Remarks
ON
HODGKIN'S DISEASE
A PATHOGENIC AGENT IN THE GLANDS, AND ITS APPLICATION IN DIAGNOSIS

BY

M. H. GORDON, D.M., F.R.S.

CONSULTING BACTERIOLOGIST TO ST. BARTHOLOMEW'S HOSPITAL; MEMBER OF SCIENTIFIC STAFF, MEDICAL RESEARCH COUNCIL

This pathogenic agent—apparently a virus—was found in the course of an inquiry in which a group of investigators—medical, surgical, and pathological—have been co-operating at St. Bartholomew's Hospital during the past five years with the express object of defining the causal agent of lymphadenoma, and the circumstances in which it came to light have been fully described in the report of the Rose Research on that disease. The purpose of the present communication is to report briefly the chief results of the observations carried out up to date. This seemed desirable, because experience already indicates that the presence of this pathogenic agent is capable of affording useful evidence in the practical diagnosis of lymphadenoma and its differentiation from allied conditions—a task in which any further help from the laboratory cannot be otherwise than welcome.

MODE OF DEMONSTRATION

Glands removed aseptically from suspected cases of lymphadenoma are placed in a sterile test tube or bottle and taken to the laboratory. After a piece has been removed and fixed for section one or more grams of the rest are cut off, weighed on a sterilized watch-glass, and transferred to a small sterile mortar under cover. The rest of the gland may be placed in the refrigerator and frozen; it remains active there for several weeks. With a sharp pair of scissors the portion of the gland in the mortar is now cut up as finely as possible and thoroughly ground to pulp with the pestle by hand, sufficient ordinary broth being gradually added to make a 10 per cent. suspension, which may be decanted into a tube, transferred to the refrigerator, and allowed to remain there for a week. One cubic centimetre of this suspension, which should be quite free of bacteria, is next injected into a rabbit, under light anaesthesia, 0.4 c.cm. being injected slowly into the brain and the rest into a marginal vein of the ear. Further particulars of the technique are described in the Rose Report.

When suspensions of glands from cases in which lymphadenoma is in active evolution have been injected, these rabbits, after an incubation period of two to six days, may show symptoms such as head retraction, pareses, and fits—symptoms indicative of meningo-encephalitis, and similar to those excited by the viruses of dermovaccine, herpes, or psittacosis, when injected cerebrally in the same way. In the case of suspensions of lymphadenoma glands, however, the usual clinical picture presented by the rabbits is peculiar and characteristic, and takes the form of muscular rigidity combined with incoordination and ataxia, and a condition of spastic paralysis, best seen by observing their gait on the floor. These symptoms are invariably accompanied by a progressive loss of weight. The disease thus engendered in the rabbit by cerebral inoculation with lymphadenoma gland is frequently fatal in a time varying from three days to a month or more, usually in about ten days. A large proportion of the rabbits, however, slowly get well, and some of these, when recovery is complete, have been found to be immune to a second dose of the same material administered by the cerebral and venous routes in the same way and positive in a control animal. Post-mortem examination of the rabbits that die shows generally a pronounced degree of marasmus, but no naked-eye lesion of any kind, except occasionally for some slight congestion of the meninges. Bacteria cannot be demonstrated either in the blood or in the meningeal fluid. In spite of the striking clinical picture, sections of the brains of the earlier rabbits that succumbed showed, as in the case of rabbits that die after cerebral inoculation with dermovaccine, a scarcity or absence of infiltrative lesions; but with the more active lymphadenoma material that killed the rabbit in four days, definite lymphocytic meningitis was present, and a proportion of the blood vessels in the cortex and elsewhere were seen to be surrounded with a perivascular cuff of lymphocytes. This has since been confirmed in two further cases in which the rabbits either died or were killed early when the disease was at its height. There can therefore be little doubt, both on clinical and on histological grounds, as to the ability of this pathogenic agent that is present in lymphadenoma gland to give rise to meningo-encephalitis in the rabbit.

Since the Rose Report was completed an important saving of time has been found to be practicable. Glands from five further cases of lymphadenoma have been tested by injecting the broth suspensions intracerebrally into rabbits, either directly or on the same day that they were made, and the result in all has been positive. It is therefore unnecessary to wait for the gland suspension to macerate in the refrigerator for a week, although the effect appears to be rendered more certain by that procedure.

OCCURRENCE IN LYMPHADENOMA

Up to the present the pathogenic agent has proved to occur in the lymphatic gland in no fewer than nineteen out of twenty cases in which the clinical and histological evidence placed the diagnosis of lymphadenoma beyond reasonable doubt. The agent would appear to be present either in a more active state or in greater abundance in glands removed from patients in the acute stage of the disease. On the other hand, in both of two patients who had succumbed to the chronic form of lymphadenoma, the pathogenic agent was limited to a minority of the glands of the neck that showed the typical histological changes; and it could not be demonstrated in the glands elsewhere, or in the liver or spleen, in which fibrosis had occurred, but the typical histological features were absent. In one of these two cases examined after death, however, the patient had died of septicaemia from a haemolytic streptococcus.

CONTROL OBSERVATIONS

The presence of this pathogenic agent in lymphadenoma glands only came to notice after three years of negative results, in which the action of spirochaetes, tubercle of human or bovine type, and pathogenic fungi had been excluded in turn. The careful work of van Rooyen recently reported in this *Journal* (January 14th, 1933) has since excluded avian tubercle as a causative factor in lymphadenoma. In order to define the significance of this new pathogenic agent it was necessary first of all to examine in the same way glands removed from patients suffering from other diseases. This investigation has now been in progress for a period of two years, during which glands from over forty control cases, chiefly removed for biopsy, have been tested in the same way—namely, by making a 1 in 10 suspension in broth, and after maceration for a week in the refrigerator by injecting it intracerebrally and intravenously into rabbits. The changes shown by these control glands and the conditions with which they were associated were as follows:

Hyperplasia and chronic adenitis	11
Leukaemia	5
Sarcoma, including lymphosarcoma	8
Carcinoma	6
Tubercle	6
Various (normal, allergic, mycosis fungoides, etc.) ...	5
Total ...	41

In none of these control cases did the gland suspension produce any obvious effect when allowed to macerate for a week in broth and injected intracerebrally and intravenously into rabbits in the same way as the suspensions of lymphadenoma gland.

SPECIFIC DIAGNOSTIC TEST

It would appear, therefore, that the pathogenic agent in question is limited to lymphadenoma, and is specific to that disease. Accordingly, the presence of this agent can be applied as a specific test in the practical diagnosis of lymphadenoma, and it may be mentioned that the test has, in fact, been on trial for that purpose at St. Bart's for some time past with promising results. That this biological test is capable of performing useful service in the diagnosis of lymphadenoma is evident from the following incidents. (1) Until recently the proportion of cases of lymphadenoma in which glands had given positive results was seventeen out of twenty, but on closer histological examination it has been found that two of the three negative cases are not histologically identical with lymphadenoma—one being probably a variety of lymphosarcoma, the other possibly a giant-cell tumour. (2) In April, 1932, a small piece of gland was received that had been removed from the neck of a patient suspected to be suffering from sarcoma of the mediastinum. This was the only palpable gland present, and the amount of it received weighed only 0.3 gram. As it was too small for a section, the whole of it was suspended in 3 c.cm. of broth, which was allowed to macerate in the refrigerator for a week, and was then injected intracerebrally into a rabbit. After an interval of two days this rabbit developed the typical syndrome produced by lymphadenoma glands, and when the test had been repeated and confirmed a report was sent to the effect that there was not enough material for a section, but that the biological test for lymphadenoma was positive. Six months later this patient returned to hospital with enlarged glands in the groins and axillae, and one of these on removal was found to show microscopically the changes characteristic of Hodgkin's disease in the acute stage, and it also gave positive results once again when the suspension was injected into three rabbits directly it had been made. It is clear, therefore, that the presence of this pathogenic agent can perform a useful service practically in the identification of cases of lymphadenoma.

CHARACTERISTICS OF THE PATHOGENIC AGENT

By employing the rabbit's brain as reagent in the manner described, the following information has been obtained with regard to the properties of the pathogenic agent present in the glands in lymphadenoma.

Morphology

With efficient aseptic technique, suspensions of lymphadenoma gland containing the pathogenic agent are found to be quite free of bacteria or other micro-organisms recognizable by the usual procedures. By intensive staining of films after they have been suitably fixed and mordanted, however, minute, deep-staining, spherical granules or "elementary bodies" can be made out that seem to be of the same category as the well-known Paschen bodies that recent experimental evidence is fast establishing as the actual virus of vaccinia. Similar minute bodies have been seen in impression preparations and smears made from the cut surfaces of the brains of rabbits that have succumbed to intracerebral injection with suspensions of lymphadenoma gland. But while the presence of these minute bodies is suggestive, their significance has still to be proved.

Cultures

No certain growth of this pathogenic agent, either aerobically or anaerobically, has yet been obtained on artificial culture media. The increased pathogenicity, as time goes on, of broth suspensions of lymphadenoma glands when kept in the refrigerator seems to be due to the setting free of the agent by autolysis of the tissue rather than to actual multiplication ; but this point, like so many others, is still an open one.

Resistance to Desiccation

The pathogenic agent in lymphadenoma gland withstands desiccation extremely well. Glands dried in a vacuum desiccator at room temperature in the dark, then sealed in a test tube and kept in the refrigerator, preserve the agent in active condition for at least six months. A gland, taken out one month before death from an exceptionally acute case of lymphadenoma, promptly dried, and preserved in a glass-stoppered bottle kept in the dark in an ordinary cupboard in the laboratory, was found to contain the pathogenic agent in an active condition after an interval of two years.

Resistance to Heat

This fundamental property—of resistance to heat—of the agent has been tested in gland suspensions from fifteen different cases of lymphadenoma. The results are summarized in the following table, and are very consistent.

Effect of Heat on the Pathogenic Agent in Lymphadenoma Gland Suspensions

	65° C.	75° C.	80° C.	100° C.
No. of tests	17	3	5	5
No. of cases	15	3	5	4
Inactivated	0	1	5	5

As throughout, a 1 in 10 suspension of the gland in broth was used. The duration of exposure at each temperature was thirty minutes, and in each test a control was performed with the same suspension unheated in order to make sure that the pathogenic agent was present and in an active condition.

As all of the fifteen strains retained their activity after exposure for thirty minutes to 65° C., it is clear that this pathogenic agent in lymphadenoma gland possesses very pronounced thermostability. It would seem that with an exposure of thirty minutes inactivation is not effected until the temperature reaches the neighbourhood of 80° C. It must be stated, however, that, although heating for thirty minutes to 65° C. has not inactivated this pathogenic agent for the rabbit's brain in any of the examples so far tested, this degree of heating has nevertheless been repeatedly found to reduce the pathogenic activity somewhat as compared with that of the same suspension when unheated. In other words, there is evidence that exposure to heat in this way does produce some slight weakening or attenuation of the pathogenic agent.

Resistance to Disinfectants

Phenol at 37° C.—The addition of carbolic acid to the extent of 0.5 per cent., followed by incubation for twenty hours at 37° C., has been repeatedly found to kill the tubercle bacillus and other forms of non-sporing bacteria, but to be practically without effect on the activity of a suspension of vaccinia virus. Phenolation in this way is also resisted successfully by psittacosis virus. A special interest therefore attaches to the fact that four out of five examples of this pathogenic agent in lymphadenoma

gland have been found to retain their activity when carbolized in the manner described and then kept for twenty hours at 37° C. Further experiments are in progress on this matter as opportunity offers, and an attempt is being made to see how long the agent retains its activity in the presence of 0.5 per cent. carbolic at 37° C. In the first test, now in progress, the pathogenic agent is still active in both of two specimens after a week at 37° C., but the pathogenicity for the rabbit's brain is less intense than that of another portion of the same suspension free from carbolic and kept in the refrigerator over the same period.

Phenol at 0° to 6° C.—In the refrigerator all of five strains of this pathogenic agent in lymphadenoma gland to which 0.5 per cent. of carbolic was added were found to retain their pathogenicity for at least a fortnight. Here again further experiments are under way. This resistance of the pathogenic agent in lymphadenoma gland to carbolic promises to be of much practical convenience, as it should enable suspensions in broth of glands from suspected cases to be sent through the post for testing, and should eliminate trouble from the presence of contaminating bacteria such as *B. coli*, staphylococci, etc. If this should prove to be practicable, all that would be necessary would be to keep in store a 5 per cent. solution of phenol in distilled water, and to add one-tenth part of that to the broth in which the gland is placed when removed from a suspected case.

Ether.—While the addition of ether to the extent of 10 per cent. by volume, followed by storage in a glass-stoppered bottle in the refrigerator, destroys non-sporing bacteria such as cocci and *B. coli*, it does not inactivate vaccinia virus, and is a valuable method of preserving it free of bacteria for several months. Etherization in this way is also resisted by psittacosis virus. Accordingly, suspensions of glands from five cases of lymphadenoma were each divided into two parts, one of which was transferred to a stoppered test tube and received an addition of 10 per cent. of ether. The control suspensions and their etherized counterparts were stored in the refrigerator, and after 10 days the first pair were tested intracerebrally on rabbits, the others being tested in the same way after eleven, fourteen, sixteen, and seventeen days respectively. The pathogenic agent was found to be still active in all of these suspensions, but in the case of two of the etherized suspensions its pathogenicity was weakened as compared with that of their non-etherized controls. One of these five duplicate suspensions of lymphadenoma glands, where the etherized portion was found to be still active after fourteen days, was examined again after three months had elapsed ; and, while the pathogenic agent was found to be still active in the control, it had become inactivated in the portion to which ether had been added. From these preliminary observations it would therefore seem that while the pathogenic agent in lymphadenoma glands withstands the addition of 10 per cent. of ether for a period, attenuation may be produced thereby in course of time. Further experiments on this matter are in progress, and a comparison is being made of the effect of carbolic and ether respectively on the same suspensions of lymphadenoma gland. Present results suggest that the pathogenic agent resists phenol better than ether.

Filterability

Six filtering experiments have been carried out in which broth suspensions of lymphadenoma gland of proved activity for the rabbit's brain have been passed through the more permeable bacterial filters. Four of these experiments were performed with the ordinary Berkefeld V filter and two with the Chamberland L2 filter. In all instances the filtrates when injected intracerebrally into rabbits proved to be inert. While these results clearly prove that this pathogenic agent that is present in lymphadenoma glands is particulate, they do not preclude the possibility of its being a filter-passing virus, for the following reason. Dilution experiments have shown that, before filtering, these broth suspensions of lymphadenoma gland do not give a positive result on the rabbit's brain when diluted above 1 in 150, and control experiments carried out with duplicates of the filters have shown that a broth suspension of dermovaccine with over ten times that titre of active virus when graded on the rabbit's skin gave equally negative results with regard to filtrates. For these reasons the question of the filterability of this pathogenic agent in lymphadenoma gland is not yet decided.

Immune Serum

As a proportion of the rabbits injected intracerebrally with suspensions of lymphadenoma gland and giving positive results recover, it was desirable to see if the serum of these recovered rabbits is capable of inactivating the pathogenic agent *in vitro*. It may be of interest to state that preliminary experiments have shown that the serum of one of these rabbits that had recovered completely after a severe attack very definitely inactivated the pathogenic agent when left in contact with a suspension of lymphadenoma gland for two hours in a water bath at 37° C., whereas a control test carried out with serum from a normal rabbit failed to do so. Here again further experiments are in progress.

Intracerebral Inoculation of Animals other than the Rabbit

Experiments were included in the Rose Report in which broth suspensions of lymphadenoma gland of proved activity for the rabbit by the cerebral route were injected into the brains of mice, but the latter were unaffected. Since these experiments were reported, glands removed from twelve patients have been suspended in broth 1 in 10, allowed to macerate in the refrigerator for a week, and, after having been proved free of bacteria, have been injected at the same time into the brains of rabbits, guinea-pigs, and mice. Six of these patients were suffering from lymphadenoma ; the others served as controls. All the animals were lightly anaesthetized by the inhalation of equal parts of alcohol and ether. The dose given to rabbits was the standard one of 0.4 c.cm. intracerebrally, followed by 0.6 c.cm. intravenously, as described previously. With regard to guinea-pigs, preliminary experiments having shown that a cerebral dose of 0.1 c.cm. of the lymphadenoma gland suspension was too small, the dose was raised to 0.15 c.cm., and as the effect then

Comparative Pathogenicity of Gland Suspensions by the Cerebral Route

No.	Case	Condition	Rabbit	Guinea-pig	Mouse
1	P.	Syphilis (III)	Negative	Negative	Negative
2	At	Lymphosarcoma	,,	,,	,,
3	S.	Sarcoma	,,	,,	,,
4	AV	Lymphosarcoma	,,	,,	,,
5	C.	Leukaemia	,,	,,	,,
6	H.	Tubercle	,,	Died 26 days Died 38 days	,,
7	De	Lymphadenoma	Positive	Died 8 days Died 14 days	,,
8	C.	,,	,,	Died 10 days	,,
9	Si	,,	,,	Negative	,,
10	Cr	,,	,,	Died 10 days Died 10 days Died 9 days	,,
11	Di	,,	,,	Died 10 days Died 21 days	,,
12	Sn	,,	,,	Died 12 days	,,

became far more definite and constant this was adopted as the standard procedure. In the case of mice the dose was 0.05 c.cm., as more than that cannot safely be injected. The result of these observations is summarized in the preceding table.

It will be observed that none of the broth suspensions of the six control glands gave evidence of any pathogenic action when administered by the cerebral route except No. 6—the tuberculous gland which killed both of two guinea-pigs from tuberculous meningitis, with generalization evident post mortem in the cervical lymph glands and spleen. On the other hand, the broth suspensions of all the six lymphadenoma glands gave typical positive results in the rabbits, and five of them produced as well a fatal disease in the guinea-pig, chiefly characterized by pareses, occasional rigidity of the muscles of the back, and by progressive weakness and loss of weight. The clinical picture of the disease thus produced in guinea-pigs by intracerebral injection of suspensions of lymphadenoma glands, however, is far less striking and obvious than in the rabbits. Post mortem, several of these guinea-pigs showed some congestion of their subcutaneous tissues and slight but definite congestion and enlargement of their lymphatic glands in the axillae and groin. Bacteria were absent from their meningeal fluid and from their blood. Sections of the brains, however, of some of these guinea-pigs have shown the presence of lymphocytic exudate and some thickening of the meninges ; and a few of the vessels in the cortex occasionally show a cuff of lymphocytes. There would seem to be little doubt, therefore, that suspensions in broth of lymphadenoma gland where injected intracerebrally can give rise to meningo-encephalitis in the guinea-pig as well as in the rabbit. With one exception, the broth suspensions of the glands used in these experiments had been allowed to macerate in the refrigerator for one to two weeks before injection. The exception was Case No. 9, in which the only broth suspension of gland available was a hundred days old. It is interesting to note that, while this old suspension was still active for the rabbit, it failed to affect the guinea-pig. Throughout these experiments the mice, of which four to six were injected with each of the suspensions, remained unaffected.

COMMENTARY

Although the characters of the pathogenic agent present in lymphadenoma glands have not yet been fully ascertained, the available evidence leaves little room for doubt that it is a specific micro-organism belonging to the virus group. This was the view expressed in the Rose Report on the evidence then available, and it is confirmed by the further particulars obtained since and reported in the present paper—namely, its capacity to produce meningo-encephalitis in guinea-pigs, its strong resistance to phenol and ether, and the presence of a neutralizing antibody in serum from an immune animal. From its pronounced resistance to heat the present agent would appear to belong to the same category as the heat-resisting viruses of trench fever and hog cholera or swine fever, the former of which withstands heating to 60° C. for thirty minutes, but is inactivated in that time at 70° C., as proved by Major Strong and his colleagues of the Medical Research Committee of the American Red Cross by human experiments during the war. The resistance to heat of the hog cholera or swine fever virus has been carefully determined by McArthur by experiments on pigs, and he found that it is not inactivated by exposure for two hours to 65° C., and that it can occasionally withstand exposure for four hours to 70° C. The pathogenic agent that is present so constantly in lymphadenoma glands would thus appear to belong to this group of the thermostable viruses ; but final proof of this matter must be awaited until its filterability has been established and passage in series has been effected. The uniformity with which the agent has been found present in the glands of cases of lymphadenoma, and its equally constant absence in other conditions, are highly suggestive that this pathogenic agent or thermostable virus is the primary causative agent of lymphadenoma ; it is difficult to see any other explanation. In the meantime, experience indicates that its presence is capable of affording valuable information in the practical diagnosis of Hodgkin's disease.

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A BIOLOGICAL TEST IN THE DIAGNOSIS OF HODGKIN'S DISEASE

BY

C. E. VAN ROOYEN, M.B., CH.B.

ASSISTANT, BACTERIOLOGY DEPARTMENT, UNIVERSITY OF EDINBURGH

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, 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.—Leutrophils 82 per cent., eosinophils