous small patches of yellowish-white tissue scattered throughout its substance. The liver displayed similar deposits, some of which were about one and a half inches in diameter. Both pleural and pericardial sacs contained serous effusions, while the left pleura showed deposits of lymphadenomatous tissue and commencing infiltration of the diaphragm. The vertebrae were also affected, and the bone marrow of the right femur showed haemopoietic activity. Histological examination revealed the typical features of an acute case of Hodgkin's disease.

**Case IV**

A fisherman, aged 24, under the care of Dr. Goodall, had cough and pallor of one year's duration, accompanied by swellings in his neck for five to six months. X-ray examination showed enlargement of the mediastinal glands and slight compression of the trachea. The spleen was not enlarged. The Pel-Ebstein type of fever was present. The blood count was as follows: red cells, 2,590,000 per c.mm.; white cells, 7,800 per c.mm.—polymorphs 69 per cent., small lymphocytes 20 per cent. and large 8 per cent., eosinophils 3 per cent.—haemoglobin, 85 per cent. Histological diagnosis: Hodgkin's disease.

**Case V**

A packer, aged 29, under the care of Dr. Chalmers Watson, had had an enlargement of the left side of his neck for sixteen months, followed by swellings in axillae and groins. X-ray examination revealed secondary deposits in the bony pelvis, ribs, and lumbar vertebrae. Motor and sensory paralysis was present from the lower costal margin downwards, accompanied by acute backache.

*Post-mortem Appearances.*—Considerable enlargement of the lymphatic glands existed throughout the body; several of them were hard and tough in consistence. The spleen weighed 500 grams, being greatly increased in size and typical in appearance. The vertebral column showed involvement near the sixth, seventh, and eighth ribs. The brain and meninges were normal in appearance.

**Case VI**

A housewife, aged 59, under the care of Dr. Comrie, complained of breathlessness, loss of appetite, and lassitude for one month. A single painless mass of slightly enlarged lymph glands was observed in the right axilla, and there was also some slight swelling in the left groin. The spleen was not palpable. Blood count showed: red cells, 3,640,000 per c.mm.; white cells, 6,400 per c.mm.; haemoglobin, 60 per cent.; colour index, 0.8. A film revealed neither visible abnormality of the erythrocytes nor any alteration in the proportion of leucocytes. Wassermann reaction was negative. X-ray examination showed a large right pleural effusion. Clinical diagnosis: pleurisy with effusion; carcinoma or tuberculosis of the lung.
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Post-mortem Appearances.—There was considerable enlargement of the upper abdominal and tracheo-bronchial lymphatic glands, both of which were heavily infiltrated with soft, whitish tissue. The glands in the lesser omentum were similarly affected, the invading tissue extending up the porta hepatitis along the larger branches of the portal vein to involve the liver and cause thickening of the wall of the gall-bladder. The kidneys and peri-pancreatic tissue were likewise affected. The spleen was twice its normal size, and exhibited numerous whitish areas about 3 mm in diameter. In the bone marrow erythroblastic and leucoblastic reaction was present.

Histology.—There was widespread infiltration with small lymphocytes of liver, spleen, lymph glands, and bone marrow, including a subcapsular infiltration of the kidney.

Diagnosis.—The absence of a blood leucocytosis during life, accompanied by the histological appearances as described, indicated that the case was one of pseudo-leukaemia.

CASE VII

A chauffeur, aged 50, under the care of Dr. Goodall, gave a history of seven weeks’ enlargement of glands in both axillae and groins, together with a swelling in his right pre-auricular region. Illness was accompanied by progressive enlargement of the spleen, some pain in his side, and a pleural effusion. X-ray treatment gave slight relief. Blood count showed: red cells, 4,900,000 per c.mm.; white cells, 7,600 per c.mm.—neutrophils 72 per cent., basophils 0.5 per cent., eosinophils 4 per cent., small lymphocytes 9 per cent., large 14 per cent.—haemoglobin, 85 per cent.

Post-mortem Appearances.—The entire para-aortic lymphatic chain was extensively involved with whitish-looking lymphoid tissue, and also the pelvic, inguinal, supra-clavicular, cervical, and axillary glands. The tracheo-bronchial and mediastinal lymphatic groups were likewise affected, but to a lesser degree. Bilateral permeation from the axillary glands extended into each pectoralis major muscle. The spleen was slightly enlarged, and showed necrotic changes. Both pancreas and suprarenals exhibited secondary invasion.

Histology.—Normal lymphoid tissue was replaced by numerous mononucleated cells, some of whose nuclei displayed mitotic figures and other degenerative changes. The capsule of the gland was invaded.

Experimental Work

Enlarged glands removed at biopsy were collected in a sterile test tube and 2 grams of glandular tissue were placed in a sterile mortar, finely minced, and ground in 20 c.cm. of nutrient broth of pH 7.2. This 1 in 10 suspension of material was maintained in a refrigerator at -4°C. for seven days, and was then tested for sterility both aerobically and anaerobically. Post-mortem material was dipped into absolute alcohol, which was burnt off; it was then plunged into boiling
water for two seconds and allowed to dry in an incubator at 37° C. The superficial tissues were now cut away, and the central portion prepared for emulsification. This was performed after the manner described above, with the exception that either 3 per cent. ether or 0.5 per cent. phenol was incorporated in the broth as an additional precaution against contamination. After ten days in the refrigerator these suspensions were standardized to approximately the density of a Brown's standard opacity tube No. 2 by the addition of fresh broth, before being used for injection. All the material used was prepared in this manner.

**Intracerebral Inoculation of Rabbits**

The procedure adopted was similar to that used by Gordon (1932), and was briefly as follows. Animals were deeply anaesthetized with ether, the hair over the head shaved, and the skin disinfected with absolute alcohol, tincture of iodine, and ether. A short incision was made through the soft tissues of the scalp down to the pericranium at a point situated 2 mm. lateral to the sagittal suture and 1.5 mm. anterior to the lambdoidal suture. The skull was now penetrated with a mechanical drill, or alternatively by means of a trephine. A fine intradermal needle was introduced through the aperture, and about 0.45 c.cm. of tissue emulsion was inoculated into the occipital lobe of the brain to a depth of about 3 mm. The needle was quickly withdrawn to avoid regurgitation, and the skin opening was united with a horsehair suture and its edges brushed over with collodion. An intravenous dose of 0.5 to 0.7 c.cm. of the same inoculum was also administered through the ear vein.

Although the method described above gave satisfactory results, yet access to the frontal lobe of the brain gained by trephining at a point situated 2 mm. lateral to the median plane on an imaginary line joining the two lateral ocular canthi yielded equally good results. This entry was the route of choice in the case of young rabbits from 6 to 8 weeks old. The sites of inoculation into the brain in both methods were situated well behind and in front of the motor area respectively. Deliberate experimental damage to the motor area was, however, inflicted on the brains of six animals. The features of damage to this area, which were characterized by the onset of paralysis within five to twenty-four hours, and its subsequent course were carefully noted.

**Results Obtained with Lymphadenomatous Material**

Glandular extracts of varying age were introduced into rabbits (vide supra) and their effects observed.

**Case 1.**—Suspensions which had been left for periods varying from one hour to seven days were found to be completely inactive, but after ten days in the refrigerator slight activity was noted. Two large rabbits which were inoculated showed, some three to four days later, a trace of stiffness in their hind limbs and some interference with
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their power of locomotion. At twenty days' broth suspension was next employed, and was found to be highly active. Thus four animals which were injected all showed the typical appearances of the encephalitic syndrome described by Gordon (1932), the principal features of which were the onset, after an incubation period of three to five days, of stiffness and paralysis of the hind limbs, ataxia (elicited by Gordon's "Romberg test" for rabbits), arching of the back, retraction of the head, incontinence of urine and faeces, nystagmus, and grinding of teeth. Death often occurred, but many of the rabbits passed into a chronic condition that was characterized by loss of weight and extreme muscular atrophy of their hind quarters. A few were observed to recover completely. The cranial contents of all such animals were examined for the presence of bacteria by aerobic and anaerobic methods of cultivation, but none were found at the end of six weeks' incubation of cultures. Histologically no obvious changes in the rabbits' brains could be seen.

Case 2.—Tissue emulsions became active at the end of the third week, and the typical encephalitic syndrome was obtained in six animals.

Case 3.—Pathological material removed from this acute case of lymphadenoma was found to be active after eight days in the refrigerator. It is possible that the acuteness of the condition in man bears some relation to the results obtained in the rabbit by the biological test. Thus, of eleven rabbits and one guinea-pig which were inoculated, every animal showed paralysis and ataxia on the third day, and six of them died. The site of inoculation, together with the surrounding brain and meninges, was subjected to a lengthy examination for the presence of bacteria, but none were found.

Case 4.—Although clinically and histologically characteristic of lymphadenoma, the gland removed at biopsy was unfortunately unsuitable as a specimen, first by reason of the smallness of its size, and secondly, owing to the fact that it had been subjected to intensive deep x-ray therapy prior to removal. The fragment, which was less than 1 gram in weight, was emulsified and injected intracerebrally, but no definite results were elicited. Of several rabbits and guinea-pigs inoculated, only two of the former showed a transient stiffness of their hind limbs on the fourth day after inoculation.

Case 5.—An enlarged lymph gland removed from the axilla of this patient was used for test. The gland, however, proved to be one that was extremely hard and tough in composition, owing to the presence of extensive fibrotic changes. A uniform suspension of the gland tissue could not, therefore, be prepared, since it did not readily emulsify or disintegrate after standing in the refrigerator. Twelve animals were injected at weekly intervals for six weeks with this material, while the suspension was left in the refrigerator, but no results followed. In view of these findings it is suggested that the test is not applicable in the case of tough fibrotic glands which do not readily emulsify, disintegrate, or undergo autolysis after standing at low temperatures for several weeks.
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Action of Tuberculous, Leukaemic, Sarcomatous, and other Tissues

A suspension of an enlarged cervical tuberculous lymphatic gland, prepared as in the case of lymphadenomatous tissue, was repeatedly introduced into five animals without any sign of appearance of the syndrome in question. Animals were observed for four weeks only, and then destroyed prior to the development of tuberculosis.

Similarly, glands removed from cases of pseudo-leukaemia (Case vi) and lymphosarcoma (Case vii) yielded consistently negative results throughout, suspensions varying in age from one hour to six weeks being unsuccessfully employed.

Reinoculation with Lymphadenomatous Material

Four rabbits unaffected after inoculation with non-lymphadenomatous material were reinoculated with Hodgkin's tissue obtained from three separate cases described. After an incubation period of four days all the animals showed the characteristic syndrome.

Toxins, Proteins, and Chemical Mechanical Irritants

In an endeavour to reproduce this encephalitic syndrome by other means the following substances were inoculated intracerebrally: 0.2 c.cm. of an active streptococcal toxin; 0.4 c.cm. of a killed emulsion of B. typhosus (916 million organisms); 0.7 c.cm. of sterile milk; 0.4 c.cm. of 5 per cent. sodium nucleinate with 5 per cent. aleuronat; 0.2 c.cm. of a mixture of 1 per cent. solution of quinine hydrochloride and 2 per cent. urethane; 0.4 c.cm. of 1 per cent. peptone water; 0.2 c.cm. of 1 per cent. turpentine oil; and 0.3 c.cm. of a sterile suspension of powdered glass in gum tragacanth. In each case, excepting the last, an intravenous dose of 0.4 c.cm. was also given.

Rabbits injected with turpentine and powdered glass showed signs of cerebral irritation accompanied by increasing restlessness after forty-eight hours, followed by sudden collapse and death three to four days later. The remaining animals were kept under observation for periods varying from seven to fourteen days and then examined for histological changes at the site of inoculation in the brain, but on no single occasion was it found possible to reproduce effects which bore the least resemblance to the syndrome under discussion. Indeed, some of these irritant substances could be introduced into the brains of rabbits as young as six weeks old without the slightest visible effects being produced.

From the results indicated above it was concluded that the inability of irritant substances to produce paralysis, ataxia, and muscular wasting in rabbits strongly supports the view that the changes following the introduction of lymphadenomatous material cannot be attributed to the outcome of simple traumatic and inflammatory changes produced in the brains of these animals. It would thus appear that the
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Symptoms are the result of the action of a diffusible agent present in suspensions of lymphadenomatous glands when kept at -40°C for ten days or longer. The nature of this pathogenic agent is the subject of a further investigation.

**Discussion**

From the results described above it will be noted that lymphadenomatous tissue, when ground, suspended in broth, and kept at -40°C for over ten days, produces striking effects when introduced into the brains of rabbits. It is also evident that these effects appear to be specific in character, for it has been shown that similarly prepared suspensions of lymphoid tissue removed from cases which clinically resembled Hodgkin's disease yielded negative results on test. Such cases have included glandular tuberculosis, lymphosarcoma, and leukaemia, all of which perhaps may be regarded as the commonest forms of lymphatic enlargement that may simulate Hodgkin's disease in temperate climates.

Calvert and Sanguinetti (1933), in their description of a case of Hodgkin's disease with relapsing pyrexia, have drawn attention to the extraordinary difficulties which may occasionally confront the clinician and pathologist alike when attempting to arrive at a diagnosis in certain atypical forms of lymphadenoma. Dr. Goodall's case (Case vii) illustrates a somewhat similar example. This patient's clinical history was suggestive of Hodgkin's disease, and clinical examination revealed the presence in both axillae and groins of enlarged glands whose size, consistence, and feel were not unlike those of a lymphadenomatous gland. The case, however, presented difficulties, since the histological appearance of these glands was not characteristic of lymphadenoma. Professor Drennan and Dr. James Davidson, however, regarded the condition as being one of lymphosarcoma, and were proved to be correct at necropsy. The case under the care of Dr. John Comrie (Case vi) illustrates another such example, for the diagnosis of pseudo-leukaemia was only first accurately established at post-mortem examination. In both these cases the Gordon biological test repeatedly yielded uniformly negative results throughout.

Space does not permit of a detailed account of experiments which have been directed towards ascertaining the precise nature of the agent responsible for paralysis, ataxia, and muscular wasting in rabbits. The results obtained so far indicate that the autolysis, disintegration, and cellular destruction of lymphadenomatous tissue liberates a product which is capable of producing an
encephalitic syndrome in rabbits and guinea-pigs. Neither the inoculum nor the brain of an animal which succumbs to the condition shows the presence of bacteria after lengthy periods of observation.

Whether this toxic agent be living or dead cannot be stated at the moment, but it may be said that the changes produced in rabbits cannot be interpreted on the basis of nervous changes following the introduction of a simple non-specific irritant substance into the brain. Thus it can be demonstrated that substances such as a streptococcal toxin, sodium nucleinate, aleuronat, quinine urethane, or finely powdered glass, which are capable of producing inflammatory tissue reactions, may be introduced into the brain of a rabbit with impunity. Indeed, these irritants may even be introduced in combination into the same animal on repeated occasions without the least visible effect.

The encouraging results given by this biological test are worthy of great attention, and we would suggest that glands removed at biopsy in suspected cases of Hodgkin's disease should not only be examined histologically and bacteriologically, but should also be subjected to Gordon's biological test.

**Summary and Conclusions**

Enlarged lymph glands removed from five cases of Hodgkin's disease have been subjected to the test devised by Gordon, three typical positive, one doubtful, and a negative result being obtained.

The syndrome produced in rabbits by Hodgkin's tissue cannot be elicited by the introduction of normal, lymphosarcomatous, leukaemic, or tuberculous lymphatic tissue. Neither can it be brought about by the intracerebral inoculation of dead bacteria, streptococcal toxin, sterile milk, peptone water, aleuronat, sodium nucleinate, quinine urethane, or finely powdered glass.

Gordon's biological test affords an easy method whereby lymphadenomatous tissue may be differentiated from lymphosarcomatous, leukaemic, and tuberculous tissue. In consequence of this it may be utilized as a laboratory aid to the diagnosis of Hodgkin's disease.

The writer desires to express his thanks to Dr. Alex. Goodall for the supply of clinical material; also to Professors Mackie and Drennan, and Drs. J. Davidson, W. G. Millar, and R. Ogilvie for their advice. He is deeply indebted to Dr. M. H. Gordon of St. Bartholomew's Hospital, London, for his valuable guidance and encouragement.

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RECENT EXPERIMENTAL WORK ON THE AETIOLOGY OF HODGKIN'S DISEASE*

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Recent work on the aetiology of Hodgkin's disease has served to bring fresh interest to bear on the nature of this condition.

In 1930 L'Esperance reported having isolated the avian tubercle bacillus from cases of Hodgkin's disease and claimed to have reproduced the condition in chickens. These claims have been investigated by Davidson (1933), the workers of the Rose Research on Lymphadenoma (1932), van Rooyen (1933), and others, all of whom failed to reproduce the disease in birds or to isolate the avian tubercle bacillus from human lesions in the disease. In 1930 Twort found that the subcutaneous inoculation of a guinea-pig with a suspension of Hodgkin lymphatic tissue produced after three to four days a swollen inflammatory nodule, in which no bacteria could be demonstrated by the application of ordinary methods. The workers of the Rose Research on Lymphadenoma (1932) next published an extensive series of observations on the pathology of the condition, showing that neither spirochaetes, yeasts, diphtheroids, nor acid-fast organisms were responsible for the causation of the disease.

THE ENCEPHALITIC SYNDROME IN RABBITS

Among the many findings of the last-named workers those of M. H. Gordon were perhaps of the greatest significance. He found that the intracerebral inoculation of rabbits with suitable suspensions of lymphadenomatous tissue was followed in three to four days by highly characteristic changes affecting the central nervous system of the animal. The sequence of events was: initially, slight impairment of locomotion, this being followed by progressive ataxia, muscular incoordination with spasm, spastic paralysis of the hind limbs, and frequently accompanied by incontinence of urine and faeces. The severity of these lesions produced in the

* Read in the Section of Pathology and Bacteriology at the Annual Meeting of the British Medical Association, Dublin, 1933.
rabbit were to some extent dependent on the acuteness of the case from which material was derived. Thus tissue from an acute case of Hodgkin's disease produced, in addition to the foregoing changes, violent convulsive movements, head retraction, opisthotonos, and grinding of the teeth. Animals affected to such an extent inevitably died, but in the less severe type of lesion several recovered completely, and a few passed into a chronic state in which severe muscular atrophy and wasting were much in evidence.

This "encephalitic" syndrome, as described by Gordon (1932), was found to be highly specific in character, and could not be elicited by the intracerebral inoculation of rabbits with material derived from cases other than those of Hodgkin's disease. This fact has been utilized by the writer (1933), who attempted to use this finding to form the basis of a biological test for the more accurate diagnosis of Hodgkin's disease.

The test has proved of value as an aid to the clinical and post-mortem diagnosis of this disease, particularly when the appearances presented are not entirely typical of the condition. In view of the satisfactory results obtained from the Gordon biological test, it has been proposed that material removed at biopsy and necropsy from suspected cases of Hodgkin's disease should be subjected not only to histological examination, but also to this animal inoculation test. It must be emphasized, however, that a rabbit which exhibits the encephalitic syndrome reveals no cultivable bacterium in either its brain or meninges. Hence, prior to the acceptance of a positive result in any animal, the possibility of bacterial infection being responsible for the effects manifested should first be excluded by appropriate methods of bacteriological examination.

**Its Aetiology**

From the theoretical standpoint one or other of the following causes might be responsible for the production of the condition.

(a) Trauma following damage by mechanical irritation.
(b) A specific neurotoxin present *in vivo* in lesions of Hodgkin's disease, or produced *in vitro* by changes in the tissue of the lesion after removal from the body.
(c) The action of a virus specifically associated with the disease and producing an effect on the nervous system of the rabbit.

The complexity of this problem, combined with the incomplete state of our knowledge regarding diseases produced by ultra-microscopic viruses in general, renders it extremely difficult to express a dogmatic view regard-
ing the nature of the pathogenic agent under discussion. I propose, therefore, merely to discuss the arguments relative to the above-mentioned hypotheses, guided by the experimental evidence so far advanced.

HISTOLOGICAL CHANGES IN THE RABBIT'S BRAIN

Below is a discussion on trauma and the histological changes produced experimentally following intracerebral inoculation with Hodgkin tissue and with a variety of other substances.

The chosen site of inoculation lies in the occipital lobe of the rabbit, and is situated well behind the motor area of its brain. Histo-pathological changes following the inoculation of Hodgkin tissue into this area were relatively slight in character. They revealed the occurrence of some localized haemorrhage occupying the path of the needle, and surrounded by a zone of lymphocytic infiltration. Occasionally similar infiltration of the meninges overlying the point of inoculation was also noticed, but this finding was too inconstant to be regarded as a regular feature. It may be stated that the slight histo-pathological changes produced in the experimental animal were not sufficient to account for the progressive degree of disability produced in the living animal. On the contrary, it could be shown that very severe damage could be inflicted to the brain tissue of the occipital lobe without in any way reproducing Gordon's encephalitic syndrome.

Such experimental damage may be deliberately produced by the intracerebral introduction into this area of chemical and mechanical irritants, such as turpentine, aleuronat, sodium nucleinate, quinine-urethane, or powdered glass. Likewise, the use of milk, dead bacteria—for example, *H. pyocyaneus*, *B. typhosus*, and *B. anthracis*—carbon particles, or human lymphoid tissue affected with a variety of different pathological conditions, could similarly be injected intracerebrally without in any way reproducing the syndrome under discussion. Histologically, however, the brain of an animal injected with any of the irritant substances mentioned above reveals extensive damage, changes such as severe haemorrhage, necrosis, degeneration, and cellular infiltration being evident microscopically in the brain of a rabbit which was apparently in good health prior to necropsy.

It is thus clear that the encephalitic syndrome cannot be regarded as the outcome of gross changes produced in a rabbit following the introduction of non-specific irritant substances into the brain of that animal.
TOXIN OR VIRUS?

The only unequivocal proof of the existence of a living virus is by means of its demonstration in vivo and in vitro, the former by serial propagation in the living animal and the latter as evidenced by its multiplication in artificial culture.

Neither condition has been fulfilled in the case of this pathogenic agent. The weight of experimental evidence so far advanced is equally capable of indicating the cause of the encephalitic syndrome to be the outcome of specific toxic activity or to its being that of a living virus. Thus the ability of this substance to withstand desiccation, to resist 78° C. for half an hour, to be annulled by the action of weak antiseptics, and occasionally to give rise to immunity phenomena (Gordon, 1932-3) is a common property of both a virus and a toxin. This agent has been further stated by Gordon to be non-filterable, but the writer has latterly been able to demonstrate in a series of preliminary experiments that filtration could be effected under certain conditions. He also observed that tissue suspensions appeared to be most active when suspended in broth ranging from pH 6.2 to pH 7.2. This aspect of the subject is under course of investigation and will be dealt with later.

CONCLUSION

It can be stated that the encephalitic syndrome produced in the rabbit is specific for Hodgkin lymphoid tissue, and may consequently be utilized as a biological test for the diagnosis of the condition. The precise nature of the syndrome produced in the rabbit is not the outcome of a simple traumatic and inflammatory change produced in the brain of the animal, but is probably due to the action of either a specific toxin or a filterable virus, derived from the human subject suffering from Hodgkin's disease. The present available data regarding the nature of this pathogenic agent neither confirm the possibility of these effects being the results of a specific toxic activity nor exclude them from being due to the action of a filterable virus.

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SOME PROPERTIES OF THE ENCEPHALITOGENIC AGENT IN LYMPHADENOMATOUS TISSUE WITH FURTHER OBSERVATIONS ON GORDON'S BIOLOGICAL TEST IN THE DIAGNOSIS OF HODGKIN'S DISEASE *

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Much interest has been aroused in the aetiology of Hodgkin's disease by the work of Gordon (1933), who found that rabbits injected intracerebrally with lymphadenomatous tissue frequently developed highly characteristic nervous lesions. This reaction applied as a biological test has proved valuable in the differentiation of Hodgkin's disease from other forms of lymphatic hyperplasia (van Rooyen, 1933), but the exact nature of the pathogenic agent and its aetiological relationship to the disease is still obscure. An attempt has therefore been made to gain further information concerning its nature by a study of its behaviour towards certain physical and chemical agents, and the data obtained, together with further observations on the clinical value of the biological test, are incorporated in this paper.

Recently Friedemann and Elkeles (1933) have reported that the intracerebral inoculation of rabbits with bone marrow derived from cases of acute leukaemia and pernicious anaemia produces effects very similar to those described by Gordon. In May, 1933, the writer made a similar observation with bone marrow removed from a case of acute myelogenous leukaemia, and there was certainly a close resemblance between the experimental condition produced by this material and the syndrome resulting from the inoculation of lymphadenomatous tissue. The further question therefore arises as to whether any relationship exists between the pathogenic agent present in the bone marrow in the leucoses and that present in the lymphatic glands in Hodgkin's disease.

FURTHER RESULTS WITH THE BIOLOGICAL TEST

The following are a list of cases in various parts of the country which have been investigated by means of the biological test.

* By aid of grants received from the Science Committee of the British Medical Association and the Medical Research Council.
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Patient’s Initial</th>
<th>Specimen Supplied by</th>
<th>Institution</th>
<th>Histological Diagnosis</th>
<th>Result of Test</th>
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<td>H9</td>
<td>T.</td>
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<td>Royal Infirmary, Edinburgh</td>
<td>Hodgkin’s disease</td>
<td>Negative</td>
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<tr>
<td>H10</td>
<td>J.</td>
<td>Dr. Goodall</td>
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<td>Prof. Murray Lyon</td>
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<td>H17</td>
<td>C.</td>
<td>Dr. F. E. Reynolds</td>
<td>Stobhill Hospital, Glasgow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H19</td>
<td>B.</td>
<td>Dr. Cruikshank</td>
<td>Royal Infirmary, Glasgow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H21</td>
<td>S.</td>
<td>Dr. Goodall</td>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>H23</td>
<td>McS.</td>
<td>McE.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H25</td>
<td>H.</td>
<td>McE.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H37</td>
<td>G.</td>
<td>McE.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H40</td>
<td>C.</td>
<td>Dr. F. Bramwell</td>
<td>Royal Infirmary, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H31</td>
<td>—</td>
<td>Dr. Wm. Brown</td>
<td>Royal Infirmary, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H35</td>
<td>B.</td>
<td>Dr. Larks and Dr. McConaghy</td>
<td>Stobhill Hospital, Aberdeen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H41</td>
<td>A.</td>
<td>Dr. Goodall</td>
<td>Royal Infirmary, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H13</td>
<td>McI.</td>
<td>Dr. Conrie</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>H20</td>
<td>S.</td>
<td>Dr. Goodall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H22</td>
<td>McH.</td>
<td>Mr. Stewart</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H29</td>
<td>R.</td>
<td>Dr. Eason</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H44</td>
<td>McL.</td>
<td>Dr. Cruikshank</td>
<td>Royal Infirmary, Edinburgh</td>
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<td></td>
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<tr>
<td>H36</td>
<td>H.</td>
<td>Mr. Chiene</td>
<td>Royal Infirmary, Edinburgh</td>
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<td>H27</td>
<td>L.</td>
<td>Prof. Wilkie</td>
<td></td>
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<td>H30</td>
<td>G.</td>
<td>Dr. Eason</td>
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<td>H38</td>
<td>—</td>
<td>Prof. Ritchie</td>
<td></td>
<td></td>
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<tr>
<td>H39</td>
<td>—</td>
<td>Mr. Stirling</td>
<td>Royal Infirmary, Edinburgh</td>
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<td>H42</td>
<td>McQ.</td>
<td>Dr. J. G. McCrie</td>
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<td>G.</td>
<td>Mr. Struthers</td>
<td></td>
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<tr>
<td>H46</td>
<td>F.</td>
<td>Dr. Comrie</td>
<td></td>
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</tbody>
</table>

† Previously published. See van Rooyen (1933).
‡ \* \* \* See Ogilvie and van Rooyen (1933).
ANALYSIS AND DISCUSSION OF RESULTS

Gordon's test has, as tabulated above, been applied to clinical and post-mortem material derived from twenty separate cases of Hodgkin's disease, and fifteen of these were found to give a positive result. Other cases of lymphadeno-hypertrophy due to a variety of different pathological conditions have likewise been submitted to the test, but found to be negative. The test is exceedingly helpful as an aid to the diagnosis of Hodgkin's disease when the histological appearances are not entirely typical of the condition.

Effect following Variations in the pH of Tissue Suspensions

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>pH of Broth Used for Suspension</th>
<th>Concentration of Tissue</th>
<th>Duration of Maintenance at -4°C</th>
<th>Results following Inoculation of Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>R354</td>
<td>5.6</td>
<td>1.20</td>
<td>3 days</td>
<td>Negative</td>
</tr>
<tr>
<td>R354</td>
<td>5.6</td>
<td>1.20</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>R350</td>
<td>6.6</td>
<td>1.20</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>R351</td>
<td>6.8</td>
<td>1.20</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>R352</td>
<td>7.0</td>
<td>1.20</td>
<td>4</td>
<td>Positive</td>
</tr>
<tr>
<td>R352</td>
<td>7.0</td>
<td>1.20</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>R350</td>
<td>7.1</td>
<td>1.20</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>R369</td>
<td>7.1</td>
<td>1.20</td>
<td>14</td>
<td></td>
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<tr>
<td>R364</td>
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<tr>
<td>R352A</td>
<td>7.1</td>
<td>1.20</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>R337</td>
<td>7.6</td>
<td>1.20</td>
<td>5</td>
<td>Negative</td>
</tr>
<tr>
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<td>7.6</td>
<td>1.20</td>
<td>4</td>
<td></td>
</tr>
<tr>
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<td>7.6</td>
<td>1.20</td>
<td>8</td>
<td>Positive</td>
</tr>
<tr>
<td>R357</td>
<td>6.6</td>
<td>1.40</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>R355</td>
<td>7.6</td>
<td>1.40</td>
<td>3</td>
<td>Negative</td>
</tr>
<tr>
<td>R516</td>
<td>6.0</td>
<td>1.40</td>
<td>7 days</td>
<td>Positive</td>
</tr>
<tr>
<td>R526</td>
<td>6.0</td>
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<td>Positive</td>
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<tr>
<td>R517</td>
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<td>1.40</td>
<td>7 days</td>
<td>Negative</td>
</tr>
<tr>
<td>R518</td>
<td>8.0</td>
<td>1.40</td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td>R528</td>
<td>8.0</td>
<td>1.40</td>
<td>Incubated immediately</td>
<td></td>
</tr>
<tr>
<td>R520</td>
<td>10.0</td>
<td>1.40</td>
<td>Incubated immediately</td>
<td></td>
</tr>
</tbody>
</table>

For example, in Case H40 death appeared to be due to bronchial carcinoma with secondary metastasis in the lymphatic glands and elsewhere. Microscopical findings, however, led to considerable diversity of opinion with regard to the diagnosis, and there was much speculation as to the nature of the condition. The case, however, gave a
strongly positive reaction in the rabbit, and was ultimately proved to be one of Hodgkin's disease; for this patient had already, some years previously, been under the care of an institution elsewhere, at which biopsy had been performed and the histological section found to be typical of Hodgkin's disease. This is the second case I have encountered in which the test has proved to be of great value; the first of them has been recorded (Ogilvie and van Rooyen, 1933), and it is hoped that these findings may prove of interest to other workers.

It will be observed from the table that certain specimens of glands produced more severe lesions in the rabbit than others.

For example, Cases H33, H40, H15, and H18 all yielded a strong reaction in animals injected with emulsified glands soon after their excision from the patient. The remainder, however, had to be emulsified in broth (pH 7.1) and stored at -40°C. for seven days (at least) before a satisfactory result could be obtained in the animal. A few were entirely negative, and preliminary treatment did not influence the results.

In view of this marked variability in pathogenicity exhibited by different specimens, an attempt was made to ascertain whether the reaction was dependent on the predominance of any particular type of cell present in the lymphatic gland. Sections were therefore made from glands which gave strong, weak, and negative reactions respectively in the rabbit, and these were examined microscopically in order to detect any demonstrable differences in histopathological appearance. No significant differences, however, could be found, and attempts to correlate the syndrome with the presence of any particular type of cell seen in the histological picture described by Sternberg and Reed were unsuccessful. The syndrome does not depend on the presence of lymphocytes or immature forms of haemopoietic cells, for enlarged glands removed from cases of lymphosarcoma and leukaemia give negative results. It is not related to Sternberg-Reed giant cells, for glands containing large numbers of them appeared to be no more pathogenic to the rabbit than those which contain only a few.

This point is well illustrated in two cases of Hodgkin's disease, H9 and H40 respectively. The former of these revealed histologically numerous giant cells, but, nevertheless, repeatedly yielded a negative result (glands being excised both at biopsy and at necropsy). The latter, on the contrary, exhibited only a few of them but gave a strongly positive result.
The presence of eosinophilia does not appear to be of any consequence either, for the syndrome has been reproduced with tissue revealing only occasional eosinophil cells in histological sections. The result noted with bone marrow may help to throw some light on the problem, but the subject requires further study.

Observations on the Bone Marrow in a Case of Acute Myelogenous Leukaemia

During the course of this work different tissues derived from various pathological conditions have been introduced into the brains of rabbits, including bone marrow derived from two cases of Hodgkin's disease. Only one result of any significance was obtained, and this is recorded below. It concerns the bone marrow removed from the femur in a fatal case of acute myelogenous leukaemia. The specimen was one which exhibited chloromatous change and contained little or no visible fat. The following clinical and post-mortem notes describe the subject from which the specimen was procured:

C. F., female, aged 35, was under the care of Professor Bramwell at the Royal Infirmary, Edinburgh. She complained of progressive anaemia for several weeks, bleeding, ulceration, and swelling of gums. Metrorrhagia and menorrhagia had occurred during last ten days. There was ecchymosis on trunk and legs. Red blood cells 1,900,000 per c.mm., haemoglobin 33 per cent., colour index 0.8; white blood cells 7,000 per c.mm.—reticulocytes 1 per cent., polymorphs few, small lymphocytes increased, myelocytes and megaloblasts numerous. Post-mortem examination revealed leucoblastic reaction and deposit of chloromatous pigment in the marrow. Cloudy swelling of myocardium, liver, and kidneys was observed. Petechial haemorrhages into the epicardium and gastric mucosa were seen. The spleen was slightly enlarged, and gave a positive prussian-blue reaction. Of eight rabbits injected intracerebrally with this tissue after three to four days all developed a spastic condition of their hind limbs, which closely resembled the encephalitic syndrome of Gordon. On the other hand, a lymphatic gland removed from the same patient gave a negative result in the biological test.

Whether the encephalitogenic agent in bone marrow described by Friedemann and Elkeles (1933), and also by the writer of this paper, is identical with that present in lymphadenomatous tissue, can only be decided after further investigation. It is hoped that information supplied in this paper concerning some physical properties of the latter might offer help towards a solution of the problem.
PROPERTIES OF THE ENCEPHALITOGENIC AGENT IN GLANDS AFFECTED WITH HODGKIN'S DISEASE

Optimum Conditions Necessary for Activation of Pathogenic Agent from Tissue: Influence of pH

Some earlier results showed that the pH of broth used for the emulsification of tissue appeared to influence the pathogenicity of suspensions in the rabbit's brain. Accordingly, in these experiments an endeavour was made to discover the optimum hydrogen-ion concentration necessary for the activation of the pathogenic agent from lymphadenomatous tissue. It has previously been noted by van Rooyen (1933) that material procured from different cases of this disease showed marked variation in their pathogenicity to the rabbit. Hence it was necessary to estimate the degree of activity of each specimen of gland by the preliminary inoculation of rabbits with varying dilutions of tissue suspensions. An approximate estimation of the minimum pathogenic dose to the rabbit could thus be arrived at for each specimen employed. The proportion of tissue present in the emulsions varied from 1 in 20 to 1 in 40 parts per c.cm., of which the dose administered was 0.4 c.cm. intravenously and 0.4 c.cm. intracerebrally. Desiccated material derived from different cases of Hodgkin's disease was weighed, emulsified, and diluted in broth varying in range from pH 5 to pH 10, and then used for intracerebral inoculation (vide supra). Animals were injected both immediately after each suspension was prepared and after it had stood for periods varying from three to twenty-three days in a refrigerator at -4°C. The latter procedure was adopted in order to elicit further information concerning the nature of changes which occurred in these tissue suspensions when maintained at low temperatures, for varying periods of time, as in Gordon's original technique.

TECHNIQUE OF pH ESTIMATION

The method adopted was to obtain varying degrees of pH by adding N/20 NaOH to 5 c.cm. of buffered phosphate broth; 0.5 c.cm. of a 0.01 per cent. aqueous solution of phenol-red was used as indicator, and, by comparison with a standard set of indicator tubes readings were made over the range pH 6.6 to pH 8. Above and below these figures a universal indicator and bromthymol-blue were used as indicators. The above method could not be used when working with smaller quantities of fluid, and the pH of these had to be ascertained by means of a B.D.H. capillator outfit, phenol-red, brom-cresol-purple, and thymol-blue being used as indicators in order to obtain the desired pH range.
The above results strongly indicate that the agent present in Hodgkin lymphatic tissue only exhibits maximum activity within a comparatively narrow range of pH (6.8 to 7.3). For example, whilst a 1 in 40 suspension of gland tissue emulsified in broth of pH 7 was found to be active both immediately and after three days, tissue suspended in broth of pH 5.6 or pH 8 produced no effect in the rabbit when tested under identical conditions.

The same common characteristic has been demonstrated in tissues derived from three different cases of Hodgkin's disease. Efforts made to reactivate tissues which had been previously rendered inactive by suspension in broth of pH 5 or pH 8 (vide supra), by the addition of fresh alkali or acid, were without success. It was therefore concluded that this inactivation was an irreversible reaction.

Reduction in Pathogenicity of Tissue Emulsions to the Rabbit by the action of Sodium Hydroxide, Sodium Bicarbonate, Ammonia, and Ammonium Carbonate

In view of the preceding experiments concerning the influence of pH on this agent, attention was next paid to the effect of treating highly pathogenic suspensions of Hodgkin lymphatic tissue with certain alkalis, prior to the intracerebral inoculation of rabbits.

Allusion has already been made to the difficulties encountered in attempting to arrive at an accurate standard of dosage for the rabbit. Accordingly, it was possible only to compare the relative severity of changes produced in two rabbits, the one inoculated with the usual material and the other with the same tissue after it had been treated with alkalis.

It was thus observed that the treatment of a highly pathogenic suspension of tissue with 1 per cent. NaOH for twenty-four hours at -4° C. resulted in considerable loss of pathogenicity for the rabbit. The effect was even more noticeable with less active tissue which could be inactivated by 0.5 per cent. liquid ammonia or ammonium carbonate within twelve to twenty-four hours. The effect appears to be quantitative in nature, for it depends on the activity of the tissue, the concentration of alkali, and the duration of its action. As previously, attempts to reactivate inactive material by neutralization with acid were unsuccessful.

Filtration Experiments

Previous experiments to demonstrate the filterability of this pathogenic agent were unsuccessful. Since my find-
ings have suggested that a comparatively narrow range of hydrogen-ion concentration is necessary for the satisfactory demonstration of this pathogenic agent, it is probable that variations in pH resulting from the act of passage through an earthenware candle may have been sufficient to account for the inert filtrates reported by Gordon (1932-3). I have overcome this difficulty by the employment of specially treated filter candles, and have been able to demonstrate the filterability of Gordon's pathogenic agent to the rabbit.

**Technique of Filtration**

Two types of filters were used, the one a Berkefeld (British), and the other a Seitz (EK) fine-pore asbestos disk 1½ inches in diameter. The candle of the former and the disk of the latter were first treated with buffered phosphate broth of pH 6.7, by allowing the fluid to act on the two elements for twelve hours at 26°C. They were then put into use. Material used for filtration was a desiccate of lymphatic tissue derived from a typical case of Hodgkin's disease that had previously yielded a strongly positive biological reaction. One gram of tissue was emulsified in 10 c.c.m. of sterile broth of pH 7.1, and then placed in a refrigerator at -4°C for seven days. Thereafter it was centrifugated at 1,500 revolutions per minute for twelve minutes, and the supernatant fluid withdrawn with a sterile pipette. To this was added 0.5 c.c.m. of an emulsion of *B. prodigiosus* prepared in broth of pH 7.1 standardized to approximate the density of Brown's opacity tube No. 1, and 0.5 c.c.m. of a similar suspension of *B. melitensis*, which was also added. The mixture was then divided into two equal parts of 5.5 c.c.m., and passed through each filter, a negative pressure of 350 mm. of Hg being applied for a total duration of twelve minutes at room temperature (20°C). Both filtrates were clear in colour, contained neither *B. melitensis* nor *B. prodigiosus*, and 0.4 c.c.m. of each was accordingly introduced intracerebrally and intravenously into four rabbits.

The animals recovered from the operation and remained in good health for a period of four days, after which they all developed the typical encephalitic syndrome. The brain of each animal was then removed and subjected to bacteriological examination. As no growth could be obtained from these by aerobic or anaerobic methods of cultivation after three months, it was concluded that the pathogenic agent was definitely filterable and could pass through both a Berkefeld (British) and the finest-pore Seitz filter. This experiment was repeated thrice with material procured from three different cases of Hodgkin's disease. The most active filtrates were obtained from lymphatic tissue that normally gave a strong positive biological test in the rabbit (van Rooyen, 1933), the
material being suspended in high concentration in buffered phosphate broth of pH 7.1, and the pH of the filtrate being kept below pH 7.1.

THE ACTION OF ADSORPTIVE AGENTS

In these experiments emulsions of Hodgkin lymphatic tissue were first treated with adsorptive agents, and then injected into rabbits. Some interesting facts have been obtained, but these are of limited value, for only the behaviour of the agent at pH 7.1 could be investigated. This has been unavoidable, because the range of pH over which this agent exhibits activity is small (pH 6.8 to 7.3), and consequently it was not possible to conduct experiments on the effect of adsorbents at different hydrogen-ion concentrations.

Technique

Two and a half cubic centimetres of a 1 in 20 emulsion of Hodgkin lymphatic tissue prepared in broth of pH 7.1 containing 0.25 per cent. phenol was added to each of four different conical flasks containing 0.2 gram of sterile kieselguhr, pulverized vegetable carbon particles, calcium sulphate, and emulsified normal rabbit’s brain. Also a fifth flask containing 45,770 millions of dead B. typhosus, a sixth with 37,870 millions of dead B. coli, and a seventh empty one, which acted as the control. The number of bacteria were calculated as follows: twelve twenty-four-hour agar slope cultures of each organism were heated to 65° C. for thirty minutes, emulsified in 10 c.cm. of 0.86 per cent. physiological saline solution of pH 7.1, standardized to approximate the density of Brown’s opacity tube No. 10, and 10 c.cm. of the emulsion centrifugalized to yield the desired number of organisms. All seven flasks were mounted on a slowly oscillating electrical shaking machine, and placed in an incubator at 37° C. for four hours. Thereafter the contents of each flask were transferred with a sterile pipette into separate test tubes, and centrifugalized at 2,100 revolutions per minute for twenty minutes; the supernatant fluid was withdrawn from each tube, divided into two equal parts, and injected into two rabbits in the usual manner.

Of the fourteen animals injected, the two inoculated with control material developed the syndrome and died after five to seven days: likewise also did those injected with material treated with B. typhosus, B. coli, and calcium sulphate respectively. Slight loss of pathogenicity followed adsorption by normal rabbit brain, for these animals developed a less severe lesion than did the controls. Greater loss was observed after treatment with carbon, as one of the rabbits developed only a slight illness lasting two days and the other escaped completely. After
treatment with kieselguhr, however, tissue suspensions of Hodgkin lymphatic tissue appeared to be completely non-pathogenic to the rabbit. The above experiment was repeated three times with clinical material supplied from institutions in Edinburgh, Glasgow, and London, and in each case the same result was obtained. It was therefore concluded that the pathogenic agent contained in these glands could be readily adsorbed in neutral solutions by treating with kieselguhr, less so by treating with carbon particles, and least of all by normal rabbit brain.

**Effect of Desiccation**

Gordon (1932) stated that the agent was capable of withstanding desiccation, and might be concentrated and rendered more active in glands by first drying them. This finding has been confirmed by the writer in the case of material from Case H28, which showed increased pathogenicity in the rabbit's brain after it had been desiccated in vacuo over P_2O_5 at 0°C for four to six weeks. In consequence of these results, a number of specimens of lymphatic tissues removed from cases of pseudoleukaemia, lymphosarcoma, and two cases of Hodgkin's disease (H9 and H10, which gave a negative result in the rabbit even after prolonged refrigeration) were desiccated, re-emulsified, and then used for inoculation, but with negative results throughout.

**Effect of Freezing Tissue Emulsions to 190°C Below Zero**

In order to gain information concerning the action of extreme cold *per se* on this pathogenic agent, material derived from four different cases of Hodgkin's disease was investigated in the following way.

**Technique**

Tissue suspensions were put up in hard glass test tubes and then placed in a small Dewar vacuum flask. A pure specimen of liquid air was next siphoned off from its container into the flask. After twelve minutes' immersion in the fluid, suspensions were withdrawn and set aside to melt at 17.4°C.

On one occasion a desiccate of Hodgkin lymphatic tissue was frozen to -190°C for twelve hours, after which it was emulsified in broth of pH 7.1, allowed to stand at -4°C for seven days, and then used for inoculation.

The material appeared to be unaffected by such a degree of exposure to cold. It still retained its pathogenicity to the rabbit, and reproduced the characteristic syndrome after the usual incubation period of three days.
§ 11 §

EFFECT OF X RAYS ON THE PATHOGENIC AGENT

Investigations were conducted towards ascertaining whether these rays produced, in vitro, any destructive effect on the agent in question.

Small fragments of desiccated tissue weighing from 230 to 350 mg. were placed in a quartz glass test tube (98 per cent. SiO₂ in composition), and then exposed for thirty-five minutes at a distance of 26 cm. to the rays emitted from a Muller W anticathode, utilizing 5 mA at 80 kV. A variety of different dosages were employed in the tests, and the greatest of them was an exposure of 4,545 r, approximately equivalent to ten unit skin doses.

As far as could be gathered from the results following rabbit inoculation, it did not appear that irradiation produced any diminution in the pathogenicity of the material to the rabbit. It was therefore concluded that the agent in these glands responsible for the encephalitic syndrome in rabbits was capable of withstanding considerable doses of x rays.

INSUSCEPTIBILITY OF RABBITS TO REINOCULATION AFTER RECOVERY FROM SYNDROME

Several animals which had recovered from the syndrome were allowed to regain their normal health and weight and then reinoculated with the same material as was used for their first injection. The period which elapsed between the two operations varied from a fortnight in some cases to three months in others. On no occasion, however, was it possible to demonstrate immunity to the second dose; indeed, so far as could be observed, it appeared that the rabbits were even more susceptible to it than before.

HISTOLOGICAL CHANGES IN BRAINS OF AFFECTED RABBITS

As previously stated, rabbits affected with the encephalitic syndrome only showed a slight leucocytic infiltration at the site of inoculation, and occasionally similar changes in the meninges overlying this area. The specificity of the latter was dubious, as its presence was comparatively infrequent, and was sometimes met with in normal rabbits. In a few animals, however, in addition to the foregoing changes, marked perivascular, round-cell infiltration, and "cuffing" was found to occur around blood vessels. The response appeared to be evanescent in type, for these changes were usually best demonstrable about the seventh to tenth days after injection, and apparently disappeared rapidly, irrespective of the state
of the animal. No evidence of *Encephalitozoon cuniculi* infection could be found, and consequently the changes were not regarded as being of a spontaneous character. A search for inclusion bodies was carried out by removing the brains of rabbits which had been paralysed for three to six and twelve days respectively, fixing these in Bouin's solution and staining them by Mann's method, but none were found. A few animals which had recovered from the syndrome were examined after six to eight weeks, but the results were negative.

**INTRACEREBRAL INOCULATION OF CATS, DOGS, AND FERRETS**

Five kittens and two puppies were injected intracerebrally and intraperitoneally with suitable suspensions of lymphatic tissue, but no results were observed to follow after three months' observation. Fifteen ferrets were injected intracerebrally, intraperitoneally, and intracon- junctivally with similar material. Twelve of these animals died within two to four weeks, and some of them showed signs of encephalitis prior to death. The animals, however, had not been quarantined before the test in order to exclude ferret distemper, and no definite statement can be made yet regarding the significance of the results. It was also noted, in some of the ferrets which had been injected intracutaneously, that a small inflammatory nodule developed at the site of inoculation after five to six days, similar to that described by Twort (1930) in the guinea-pig. This part of the work is still in progress.

**NATURE OF THE ENCEPHALITOCENIC AGENT: DISCUSSION**

Extended observations on Gordon's test have again proved it to be a reliable laboratory method for the diagnosis of Hodgkin's disease. The specificity of the reaction with lymphatic tissue obtained from this condition appears to be a well-established fact, but the precise nature of the agent—whether a virus, toxic substance, or enzyme—responsible for the syndrome in rabbits is quite obscure. The effects are not due to trauma (van Rooyen, 1933), but seem to result from some pathogenic entity present in lymphoid tissue affected with Hodgkin's disease, and to be demonstrable experimentally by the intracerebral inoculation of rabbits.

In his earlier work Gordon (1932) found it was necessary to place tissue emulsions in a refrigerator at −4° C. for several days before they showed pathogenicity. Since then many other cases of Hodgkin's disease have been investigated, and several have been encountered, both by
Gordon (1933) and by the writer, in which tissue has been found to be active when immediately emulsified. This may be due to the fact that the quantity of the agent present in different pathological specimens varies greatly. Thus, when present in large amount in any one case, the immediate trituruation and emulsification of the tissue may liberate a sufficient quantity to produce lesions in the rabbit. This presupposes that the pathogenic principle is intracellular either in origin or distribution. It has, in fact, been observed that feebly pathogenic emulsions of tissue may be rendered more active by allowing rapid cytolysis to occur—for example, by maintaining it at 37°C. for two to three days, or by intense freezing in liquid air followed by rapid thawing. The fact that fresh tissues may prove active excludes the possibility that the agent is a product of \textit{in vitro} autolysis. The diffusibility of the agent is indicated by the positive results obtained with centrifugalized and cell-free suspensions; and should it be of particulate nature, then the particles must be of ultra-microscopic dimensions. The filterability of this agent has been clearly demonstrated.

The work of Friedemann and Elkeles (1933) on bone marrow may throw some light on the problem, and it is of considerable interest, since Medlar (1931) has regarded Hodgkin's disease as a megakaryoblastoma and a tumour having its origin in bone marrow. This might suggest that the pathogenicity of lymphatic tissue is related to its cellular content, but so far no such correlation has been found to exist; moreover, further work is required to ascertain whether the encephalitogenic agent in bone marrow is identical or not with that demonstrated in Gordon's test.

Another question for consideration is the aetiological relationship of this encephalitogenic agent to the clinical condition, but no evidence is available at present which affords any definite indication that it is causally related to Hodgkin's disease, except for its striking association with the disease. Meanwhile, the demonstration of such an agent in bone marrow (normal and pathological) limits the significance to be attached to the encephalitogenic property of lymphadenomatous tissue.

In conclusion, there remains the problem concerning the exact nature of the pathogenic principle in lymphatic tissue, and some information has been obtained on its characters by a study of its behaviour and susceptibility towards certain physical effects. Thus, it exhibits great resistance towards cold and $x$ rays, displays maximum activity in solution at $pH$ 7.1, can be readily adsorbed by kieselguhr, carbon particles, and normal rabbit brain,
and is definitely filterable. It has also been shown by Gordon to be capable of withstanding temperatures up to 70°C for half an hour.

The significance of these facts cannot readily be assessed, because many of the features alluded to above are those which are common to both viruses and toxins, though the peculiar nervous syndrome produced in the rabbit, by analogy with other virus diseases of the central nervous system, might seem to favour the virus hypothesis. On the other hand, the failure so far to transmit the experimental disease in animals, and the absence of immunity after recovery do not support the view that the active principle is of virus nature. Finally, there is the possibility that the substance may be of the nature of an enzyme, producing specific damage to the central nervous system.

The problem is one of the greatest interest and practical importance, and the data given in this paper are put forward as a further contribution towards its study. Whether or not the encephalitogenic agent is directly related to Hodgkin’s disease, the fact remains that it is of clinical importance in the diagnosis of the condition, and as a new pathogenic principle merits the most careful investigation.

CONCLUSIONS

1. Twenty cases of Hodgkin’s disease and thirteen other conditions of lymphadenoid hypertrophy have been investigated. Gordon’s biological test gave positive results in fifteen (75 per cent.) cases of Hodgkin’s disease, and was found to be negative in the others.

2. The occurrence of a reaction in the rabbit closely simulating Gordon’s encephalitic syndrome has been observed to follow intracerebral inoculation with bone marrow derived from a case of acute myelogenous leukaemia.

3. The encephalitogenic agent in Hodgkin’s disease has been found to exhibit the following properties: (a) The maximum quantity is liberated from Hodgkin lymphatic tissue when buffered phosphate broth of pH 7.1 is used for its emulsification. (b) Alkalis, such as sodium hydroxide, sodium bicarbonate, ammonia, and ammonium carbonate, may all cause considerable reduction in the pathogenicity of active material. (c) Tissue emulsions have been frozen to -190°C for ten minutes and tissue desiccates for twelve hours, without inactivation. (d) The agent resists exposure to ten unit skin doses of x rays. (e) It can be readily adsorbed in neutral solutions by kieselguhr, less so by carbon particles, and least of all by normal rabbit brain. (f) It can be passed through Berkefeld (British) and Sietz (EK) filters.
The writer desires to thank the clinicians and pathologists at the Royal Infirmaries of Edinburgh and of Glasgow, and at other institutions in Scotland and England, who so willingly co-operated in the investigations. He is greatly indebted to Drs. J. Duncan White and C. M. Scott for their assistance in connexion with the x-ray experiments, and to Professors Mackie and Drennan for their valuable advice and helpful criticism.

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A CASE DEMONSTRATING THE VALUE OF GORDON’S TEST IN HODGKIN’S DISEASE

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RENEWED interest has been aroused in Hodgkin’s disease by Gordon’s description of a biological test which may be carried out with lymphadenomatous tissue obtained at either biopsy or autopsy. The value of this test is demonstrated by the following case in which the findings, even post mortem, still left the diagnosis in doubt.

CLINICAL HISTORY

A married woman, aged 24, with one child, took a “cold” in November, 1931, and developed a troublesome dry cough which was accompanied by pain over the sternum. Thereafter she noticed that she was losing weight, and complained of increasing weakness. Early in 1932 a swelling appeared in the right supravaculicular region. As she could not get rid of the cough she went to her doctor in February. He referred her to the Royal Infirmary, Edinburgh, for X ray examination, and in consequence of the findings at this examination she was admitted to the Infirmary on May 4th.

She had had several attacks of pain in the right iliac fossa during the past three years. In the family history there was nothing of interest.

Examination.—She was of medium stature, sparsely nourished, and looking on the whole fairly healthy. Her temperature was 98° F.; her pulse-rate 74; and her respiration-rate 20. She had a cough, but no sputum or breathlessness; she had had some pain behind the sternum. The thorax was well formed; there was slight fullness on the right side at the root of the neck. In the posterior triangle on the right side some discrete, hard lumps about the size of walnuts were felt. They appeared to be continuous with a mass which disappeared behind the sternum. There were no more lumps on the left side. There was greater dullness than normal over the sternum, and this dullness was also present for 2 in. on each side of the median line in the first, second, and third interspaces. Over the rest of the chest the note was normal. Vesicular breathing was heard all over both lungs, and vocal resonance was not increased. Nothing unusual was noted in the circulatory system, except abnormal faintness of heart sounds in the aortic and pulmonary
areas. The lymph glands were enlarged in the right posterior triangle of the neck, but not elsewhere. The alimentary, genito-urinary, and hemopoietic systems showed nothing of note. The urine was normal, and the Wassermann reaction of the blood negative. Radioscopy showed in the upper anterior mediastinum a large shadow of even density and irregular outline. The trachea was centrally situated and not compressed.

Clinical Diagnosis and Treatment.—In view of the clinical and radiological evidence a diagnosis of sarcoma of the thymus gland was made, and a course of deep X ray therapy instituted. In consequence of this treatment the neoplastic mass underwent a rapid and pronounced diminution in size. The patient was discharged on May 21st and given instructions to attend the radiological department as an out-patient. Her general condition was then good.

State on Readmission.—She was readmitted on Feb. 22nd, 1933, with a history of increasing malaise, weakness, and dyspnea, giddy turns, cough with little sputum, and loss of weight. Dullness was present below the clavicle to the second rib, and tranversely from an inch to the right of the midline to 3 in. to the left. Posteriorly there was a midline area of dullness about 4-5 in. broad. Over these regions vocal resonance and fremitus were greatly increased, and breathing was bronchial in type with no accompaniments. The supraclavicular glands were palpable on both sides, round and shotty, largest on the left. Post-cervical glands were also palpable. One gland in each axilla was enlarged to the size of a big walnut, but none were felt elsewhere. X ray examination now revealed definite enlargement of the mediastinal mass, together with diffuse opacity of the upper lobe of the left lung.

Termination of Illness.—While in hospital the patient developed a large bilateral pleural effusion. Repeated aspiration had no effect in reducing the amount of fluid, which showed numerous red blood corpuscles and lymphocytes with a fair number of endothelial cells and polymorphonuclears, but no organisms. She rapidly became weaker and more breathless, and died on June 3rd.

Post-mortem Examination and Diagnosis

Both pleural and the pericardial cavities contained large quantities of greenish serous fluid. In association with the parietal layer of the pericardial sac on the left side, there was, just above the diaphragm, a large mass of nodular, very firm, pale yellowish-white tissue. The peritoneum, larynx, and trachea were healthy.

The pleura of the left lung was smooth and glistening over the upper lobe, wrinkled over the lower lobe. The upper lobe was uncollapsed and more or less uniformly consolidated; the lower lobe was unduly collapsed. Section revealed that the consolidation of the upper lobe was due to extensive infiltration of the tissue by new growth, which seemed to have originated in the region of the hilum and spread peripherally. The margin of the infiltrated zone was irregular, but fairly well defined. The infiltrated area was greyish-yellow in colour, with here and there a congested hemorrhagic patch. The surrounding lung was rusty-red in colour. The lower lobe
was congested. The right pleura was unduly wrinkled, and the right lung collapsed, congested, and moderately oedematous.

A group of enlarged glands was present at the base of the neck on the left side. These glands were fairly discrete and very firm, and presented on section pale yellowish-white, more or less homogeneous surfaces; no necrosis or caseation was evident. A few enlarged glands were also present in the anterior mediastinum, and there was a chain along the vertebral column in the posterior mediastinum. Another large mass of glands was present along the lesser curvature of the stomach, and the para-aortic group of glands was markedly enlarged. The heart showed brown atrophy. The liver and spleen showed well-marked chronic venous congestion. The kidneys also showed chronic venous congestion.

The macroscopical findings necessitated a consideration of (1) Hodgkin's disease; (2) carcinoma of lung (bronchial or alveolar); and (3) mediastinal sarcoma. Points in favour of Hodgkin's disease were the age of the patient, the excellent response to irradiation, the widespread lymphatic involvement and the character of the glandular masses (discrete, non-necrotic, non-caseous). Points against this diagnosis were the invasion of the left lung, the presence of a large mass in association with the pericardial sac, and the absence of splenic and hepatic involvement. Bronchial carcinoma was favoured by the character of the growth invading the upper lobe of the left lung. The situation of this apparent neoplasm also suggested the possibility of a sarcoma of mediastinal origin.

**BIOLOGICAL TEST**

Although the weight of the evidence was in favour of Hodgkin's disease, it was impossible to be certain of this diagnosis without microscopical examination of the tissues. While ordinary paraffin sections were being prepared it was decided to employ and test the efficacy of the recently described Gordon biological test for Hodgkin's disease.

In order to avoid superficial contamination, enlarged lymphatic glands removed from both neck and abdomen were flamed in absolute alcohol and plunged into boiling water for two seconds. The superficial loose tissues were then clipped away, and the central portion prepared for emulsification. About 2 g. of tissue were then ground in 20 c.cm. of broth (pH 7.1) and a part of it used for the immediate inoculation of rabbits, whilst the remainder was stored at -4° C. in the refrigerator for later use.

Three rabbits were prepared for intracerebral inoculation, and approximately 0-36 c.cm. of the suspension was introduced into the occipital lobe of each animal to a depth of 3 mm., accompanied also by the administration of an intravenous dose of 0.5 c.cm. into the auricular marginal vein, conducted after the manner described by van Rooyen.

The animals withstood the operation satisfactorily and were apparently in good health after 36 hours. At the
end of 48 hours, however, all three rabbits showed slight signs of impaired locomotion. This was followed next day by spastic paralysis of the hind limbs and marked ataxia with incoordination on attempted movement. The last-named features were more readily demonstrable by the application of Gordon's Romberg test for rabbits.¹

Tissue suspensions which had been allowed to stand at — 4° C. in the refrigerator for varying periods of time up to three weeks were used for the inoculation of 12 additional rabbits. Eleven of these developed the characteristic syndrome described by Gordon¹ within three days. Four rabbits succumbed to the illness, three recovered completely, and four passed into a chronic condition characterised by muscular atrophy of the hind-quarters, loss of weight and hair, and incontinence of urine and feces.

Bacteriological examination by aerobic and anaerobic methods of cultivation failed to reveal the presence of any organism in the brains of animals which had died from the condition. Media were kept under observation and remained sterile for four weeks.

The above results favoured the conclusion that the case from which the glands had been taken was one of Hodgkin's disease.

Microscopical examination of tissue from cervical and para-aortic lymph glands, pulmonary growth, and pericardial mass was carried out a week after the autopsy. The histological appearances were in all cases similar. The tissue consisted in the main of a mass of reticulo-endothelial cells of all sizes up to small giant-cells of the typical Hodgkin type. Among this heterogeneous mass of cells were scattered numerous lymphocytes. The background consisted of finely granular, eosinophilic, necrotic-looking material. Here and there fairly large areas of tissue had undergone complete necrosis. This was especially so in the case of the pulmonary growth. Occasionally evidence of slight fibrosis was apparent, but this was not at all an outstanding feature. The condition was histologically one of acute "malignant" Hodgkin's disease.

SUMMARY

The Gordon biological test was applied to a case in which the clinical and post-mortem naked-eye findings were not conclusive of Hodgkin's disease. The test gave a positive result in 48 hours and thus substantiated a diagnosis of lymphadenoma. This was corroborated a week later by microscopical examination of the tissues.

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Value of Gordon’s Test in Diagnosis of Mediastinal Hodgkin’s Disease

REPORT OF TWO ILLUSTRATIVE CASES

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VALUE OF GORDON’S TEST IN DIAGNOSIS OF MEDIASTINAL HODGKIN’S DISEASE

REPORT OF TWO ILLUSTRATIVE CASES

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Recently we\(^1\) reported a case in which the clinical and postmortem naked-eye observations were not conclusive of Hodgkin’s disease. The efficacy of Gordon’s\(^2\) test (1932-1933) as a means of diagnosing lymphadenoma was tried out with postmortem material obtained from this case. The test gave a positive result in favor of lymphadenoma and this was corroborated shortly after by microscopic examination of the tissues. The first case to be described here is similar but even more instructive, since it demonstrates the value of the new test as a diagnostic procedure in circumstances in which not only the clinical and naked-eye observations but even histologic examination of the tissues left an element of doubt in the diagnosis. This case is also interesting in that it illustrates the change in cell structure which the Hodgkin’s lesion may undergo in the course of two or three years. Finally, there is appended a note on a case of bronchial carcinoma together with the result of Gordon’s test in that condition. This note is added, since bronchial carcinoma is a lesion that must sometimes be considered in the differential diagnosis of mediastinal Hodgkin’s disease.

REPORT OF CASES

CASE 1.—Clinical History.—A man, aged 50, a miner, admitted to the Royal Infirmary, Edinburgh, Nov. 15, 1933, under the care of Prof. Edwin Bramwell, complained of weakness and loss of weight (with a duration of nine weeks), dyspnea and cough (six weeks), anorexia and sweating (three weeks). The patient was well until about nine weeks before admission, when one day he had an attack of shivering and went home to bed. He did not stay in bed and during the next three weeks he had repeated slight shivers, sometimes two or three in a day. He felt himself getting weaker and people remarked that he was not looking well. Six weeks before admission he was compelled to take to bed because of weakness, and about the same time he began to have a cough and to be short of breath. He never saw any blood in his sputum, which was yellowish, scanty and difficult to expel. The bowels were constipated and during this time he lost weight rapidly. His appetite was poor. During the three weeks prior to admission he sweated profusely when asleep and latterly his sleep had been disturbed.

Two years before he had a swelling removed from the left side of the neck. This swelling had been present for four years, during which time it had enlarged slowly and latterly it had been red. About a year before admission he had noticed further swellings in the right side of the neck, but he did not think these had grown much lately. They had never been painful or tender.

Previous illnesses included typhoid and rheumatic fever. There was no family history of tuberculosis, but the patient lived in a small, insanitary and overcrowded house. He used to take a fair amount of alcohol.

Physical Examination.—The man looked ill, pale and emaciated but was cheerful and optimistic. There was considerable myotatic irritability. The skin was loose and atonic and the hair shabby and dry. The temperature was 101.4 F. The breathing was mainly abdominal and at the rate of 32 per minute. The chest was well formed but poorly clothed and moved equally on the two sides. There was no definite impairment of the percussion note, but a suggestive area of dullness was present over the middle zone of the right lung. The breath sounds on both sides were harsh and vesicular, with sibilant rhonchi toward the end of inspiration and some during expiration. Sputum was negative for the tubercle bacillus. A roentgenogram of the chest revealed a slight deviation of the heart and mediastinum to the right side, with infiltration of the medial part of the upper, middle and lower zones of the right lung; there was also thickening of the pleura in the lesser and greater fissures. The pulse was 136 per minute and regular in time and force. The blood pressure was 119 systolic, 65 diastolic. The heart sounds were pure but feeble. The abdomen was rather prominent. On the skin of the
abdomen and lower part of the chest there was a yellow scaly lesion, which had been present since the Boer War. There was no abdominal rigidity or tenderness. No abnormal swellings were present and there was no enlargement of the liver or spleen. Many enlarged glands were present in the neck and axillae. The Wassermann reaction was negative. There was nothing to note in the nervous system.

Clinical Diagnosis and Termination of Illness.—In view of the clinical and radiologic evidence, a diagnosis of pulmonary tuberculosis was made. The patient unfortunately went rapidly downhill and died six days after admission.

Postmortem Examination.—Macroscopic: The body was somewhat emaciated. The pericardial sac contained a small quantity of clear serous fluid. The left pleural sac contained half a pint of slightly blood-stained fluid; the right contained a few ounces of similar fluid. The peritoneal cavity was healthy. In the right bronchus just beyond the bifurcation of the trachea there was a new growth in the shape of firm, whitish, slightly raised plaques. The growth extended down into the main branch of the right lower lobe and had actually spread for a short distance into the substance of this lobe. It extended upward into the trachea for 2 or 3 inches and also down the left bronchus and its larger branches. Both lungs were voluminous and emphysematous and showed marked carbon pigmentation. Numerous deposits of white tissue were scattered over the surfaces of both lungs. Patches of bronchopneumonia were present in the upper lobe of the right lung. The rest of the lung and also the left lung showed congestion, and the bases of both lungs were edematous. Large masses of glands were found at the roots of the lungs. These extended up the trachea and communicated with similar large glands in the anterior triangles of the neck. Masses of glands were found in both axillae, along the aorta and the common iliac vessels, at the porta hepatis, and along the superior border of the pancreas. In all these situations the glands were discrete, firm and elastic in consistency, and on section showed a whitish marbled surface. The heart was globular, owing to dilatation of all chambers. The myocardium was very pale and soft. The coronary vessels and the aorta showed slight atheroma. The esophagus, stomach and intestine were free from pathologic change. The liver was of average size but pale. It was dotted throughout by small white deposits, the largest of which was a centimeter in diameter. The spleen was three times its normal size and nodular on the surface. On section it presented a dark red surface, throughout which were scattered nodules of white tissue. The pancreas showed nothing of interest. The genito-urinary system, beyond the abnormal pallor of the kidneys, was normal. A large deposit about 2.5 cm. in diameter was found in the left parietal bone toward the vertex. It was similar in appearance and consistency to the glandular masses elsewhere. It involved the
whole thickness of the skull but not the underlying dura. Other deposits were seen in several vertebrae (third, fourth, tenth and twelfth thoracic and first and second lumbar). The brain and its meninges were healthy, but the cerebral vessels were markedly atheromatous. The yellow marrow at the middle of the femur was replaced by whitish tissue.

Microscopic: Tracheal and pulmonary growths consisted of polyhedral cells with a moderate amount of clear cytoplasm and a nucleus varying in size and chromatin content. These cells were for the most part distributed indiscriminately, but occasionally, especially in the pulmonary growth, they tended to assume a palisade arrangement, though no actual acini were formed. In the tracheal growth, cells were occasionally seen that were larger than the others with a single lobulated nucleus or with from two to six nuclei irregularly arranged toward the center of the cell. No such cells were observed in the pulmonary growth. Special staining revealed the presence between the cells of a fine supporting reticulum. This malignant-looking tissue was actively invading the mucosa of the trachea on the one hand and the alveoli of the lung on the other. Many mitotic figures were present throughout the tissue of both lesions.

In one of the lymph glands examined only a few small foci of lymphoid tissue were left. The remainder of the tissue presented appearances similar to those of the tracheal lesion, though small giant cells with single lobulated or several nuclei were rather more numerous, and there was no palisade arrangement. Between the cells was a very definite fine reticulum. In another gland widespread necrosis had occurred with hemorrhage and the formation of hemosiderin.

In the liver small foci of new growth had developed in relation to some of the portal tracts. The microscopic appearance of these and also of the growths in the spleen, marrow, skull and vertebrae was similar to that of the trachea and lymph glands already described.

Biologic Test.—A large gland removed from the left axilla was freed from superficial contamination by flaming with absolute alcohol, immersion in boiling water for two seconds, and subsequent removal of loose surrounding tissue. The gland was then divided with a knife and a portion (about 1 Gm.) was removed from the center. This was finely divided and thereafter emulsified with pestle and mortar in 20 cc. of broth of pH 7.1. The emulsion was divided into two parts: one of these was used for the immediate intracerebral inoculation of three rabbits; the other was allowed to stand for seven days in a refrigerator at —4 C. and then was used for the inoculation of three additional rabbits. Inoculation consisted in the injection of 0.35 cc. of the suspension into the occipital lobe of each animal to a depth of 3 mm. This was accompanied by the administration of an intravenous dose of 0.5 cc. into the auricular marginal vein.
The three animals that were inoculated immediately with gland emulsion showed after four days signs of only slight ataxia, from which they rapidly recovered. The other three rabbits injected with emulsion which had been refrigerated for a week showed signs of gross nervous damage. These signs consisted of ataxia and incoordination setting in on the third day and progressing rapidly during the next few days to complete paralysis of the hind quarters with retraction of the head and nystagmus. On examination by aerobic and anaerobic methods of cultivation the brain and meninges of these animals yielded no growth. The test was therefore regarded as positive in favor of Hodgkin's disease.

**COMMENT**

The diagnosis of this case remained in doubt even after a naked-eye study of the organs and histologic examination of the various lesions. The main interest centers round the biologic test and the help it gave in determining the diagnosis.

The clinical diagnosis was pulmonary tuberculosis. Post mortem the diagnosis lay between (1) bronchial carcinoma and (2) Hodgkin's disease. The former was favored by (a) the age of the patient (59 years), (b) the presence of tracheal and bronchial lesions invading the right lung, (c) the presence in the pulmonary growth (right lung) of more or less columnar cells arranged in rows in the midst of an otherwise spheroidal-cell tissue, and (d) the indeterminate histology of the lesions in other organs. Points in favor of lymphadenoma were (a) a history of cervical glandular swellings for six years, (b) widespread lymphatic involvement (post mortem) and the character of the glandular masses, (c) involvement of the liver and spleen, (d) the presence in all the lesions of a fine but definite supporting reticulum, and (e) the occurrence in most lesions of cells like Hodgkin giant cells.

Although the weight of evidence was undoubtedly in favor of lymphadenoma, there yet remained an element of doubt. Consequently the result of Gordon's test was anticipated with interest and, as already indicated, it was positive. Since, moreover, bronchial carcinoma yields a negative biologic test (case 2) the lymphadenomatous character of this case seemed definitely established.

To complete the history, it should be stated that the gland which had been removed from the neck two years before death (1931) and examined elsewhere
was later traced. It showed lymphoid hyperplasia with loss of gland architecture and in places proliferation of the endothelial cells, among which were a few giant cells with a single lobulated nucleus or two or three nuclei. The condition was histologically one of early Hodgkin's disease. The case is thus also noteworthy as illustrating how the lymphadenomatous lesion, from being more or less characteristic, may in the course of time develop very atypical features and assume malignant characters.

Case 2.—Clinical History.—A man, aged 61, a railway porter, admitted to the Royal Infirmary, Edinburgh, under the care of Prof. W. T. Ritchie, Oct. 17, 1933, had had pain in the upper part of the left chest anteriorly for the past six months. For the past two months there had been a painful, tender swelling about 3.5 cm. in diameter over the second left costal cartilage. Until a fortnight before admission he had been in good health except for the painful swelling, but since then he had felt weak and breathless on exertion and had noticed that his ankles were swollen, particularly in the evening. He had had a slight cough for years.

Physical Examination.—The patient had an ashen complexion with a cyanotic tinge. Two firm tender lumps, each about 3 cm. in diameter, were present under the skin over the second left rib. The chest expansion was diminished. In the lower part of the right lung there were areas of low-pitched bronchial breathing with moist accompaniments, and in the left lung there was dulness with high-pitched bronchial breathing and whispering pectoriloquy. The cardiovascular, alimentary, genito-urinary and nervous systems showed nothing of interest. There was a slight degree of secondary anemia.

Postmortem Examination.—The lungs were moderately emphysematous and showed some basal congestion. The right lung was otherwise healthy. In the left bronchus just beyond the bifurcation of the trachea there was a nodule of neoplastic tissue in the process of invading the adjacent lung substance. The nodule was continuous in the anterior mediastinum with a large, firm, creamy yellow new growth consisting apparently of enlarged lymphatic glands. The upper part of the left lung was collapsed and heavily infected, owing to bronchial obstruction. The mediastinal mass also extended through the intercostal spaces to form two nodules below the left pectoralis major. The right kidney contained a single large mass of tumor tissue similar to that described. The left lobe of the prostate gland had in it a nodule which superficially resembled the tumors elsewhere. The liver showed marked chronic venous congestion. The spleen exhibited no noteworthy abnormality. The abdominal lymphatic glands, with the exception of one on the right renal vein, showed no malignant involvement.
Microscopic examination of the thoracic mass proved it to consist of adenocarcinomatous tissue. Much of the tumor was very undifferentiated, but acini occurred here and there. The neoplasm had induced the formation of fairly abundant stroma. The lymph glands and right kidney showed invasion by similar tissue. A prostatic nodule was adenomatous in character and macroscopically did not resemble the other neoplasms.

Macroscopic and microscopic observations together indicated a bronchial carcinoma.

**Biologic Test.**—Three rabbits were inoculated after the manner already described with tissue taken from the enlarged lymph glands—one immediately, October 19 (the day of the autopsy), a second on November 1, and a third on November 8. The material used to inoculate the last two rabbits was kept in a refrigerator at —4 C. All the animals remained normal, and the test was therefore regarded as negative.

**SUMMARY**

In case 1 the clinical and postmortem (macroscopic and microscopic) observations, while favoring Hodgkin's disease, did not conclusively support such a diagnosis. Gordon's biologic test was applied. The test gave a positive result, thus supporting a diagnosis of lymphadenoma. Case 2 is one which macroscopically resembled Hodgkin's disease. It yielded a negative biologic test and was ultimately proved by histologic examination to be a typical bronchial carcinoma.

These cases illustrate the value of Gordon's test as a diagnostic procedure in circumstances in which Hodgkin's disease is suspected.

Teviot Place.
RELATIONSHIP OF JOCHMANN'S AND OTHER ENZYMES TO THE ENCEPHALITOGENIC AGENT IN LYMPHADENOMATOUS LYMPHATIC GLANDS

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It has been shown by Gordon (1932-3) and van Rooyen (1933-4) that the intracerebral inoculation of rabbits with emulsions of lymphadenomatous tissue is followed by highly characteristic nervous lesions in the animal. Thus, after a definite incubation period lasting from three to four days, the animal develops signs of ataxia, incoordination, muscular spasms, head retraction, and generalized encephalomyelitis with usually a fatal result. A number of animals, however, that are affected to a lesser degree pass into a chronic state of disease, in which progressive atrophy of muscles and general emaciation are the principal features.

Such effects as these cannot be produced by the intracerebral inoculation of lymph glands affected with lymphosarcoma, leukaemia, carcinoma, tuberculosis, or syphilis, and thus the phenomenon has served as a biological test for the diagnosis of Hodgkin's disease. The value of the test has been adequately demonstrated by several workers (see van Rooyen, 1933-4; Ogilvie and van Rooyen, 1933-4; Gow, 1934; and van der Hoeden and Hulst, 1934), and consequently no further reference need be made to this aspect of the subject in the present paper.

Although the encephalitogenic effect in the rabbit of lymphadenomatous tissue appears to be a well-established
fact, considerable dubiety exists with regard to the nature of the pathogenic agent present in this tissue. Gordon (1933) has suggested that the behaviour of this agent is analogous to that of certain viruses in man and animals. Van Rooyen (1934) has concluded that the effects produced in the rabbit resemble more those of toxic action than of a virus infection, and has also pointed out that inability to transmit the condition from rabbit to rabbit, and the absence of intracellular inclusion bodies and of any immunity reaction, renders it difficult to assign this encephalitogenic agent to the infective viruses.

Further light has been thrown on the problem by the work of Friedemann (1934), who showed that the effects in the rabbit of intracerebral inoculation of lymphadenomatous tissue may also be produced by the inoculation of emulsions of normal splenic tissue and bone marrow, and of normal leucocytes. He showed that similar materials derived from cases of acute leukaemia and pernicious anaemia may likewise yield the characteristic effects. The encephalitic reaction has been found to be particularly marked with a specimen of bone marrow derived from a case of acute myelogenous leukaemia in which there were also chloromatous changes (van Rooyen, 1934).

The question has therefore arisen whether the agent present in lymphadenomatous tissue is identical with that present in these tissues, and whether, as Friedemann points out, both agents can be identified with the proteolytic enzyme extracted by Jochmann and Lockemann (1908) from leucocytes, splenic tissue, and bone marrow.

Thus it has been clearly shown that the characteristic encephalitic syndrome can be produced by the intracerebral inoculation of a product derived from normal tissues and cells; but, on the other hand, we have obtained evidence to show that the encephalitogenic agent in lymphadenomatous glands is not identical with Jochmann's enzyme, although both of them exhibit the same degree of resistance towards certain physical and chemical agents. We have also ascertained whether certain bacteria and vaccinia virus can withstand these agents.

The extraction and properties of the enzyme have been fully described by Jochmann and Lockemann (1908), and Jochmann (1913), and further reference has been made to its presence in tissues by Friedemann (1934). We have carried out a number of experiments with tissues extracted according to the method adopted by these workers for separating the ferment, and the extracts have been
carefully examined for proteolytic properties and their ability to produce pathogenic effects when inoculated into the brain of the rabbit.

**Technique for Extraction of Jochmann's Enzyme**

We have employed the same method as was adopted by Friedemann (1934) for this purpose. It is as follows.

Tissue is weighed, placed in an oven at 55° C. for forty-eight hours, and 5 volumes of a mixture consisting of 2 volumes of absolute alcohol to 1 volume of ether added. After twenty-four hours at room temperature the supernatant fluid is discarded and the residue desiccated to dryness in vacuo. The desicate is thereafter triturated in a 50 per cent. aqueous solution of glycerol, allowed to stand for twenty-four hours at room temperature, centrifugalized at 2,500 revolutions per minute for twelve minutes, and the supernatant fluid withdrawn with a sterile pipette and added to an alcohol-ether mixture of the previous proportions. After twenty-four hours at room temperature the precipitate is removed by centrifugalization, resuspended in 2 to 3 c.c.m. of 0.86 per cent. saline, and used for injection.

The alternative method (advocated by Friedemann, 1934) for the extraction of the enzyme by the use of acetone and filter paper was also tried, and found to give similar results. Unextracted tissue emulsions were also used for inoculation experiments; these constituted 1 in 10 suspensions obtained by finely mincing and grinding material in broth of pH 7.1 and thereafter allowed to stand for seven days in the ice-chest prior to injection. As a precautionary measure against bacterial contamination those tissues which had been obtained at necropsy were initially subjected to the superficial application of heat and alcohol and then triturated in 0.5 per cent. phenol broth according to the method described by van Rooyen (1933). In some cases the broth emulsions were also heated at 60° C. for sixty minutes to exclude all possibility of bacterial contamination.

The technique used for intracerebral inoculation of rabbits was similar to that described by van Rooyen (1933), and, stated briefly, consisted of injecting 0.4 c.c.m. of tissue emulsion into the occipital lobe of the animal to a depth of 3 mm. from the skin surface. The site chosen for perforating the skull was approximately 2 mm. equidistant from the right (or left) lambdoidal suture posterolaterally and the sagittal suture medially.

**Interpretation of Results**

Only those animals which exhibited the characteristic syndrome described by Gordon (1932) have been regarded as yielding positive results. The features of this condition are well known and require no further description, but we would emphasize that the incubation period of
three to four days prior to the development of the syndrome and the absence of any cultivable bacterium in the brain of such an affected animal are perhaps the most striking features of the encephalitic condition. We would also like to place on record that "spasmodic contractions of the muscles of the neck, distorted position of the head, frequent rotations around the body axis" (Friedemann and Elkeles, 1933) are not a characteristic feature of Gordon’s syndrome. In our experience, gleaned from the results of intracerebral inoculation in over 1,000 rabbits, such signs are the outcome of brain injury.

**Effect of the Jochmann-Lockemann Method Applied to Histologically Typical Glands**

Twelve enlarged lymphatic glands removed from cases of clinically and histologically characteristic Hodgkin’s disease, which had given a positive reaction on intracerebral inoculation, were treated by the Jochmann-Lockemann method and then tested in the same way. On every occasion the product obtained after extraction produced the same effect in the rabbit as was noted with freshly emulsified suspensions of similar tissue. In the case of two freshly removed glands which yielded a weakly positive effect when emulsified in broth, an appreciably greater reaction in the animal was elicited after extraction. But the feebly enhanced effects of concentration by this method were not as great as that obtained with broth emulsions prepared from similar glands which had been first subjected to desiccation. The experiments have, however, demonstrated that the encephalitogenic agent is capable of withstanding consecutively 55°C for forty-eight hours, alcohol (2 volumes) and ether (1 volume) for forty-eight hours, and 50 per cent. glycerol for twenty-four hours’ duration.

**EXPERIMENTS ON LYMPH GLANDS FROM TYPICAL CASES YIELDING A NEGATIVE BIOLOGICAL TEST**

Elsewhere van Rooyen (1933) has pointed out that this test was negative in 25 per cent. of cases of clinically and histologically characteristic Hodgkin’s disease. It was, moreover, suggested that the absence of the reaction in such cases may possibly have been due to the effects of repeated irradiation or excessive fibrosis of the glands examined. Further work has been done on this aspect of the subject, and an attempt made to ascertain whether the extracts obtained from negatively reacting glands by the Jochmann-Lockemann procedure may yield positive reactions.
Six enlarged Hodgkin lymph nodes of over 2 mg. in weight, which had previously given a negative reaction, were extracted by the Jochmann-Lockemann technique, and the extract was injected intracerebrally into rabbits. Fifteen rabbits were injected with such emulsions, and the dosage varied from 0.5 to 1 c.cm. in size, but, in spite of the employment of such comparatively large amounts, no effects were produced. These results have indicated that the preliminary application of the Jochmann-Lockemann technique to lymphatic glands giving a negative biological test fails to extract an encephalitogenic product from them.

Examination of Lymphatic Glands affected with Lymphadenoma and other Pathological Conditions

In order to ascertain whether Gordon's syndrome is due to Jochmann's proteolytic enzyme, we have investigated a number of lymphatic glands affected with a variety of different diseases, including Hodgkin's disease, for the presence of this enzyme. Thus twenty-four enlarged lymphatic glands have been examined for proteolytic action on coagulated serum (see Müller and Jochmann, 1906) in gelatin and litmus milk, and the results tabulated as follows.

TECHNIQUE

This test consisted in placing a large loopful of the tissue extract (vide supra) on the surface of a Petri dish containing Loeffler's coagulated serum medium which was incubated for forty-eight hours at 55° C., and then examined for liquefaction. Positive results were signified by the formation of a crateriform area of depression with liquefaction at the site of addition to the medium (see Müller and Jochmann, 1906). To 5 c.cm. of litmus milk medium 1 to 2 c.cm. of the tissue extract was added, which was then incubated at 55° C. for three days. In the case of post-mortem material the presence of bacterial contamination was excluded by appropriate methods of examination as a precautionary measure. The slightest trace of redness in the indicator as compared with the uninoculated control was regarded as a positive result, and the appearance of marked acidity with digestion of the milk was interpreted as a strongly positive reaction. Then 0.5 c.cm. of tissue extract was added to a tube containing 2.5 c.cm. of gelatin, incubated at 55° C. for forty-eight hours, and allowed to stand for one day at room temperature (15° to 20° C.). The amount of liquefaction produced was measured by means of a calibrated pipette, and any bacterial growth was excluded by cultural examination. The results obtained were found to be identical with those noted in the case of coagulated serum, and consequently they have not been incorporated in the tables.
The findings in Table I indicate that Jochmann's enzyme is absent in lymphadenoma and the other conditions studied. They also show that the encephalitogenic action of Hodgkin lymph nodes cannot be identified with Jochmann's enzyme; thus several glands were found to be markedly encephalitogenic, but possessed no apparent proteolytic activity. From the results obtained with splenic and bone marrow tissue (Table II) it will be

<table>
<thead>
<tr>
<th>Case</th>
<th>Histology</th>
<th>Result of Intracerebral Inoculation of Broth Emulsion (Gordon Syndrome)</th>
<th>Extract prepared by Jochmann-Loeffler Method</th>
<th>Proteolytic Action of Extracts on Loeffler's Serum</th>
<th>Löffler's Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>H 12</td>
<td>Hodgkin's disease</td>
<td>Positive</td>
<td>Positive</td>
<td>No liquefaction</td>
<td>No change</td>
</tr>
<tr>
<td>H 13</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>G</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>H 40</td>
<td>&quot;</td>
<td>Strongly positive</td>
<td>Negative</td>
<td>No change</td>
<td>&quot;</td>
</tr>
<tr>
<td>E</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>J</td>
<td>&quot;</td>
<td>Positive</td>
<td>Positive</td>
<td>Slight acidity</td>
<td>No change</td>
</tr>
<tr>
<td>NH 2</td>
<td>&quot;</td>
<td>Negative</td>
<td>Negative</td>
<td>No change</td>
<td>&quot;</td>
</tr>
<tr>
<td>NH 3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>(?) Positive</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>F</td>
<td>Case clinically resembling lymphadenoma but unconfirmed on histological examination, and no diagnosis given</td>
<td>Negative</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>C</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Acid with digestion of milk</td>
<td>No change</td>
</tr>
<tr>
<td>D</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>G 2</td>
<td>Reticulosarcoma</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>NH 7</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>LL 2</td>
<td>Lymphatic leukaemia</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>LL 3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>LL 4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>NH 8</td>
<td>Secondary carcinoma</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>I</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Large area of liquefaction</td>
<td>Acid with digestion of milk</td>
<td>No change</td>
</tr>
<tr>
<td>C</td>
<td>Undifferentiated carcinoma</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Slight acidity</td>
<td>No change</td>
</tr>
<tr>
<td>H</td>
<td>Caseating tuberculosis</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>F</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>B</td>
<td>Chronic tuberculosis</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Table I.—Examination of Lymphatic Glands for Jochmann's Enzyme correlated with Pathogenic Effect on Intracerebral Inoculation of Tissue Emulsions and Tissue Extracts
<table>
<thead>
<tr>
<th>Case</th>
<th>Pathological Condition</th>
<th>Tissue</th>
<th>Intracerebral Inoculation of Broth Extract</th>
<th>Intracerebral Inoculation of Jochmann Extract</th>
<th>Proteolytic Action of Jochmann Extract on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Loeffler's Serum</td>
</tr>
<tr>
<td>B (W.G.H.)</td>
<td>Myelogenous leukaemia</td>
<td>Bone marrow</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>B (W.G.H.)</td>
<td></td>
<td>Spleen</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>LL 1</td>
<td>Lymphatic leukaemia</td>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL 2</td>
<td></td>
<td>Spleen</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL 3</td>
<td></td>
<td>Bone marrow</td>
<td></td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>LL 4</td>
<td></td>
<td>Spleen</td>
<td>Positive</td>
<td>Positive</td>
<td>Active liquefaction</td>
</tr>
<tr>
<td>LL 4</td>
<td></td>
<td>Bone marrow</td>
<td></td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>A</td>
<td>Removed at necropsy from patients dying of various conditions, none of which were blood diseases</td>
<td>Spleen</td>
<td>Positive</td>
<td>Positive</td>
<td>Liquefaction</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>No liquefaction</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Negative in acid, neutral, and alkaline media.
observed that though the broth extracts generally reproduced the encephalitic syndrome, the corresponding extracts obtained by the Jochmann-Lockemann method were by no means always active. Further analysis of the results reveals that there was no relation between proteolytic activity on the one hand and encephalitogenic effect on the other. From these experiments we have concluded that the encephalitogenic agent which occurs in lymphadenomatous lymphatic glands also occurs in the spleen and bone marrow.

Our experiments with human pus and leucocytes have confirmed the early work of Müller and Jochmann (1906), who drew attention to the marked proteolytic activity of polymorphonuclear leucocytes derived from normal blood and inflammatory exudates. It will again be observed, however, that there was no correlation between proteolytic activity and encephalitogenic effect; thus in four cases the Jochmann-Lockemann extracts were found to be non-pathogenic to the rabbit, but possessed definite proteolytic powers. Further, this method of extraction proved detrimental to the pathogenic agent, since only three out of seven cases yielded a positive result with Jochmann-Lockemann extracts, whereas positive results were obtained in all cases by the use of broth extracts. Similar results have been noted by Gordon (1934).

Müller and Jochmann (1906) state that "pancreas induces the quickest and by far the most intensive digestive action on Loeffler's serum at 55° C." In our experiments with

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical Condition</th>
<th>Intracerebral Inoculation of Broth Extract</th>
<th>Intracerebral Inoculation of Jochmann's Extract</th>
<th>Proteolytic Action of Jochmann's Extract</th>
<th>Loeffler's Serum</th>
<th>Litmus Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyogenic skin abscess</td>
<td>Positive</td>
<td>Negative</td>
<td>Active liquefaction</td>
<td>Loeffler's Serum</td>
<td>Very slight action</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>No action</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Positive</td>
<td>Active liquefaction</td>
<td>&quot;</td>
<td>Slight action</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Liquefaction</td>
<td>&quot;</td>
<td>Marked action</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Negative</td>
<td>Liquefaction</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>Normal leucocytes†</td>
<td>Positive</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>Marked action</td>
</tr>
</tbody>
</table>

* Sterilized by heating at 60° C. for sixty minutes.
† Obtained by differential centrifugalization of normal human blood which had been citrated.
human pancreatic tissue we found that, while the Jochmann-Lockemann extracts induced active proteolysis on Loeffler's serum and in litmus milk, in no instance did the characteristic syndrome described by Gordon appear

<table>
<thead>
<tr>
<th>Case</th>
<th>Intracerebral Inoculation of Broth Extract</th>
<th>Intracerebral Inoculation of Jochmann Extract</th>
<th>Proteolytic Activity of Jochmann Extract on Loeffler's Serum</th>
<th>Litmus Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative</td>
<td>Negative</td>
<td>Marked liquefaction</td>
<td>Slight action</td>
</tr>
<tr>
<td>2</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>3</td>
<td>..</td>
<td>..</td>
<td>Liquefaction</td>
<td>Marked action</td>
</tr>
<tr>
<td>4</td>
<td>..</td>
<td>..</td>
<td>Marked liquefaction</td>
<td>..</td>
</tr>
<tr>
<td>5</td>
<td>..</td>
<td>..</td>
<td>Liquefaction</td>
<td>..</td>
</tr>
<tr>
<td>6</td>
<td>..</td>
<td>..</td>
<td>Marked liquefaction</td>
<td>..</td>
</tr>
<tr>
<td>7</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
</tbody>
</table>

in the rabbits injected with this material. On several occasions when injections of 0.6 c.cm. were given intracerebrally, the animals died either immediately or within forty-eight hours with signs of paralysis which rapidly developed within five hours of the injection. Reduction of the amount injected to 0.3 to 0.4 c.cm. gave uniformly negative results, the animals remaining unaffected by such dosages. A 1 in 10 concentration of pepsin solution (B.P.) in 0.2 per cent. hydrochloric acid, and in neutral solution, was also injected intracerebrally into rabbits, but no significant changes were observed.

In Vitro Effect of Human Tissue Extracts on Rabbit Brain Tissues

Friedemann (1934) has drawn attention to the lytic action of Jochmann-Lockemann extracts of spleen and bone marrow on emulsions of rabbit brain as a possible interpretation of the occurrence of Gordon's syndrome in the rabbit. We have investigated this action in the following manner.

The brains of several rabbits were removed aseptically and ground up in an equal quantity of normal saline to make a dense uniform emulsion; 4.5 c.cm. amounts of this emulsion, together with 0.5 c.cm. of extracts of various tissues obtained by the Jochmann-Lockemann method, were placed in sterile stoppered tubes and incubated at
55°C for three days. The control consisted of 4.5 c.cm. brain emulsion with 0.5 c.cm. normal saline.

**Table V.** *In Vitro Effect of Tissue Extracts on Rabbit Brain Tissue*

<table>
<thead>
<tr>
<th>Material</th>
<th>Result of Intracerebral Injection of Extract in Rabbit</th>
<th>Effect on Brain Emulsion after 24 Hours</th>
<th>48 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gland affected with lymphadenoma</td>
<td>Positive</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Gland affected with lymphosarcoma</td>
<td>Negative</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Normal pancreas</td>
<td>...</td>
<td>Progressive liquefaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>...</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

In those tubes containing pancreatic extract there was progressive digestion, whereas in the tubes containing extracts of lymphadenomatous and lymphosarcomatous glands and in the control tube no such change had occurred after incubation. This result shows quite clearly that gross proteolytic action on the brain plays no part in the effect produced by Gordon's agent.

**Experiments with Lymphoprotease and Leucoprotease**

It has been stated by Jochmann, and subsequently alluded to by Friedemann, that Jochmann's proteolytic enzyme is also to be found in the spleen, bone marrow, and leucocytes of normal dogs. Opie (1905-6-7) has studied the proteolytic property of dog tissues in great detail, and has shown that such activity is attributable to the presence of two proteolytic enzymes. The first, derived from the polymorphonuclear leucocytes and acting in an alkaline or neutral medium, he has called "leuco-protease." The second, which he termed "lymphoprotease," is derived from the mononuclear macrophages, and acts in an acid medium.

We have been able to confirm these observations, and have investigated the possible encephalitogenic action of the two enzymes when injected intracerebrally into the rabbit.

**Technique**

Opie's method of obtaining the two enzymes has been used, and is briefly as follows. A healthy dog was injected intrapleurally on each side with 10 c.cm. of a 10 per cent. suspension (by weight) of aleuronat in a 3 per cent. solution of starch. An inflammatory reaction ensued and a pleural exudate formed. After two days part of this exudate was
withdrawn with needle and syringe. At this stage the cellular content of the exudate consisted mainly of polymorphonuclear leucocytes, and, as Opie has shown, contained the enzyme leucoprotease. At the end of six days from the date of inoculation the dog was killed and the pleural exudate removed. Microscopically its cellular content consisted of a high percentage of large mononuclear cells. The lymph glands adjacent to the site of inflammation—for example, the substernal glands—were markedly enlarged, and microscopical examination showed that the sinuses were crowded with large mononuclear cells. The cells of the pleural exudates were separated by centrifugation, washed with normal saline, and finally suspended in nine times their volume of 0.85 per cent. saline. The lymph glands were thoroughly minced and triturated with three times their volume of 0.85 per cent. saline. All three suspensions were placed at 55° C. for twenty-four hours, and then tested for their proteolytic and encephalitogenic activity.

Table VI—Experiments with Tissue Enzymes from Dogs

<table>
<thead>
<tr>
<th>Nature of Specimen</th>
<th>Encephalitogenic Action in Rabbit</th>
<th>Proteolytic Action on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loedler's Serum</td>
<td>Gelatin</td>
</tr>
<tr>
<td>Pleural exudate after 2 days</td>
<td>Nil</td>
<td>+ + +</td>
</tr>
<tr>
<td>Pleural exudate after 6 days</td>
<td>+ + +</td>
<td>++ +</td>
</tr>
<tr>
<td>Glands ...</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Bone marrow I</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone marrow II</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Normal leucocytes</td>
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The exudates were found to be innocuous when introduced into the brain of the rabbit. In the case of the gland emulsion, however, results were obtained similar to those described with extracts of human pancreas (vide supra). Thus intracerebral injection with 0.5 c.cm. of a 1 in 3 concentration of gland emulsion caused paralysis and death within twenty-four hours, but if the dose were diminished to 0.25 c.cm. the rabbit remained unaffected. These results show, therefore, that no relation exists between the encephalitogenic agent under discussion and the enzymes leucoprotease and lymphoprotease described by Opie. Likewise, emulsions of spleen and bone marrow, and extracts of these tissues obtained by the Jochmann-
Lockemann method, were actively proteolytic, but non-pathogenic to the rabbit.

Resistance Exhibited by Certain Bacteria to the Jochmann-Lockemann Method of Tissue Extraction

It has been stated by Friedemann (1934) that the procedure adopted for the extraction of Jochmann's enzyme from tissues was adequately rigorous for the destruction of most forms of micro-organic life which might have been present in tissues before treatment by this method of extraction. We have investigated the precise degree of lethal effect exerted by such extraction when applied to a variety of different bacteria, and have noted that certain organisms resist such treatment.

TECHNIQUE

Aerobic five-day cultures of B. proteus, B. pyocyaneus, B. anthracis, B. anthracoides, B. mycoides, Streptococcus faecalis, Staphylococcus aureus and albus, together with anaerobic cultures of B. tetanus, B. botulinus, B. sporogenes, and Vibrion septique were treated as follows: placed in a paraffin oven at 55° C. for three days; centrifugalized at 3,000 revolutions per minute for thirty minutes; the supernatant fluid pipetted off; 15 c.cm. of a mixture composed of 2 volumes alcohol to 1 volume ether added, and allowed to stand at room temperature for twenty-four hours; thereafter tubes were recentrifugalized; the alcohol-ether mixture discarded and replaced with a 50 per cent. aqueous solution of glycerin, which was next allowed to act for a further twenty-four hours at room temperature, and then removed by differential centrifugalization. The deposited organisms were again subjected to a final treatment by alcohol and ether, and then resuspended in saline solution.

The presence of living organisms in each case was next ascertained by inoculation of blood-agar plates, which were incubated under appropriate conditions and then examined for the presence of typical colonies. The following results were obtained: B. anthracis, B. anthracoides, and B. mycoides, abundant growth after thirty-six hours; B. sporogenes and B. botulinus, numerous colonies at the end of seventy-two hours; B. tetani, Vibrion septique, Enterococcus, Staphylococcus aureus, and Staphylococcus albus, a few colonies after the same length of time. It should be mentioned in connexion with experiments on cocci that dense bacterial emulsions suspended in the minimum quantity of saline were used. A considerable amount of drying thus occurred during heating at 55° C. for forty-eight hours, similar to that which resulted when tissues were placed under identical conditions. As far as could be ascertained, therefore, from
our observations, the survival of staphylococci following exposure to the Jochmann-Lockemann method of extraction was dependent on the degree of drying to which these organisms were initially exposed. *B. proteus* and *B. pyocyaneus* failed to withstand the extraction process, and no growth was obtained after attempted cultivation. Likewise, similar results were obtained with an active strain of vaccinia virus, which was inactivated by this treatment.

These findings indicate that the method of Jochmann-Lockemann extraction is not necessarily lethal to organismal life, certain bacteria, both sporing and non-sporing, being able to survive the procedure.

**Discussion**

Three different hypotheses have been advanced in an attempt to explain the production of the encephalitic syndrome in rabbits following the intracerebral inoculation of extracts of certain human tissues. Gordon (1933-4) has suggested that the pathogenic agent belongs to the virus category; van Rooyen (1934) that it is possibly a neurotoxic substance with special affinity for rabbit brain tissue; while Friedemann (1934) concluded that it is identical with Jochmann's proteolytic enzyme. The last-mentioned worker arrived at this conclusion after a study of its distribution in the human body and of its resistance to certain chemical substances. This theory has formed the subject of our investigations.

F. Müller (1888), and later E. Müller and Jochmann (1906), noted that a proteolytic enzyme was present in human leucocytes, spleen, bone marrow, and pancreas, and in pus. Jochmann (1906) further demonstrated that it was present in these organs in the monkey, and to a lesser degree in the dog. They found that it could be effectively demonstrated by its ability to liquefy coagulated serum, a drop of the enzyme solution placed on Loeffler's serum medium producing a crateriform depression after incubation at 55°C. From their studies on the comparative proteolytic properties of white blood cells derived from cases of lymphatic and myelogenous leukaemia they came to the conclusion that in so far as leucocytes were concerned the proteolytic action was due to a ferment produced by the polymorphonuclear leucocytes.

The enzymes derived from leucocytes have been carefully studied by Opie (1905-6) in the dog. He found that two distinct enzymes were present. The first of them he names "leucoprotease," and states that it acts in
a neutral or alkaline medium. This appears to be the enzyme described by Jochmann et al., as it can be obtained from pus and withstands dehydration by alcohol and ether and extraction by glycerin. It resembles trypsin, but is less active. The other enzyme, which he calls "lymphoprotease," was obtained from the large mononuclear cells of lymph glands and inflammatory exudates—the macrophages of Metchnikoff. This enzyme, unlike leucoprotease, digests with greatest activity in an acid medium, being almost entirely inactive in neutral or alkaline media. It differs from leucoprotease further in that it is less resistant to the action of heat, being largely inactivated by temperatures between 60° and 70° C., and in being destroyed by drying after treatment with alcohol and ether. Hedin and Rowland (1901) demonstrated the presence of proteolytic substances in the spleen of various animals (ox, sheep, horse, and the pig); and Hedin (1904) succeeded in isolating two proteolytic enzymes: one, which acted in an alkaline medium, he termed "lieno-protease"; and the other, which acted in an acid medium, he called "lieno-β-protease."

The results recorded in this paper indicate, in the first place, that the encephalitogenic action of extracted leucocytes and other tissues on the brain of the rabbit is not due to proteolytic action per se, as extracts of human pancreas which were actively proteolytic were incapable of reproducing the characteristic syndrome in the rabbit. This result with pancreatic extracts further indicates that a lipolytic ferment plays no part in the production of the syndrome.

The question then arose as to whether the pathogenic action in the rabbit was due to a specific proteolytic enzyme, associated with the cells of the various tissues exhibiting encephalitogenic properties. A study of the various enzymes described by Müller and Jochmann, Opie, and Hedin in these tissues led us to the conclusion that none of these enzymes could be regarded as Gordon's encephalitogenic agent. For example, various Jochmann-Lockemann extracts of Hodgkin lymph glands, which yielded a strongly positive encephalitogenic action, were apparently devoid of proteolytic activity according to the standards adopted. The same was true of several specimens of bone marrow and spleen when tested in alkaline, acid, and neutral media respectively. On the other hand, several Jochmann-Lockemann extracts of pus which were actively proteolytic contained no encephalitogenic agent. Likewise, in material derived from the normal organs and inflammatory exudates of dogs,
proteolytic enzymes, active in acid, neutral, and alkaline media, were obtained; but these proved to be incapable of producing the typical syndrome in the rabbit.

We do not propose in this communication to enter into a discussion of the neutralizing properties of normal serum on the enzymes and pathogenic agent under discussion. We would draw attention to the fact, however, that whereas Gordon's agent, derived from lymphadenomatous glands, does not appear to be readily neutralizable, the anti-ferment action of normal serum is well recognized. Opie (1907) has further shown that leucoprotease is easily neutralizable by such serum. This aspect of the subject is in course of investigation, and will be referred to at a later date. The question of the vital nature of the encephalitogenic agent in the light of its resistance to a somewhat drastic mode of treatment has also been investigated. Comparative experiments with a number of different bacteria have led us to the conclusion that its resistance to the Jochmann-Lockemann procedure does not necessarily exclude the possibility of its being a living organism.

After full consideration of the foregoing experimental data, we have concluded that Gordon's agent is not identical with Jochmann's enzyme. Neither is it identical with the several proteolytic enzymes described by Opie, Hedin, and their co-workers. Further, it is of importance to note that the property of leucocytes to produce encephalitis in the rabbit appears to be limited to the human subject among various animal species which we have examined. It is still impossible, however, to define this unique pathogenic principle, and further work might appropriately be directed towards obtaining a more exact definition of its true nature and mode of action.

Summary and Conclusions

1. Gordon's encephalitogenic agent originally derived from lymphadenomatous tissue may occur in human bone marrow, spleen, and leucocytes (whether normal or pathological), but not in those of the dog or the rabbit.

2. This agent is capable of withstanding the Jochmann-Lockemann procedure for the extraction of proteolytic enzymes from tissues.

3. Extracts of certain tissues obtained by this procedure may be highly pathogenic to the rabbit on intracerebral inoculation (encephalitogenic) but devoid of proteolytic action.

4. Conversely, extracts of certain tissues may be
markedly proteolytic, containing the so-called Jochmann ferment, but quite non-pathogenic to the rabbit.

5. Gordon’s pathogenic agent found in lymphatic glands affected with Hodgkin’s disease cannot, therefore, be identified with Jochmann’s proteolytic enzyme.

6. Lymphatic glands affected with a variety of different pathological conditions have been subjected to the Jochmann-Lockemann method of extraction, but it has not been found possible to derive an encephalitogenic product from these glands which initially gave a negative Gordon reaction.

7. Experiments with certain bacteria have demonstrated that this method of chemical extraction does not destroy all forms of living organisms.

8. The enzymes lymphoprotease and leucoprotease obtained from the dog, and also lieno-a-protease and lieno-ß-protease, do not appear to be concerned in the production of the encephalitic syndrome following the intracerebral inoculation of tissue extracts.

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