described a rigidity of the legs as characteristic of his rabbits. He used intracerebral injections. Exactly the same thing is observed in rabbits which receive bone marrow intracerebrally. When, however, bone marrow suspension is given intrathecally, as in our original experiments, flaccid paralysis is the characteristic feature, though occasionally rigidity is seen. When bone marrow is injected intracerebrally, then rigidity is the characteristic consequence, just as in Gordon's rabbits. It is all a matter of technique. We have found, too, that, when tissue emulsions have been heated for one hour at 65° C. and then injected, it matters little whether the intracerebral or the intrathecal route is used; rigidity of the legs is a common feature in either case. Further, Gordon reported that his agent is remarkably stable in the cold. I find that the stability of the bone-marrow agent varies with the conditions of the experiment.

If bone marrow be ground in saline and then left in the cold room (4° C.) it loses its pathogenic activity rapidly—often within an hour. Whether there is an actual destruction of the pathogenic agent under these conditions has not been determined. The observed facts are that, when bone marrow is rubbed up in saline as described, a coagulation of the fatty matter soon sets in, clumps are formed, and the presence of the agent can no longer be demonstrated. Probably the agent is gathered up in the clumps. With alteration in technique the results alter. In the presence of 5 per cent. glycerin in saline the clumping sets in very slowly and the clumps are soft and easily broken down again. If the soft coagulated material be rubbed up in a mortar the fatty matter may be removed without difficulty. Glycerinated material has now been kept for six days in the cold room, rubbed up in a mortar, spun free of cells and gross particulate matter, and has then proved pathogenic for rabbits. Both agents are alike, too, in that they withstand a temperature of 65° C. Thus we reach the conclusion that the agent found in normal human bone marrow resembles the agent found in lymphadenoma glands in every material particular. Identity cannot, however, be said to be completely and finally established as yet.

CONCLUSIONS

Studies of the agent which is present in normal human bone marrow, and which Friedemann and Elkeles found to be pathogenic for rabbits, have been continued.

It is shown that this agent cannot be a living virus, since it withstands treatment which no living virus could be expected to withstand.

It has now been demonstrated that the agent, in addition to being present in human bone marrow and spleen, is present in human leucocytes.

The peculiar species and organ distribution which is displayed by this agent has been shown to be paralleled by the distribution of the proteolytic ferment which Jochmann found in normal human leucocytes. Whether the agent and the ferment are really identical remains to be finally established.

Increased familiarity with the pathogenic agent which we have studied still reveals no material difference between it and the agent which Gordon discovered in glands taken from cases of human lymphadenoma.

My indebtedness to Dr. Gordon has been mentioned already. It is now my pleasure to acknowledge financial assistance from the Rockefeller Foundation and the hospitality extended to me by the Medical Research Council at their Mill Hill laboratories.

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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.

Biological Diagnostic Test (Gordon)

Case No.	Patient's Initial	Specimen Supplied by—	Institution	Histological Diagnosis	Result of Test
H9	T.	Dr. Chalmers Watson	Royal Infirmary, Edinburgh	Hodgkin's disease	Negative
H10	J.	Dr. Goodall	"	"	"
H12	R.	"	"	"	Positive
H14	F.	Dr. Matthews	"	"	"
H15	L.	Prof. Murray Lyon	"	"	Strongly positive
H18	H.	Prof. Murray Lyon	"	"	"
H26	H.	Prof. Fraser and Dr. Eason	"	"	Negative
H32	C.	Dr. Goodall	"	"	"
H17	C.	Dr. F.E. Reynolds	Stobhill Hospital, Glasgow	"	Positive
H19	B.	Dr. Cruikshank	Royal Infirmary, Glasgow	"	Negative
H21	S.	"	"	"	Positive
H23	McS.	"	"	"	"
H28	McL.	"	"	"	"
H33	H.	"	"	"	Strongly positive
H37	G.	"	"	"	Positive
H40	C.	Prof. Bramwell	Royal Infirmary, Edinburgh	"	Strongly positive

* By aid of grants received from the Science Committee of the British Medical Association and the Medical Research Council.

† Previously published. See van Rooyen (1933).
‡ " " See Ogilvie and van Rooyen (1933).

Biological Diagnostic Test (Gordon)—Continued

Case No.	Patient's Initial	Specimen Supplied by—	Institution	Histo-logical Diagnosis	Result of Test
H34	—	Dr. Wm. Brown	Royal Sick Children's Hospital, Aberdeen	Hodgkin's disease	Positive
H35	B.	Dr. Larks and Dr. McConaghy	City Hospital, Plymouth, England	"	"
H11	A.	Dr. Goodall	Royal Infirmary, Edinburgh	Lympho-sarcoma	Negative
H13	McI.	Dr. Comrie	"	Pseudo-leukaemia	"
H20	S.	Dr. Goodall	"	Lympho-sarcoma	"
H22	McH.	Mr. Stewart	"	Gumma	"
H29	R.	Dr. Eason	"	? tubercle	"
H44	McL.	Dr. Cruikshank	Royal Infirmary, Glasgow	Acute lymphatic leukaemia	"
H36	H.	Mr. Chiene	Royal Infirmary, Edinburgh	Lymphatic leukaemia	"
H27	L.	Prof. Wilkie	"	Lympho-sarcoma	"
H8	A.	Prof. Murray	"	"	"
H30	G.	Dr. Eason	"	Tuber-culosis	"
H38	—	Prof. Ritchie	"	"	"
H39	—	Mr. Stirling	"	Carcinoma	"
H42	McQ.	Dr. J. G. McCrie	Royal Sick Children's Hospital, Edinburgh	Leukaemia	"
H43	G.	Mr. Struthers	Western General Hosp., Edinburgh	Lympho-sarcoma	"
H46	F.	Dr. Comrie	Royal Infirmary, Edinburgh	Hodgkin's disease	Positive

† Previously published. See 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

Animal Number	pH of Broth Used for Suspension	Concentration of Tissue	Duration of Maintenance at -4° C.	Results following Inoculation of Rabbit
R354	5.6	1.20	3 days	Negative
R345	5.6	1.20	4 "	"
R350	6.6	1.20	4 "	"
R351	6.8	1.20	4 "	"
R352	7.0	1.20	4 "	Positive
R340	7.1	1.20	7 "	"
R369	7.1	1.20	8 "	"
R364	7.1	1.20	14 "	"
R349	7.1	1.20	3 "	"
R352a	7.2	1.20	23 "	"
R337	7.6	1.20	3 "	Negative
R353	7.6	1.20	4 "	"
R379	7.6	1.20	8 "	Positive
R367	6.6	1.40	3 "	"
R265	7.6	1.40	3 "	Negative
R516	6.0	1.40	Inoculated immediately	"
R526	6.0	1.40	7 days	Positive
R517	7.0	1.40	Inoculated immediately	"
R218	8.0	1.40	"	Negative
R528	8.0	1.40	7 days	"
R520	10.0	1.40	Inoculated immediately	"

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 -4° 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 follow-

ing 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 findings 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 $1\frac{1}{4}$ 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.cm. of sterile broth of pH 7.1, and then placed in a refrigerator at -4° C. for seven days. Thereafter it was centrifugalized 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.cm. 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.cm. of a similar suspension of *B. melitensis*, which was also added. The mixture was then divided into two equal parts of 5.5 c.cm., 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.cm. 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 twelve 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 P₂O₅ 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.

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 intraconjunctivally 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 ENCEPHALITOGENIC 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 trituration 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 cytotoxicity 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 *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 lymphadeno-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.

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A new edition of the *Official Guide Book of Medical Post-Graduate Work in Hungary* has just been issued by the Hungarian Medical Post-Graduate Committee (VIII, Mária-utca 39, Budapest). This excellently illustrated volume contains full details in English of the various hospitals and clinics where all kinds of medical post-graduate work can be undertaken. It is stated that all the professors and chief physicians speak English, French, or German. In addition to its main office in Budapest, the Post-Graduate Committee has subcommittees in the other university towns, and arrangements for study can be made wherever desired. Much modernization of the existing institutions has been effected in the last few years, and several new medical buildings have been erected. The *Guide Book* contains also information about places of historic interest in Hungary. There are now in that country four medical faculties, with 4,520 beds available for medical and post-graduate training, as well as 21,534 beds in the large public hospitals.

THE USE OF 2:4-DINITROPHENOL AS A METABOLIC STIMULANT

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It has been known since 1885¹ that nitrated naphthols can stimulate metabolism, and during the war attention was called to their toxic effects owing to the incidence of poisoning among workers in munition factories. A review of these effects was made by Perkins,² and they were more recently commented on by the Council of Pharmacy and Chemistry of the American Medical Association.³ Only incomplete investigations of the pharmacological action of such compounds were, however, made till 1928, when Heymans⁴ and his co-workers revived interest in the subject by a series of publications which showed that certain nitrophenols are powerful stimulators of metabolism, causing a greatly increased oxygen consumption. When a considerable dose is taken the endogenous heat production may be so stimulated as to cause a rise of temperature, and death from a lethal dose occurs during a hyperpyrexia. The lethal dose of dinitrophenol for rats has been shown by Anderson, Reed, and Emerson⁵ to lie between 30 and 50 mg. per kilo of body weight, but doses as small as 5 mg. per kilo of body weight may produce pyrexia in man, and it has been stated that the margin between the febrile and fatal dose is narrow.

During the last two years Magne, Mayer, and Plantefol,⁶ and Cutting and Tainter,⁷ have published reports on the main pharmacological actions of dinitrophenol. All these workers are agreed that the action of the drug in stimulating the metabolic rate is peripheral, due to an increased oxygen consumption in the tissues, and is independent of nervous and glandular action. In considering the source of the fuel for the increased metabolism they are also agreed that the body proteins are not broken down to any appreciable extent. The French workers, however, observing in the experimental animal under the influence of dinitrophenol a marked decrease of the carbohydrate content of the body, particularly in respect of the liver glycogen, and a corresponding rise in the blood sugar, argued that the excess metabolism was mostly at the expense of carbohydrate. The experimental and clinical studies of Cutting and Tainter, on the other hand, failed to reveal any difference between the excess metabolism due to dinitrophenol and that occurring normally. The latter workers, using this drug in doses of 3 to 5 mg. per kilo of body weight, have recommended its use in the treatment of obesity, and have recently published encouraging results from extended clinical trials in which an average daily oral dose of 0.3 gram (5 grains) of the drug was administered to 113 consecutive cases of obesity without drastic dietetic restrictions. No severe cumulative or toxic effects were produced, though most of the patients noticed a sense of increased warmth and increased sweating, while in 7 per cent. of them a skin rash occurred, and in 5.3 per cent. there was a loss of taste. These side actions cleared up quickly, without sequelae, on discontinuing the medication. Anderson, Reed, and Emerson⁵ have also treated fourteen cases of obesity, using similar doses of the drug, and obtained some slight loss in weight without much dietetic restriction. In one patient, however, they obtained a serious allergic skin and joint reaction. This patient suffered from chronic articular rheumatism, and it is suggested that individuals suffering from this complaint have a lessened resistance to the agent. Indeed, Perkins,² in his review of the poisonous effects of dinitrophenol on

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