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- α -protease and lieno- β -protease, do not appear to be concerned in the production of the encephalitic syndrome following the intracerebral inoculation of tissue extracts.

We desire to acknowledge our indebtedness to Professor T. J. Mackie for much constructive criticism and valuable advice received during the course of the investigations, also to Dr. Mervyn H. Gordon of St. Bartholomew's Hospital, London, for the interest which he has taken in our work.

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L. W. Sauer (*Amer. Journ. Dis. Child.*, January, 1935, p. 69) has treated 650 non-immune infants and young children by a vaccine which differed from all other pertussis vaccines in being made every few months from recently isolated haemolytic strains of the Bordet-Gengou bacillus. The total dosage after 6 months of age was 8 c.cm. Local and systemic reactions were negligible. Thirty-two children used as controls in twenty-five of the families contracted undoubted pertussis, whereas 170 of the injected children in these families who had been exposed escaped. No claims are made for the vaccine as a therapeutic agent, nor when exposure has already occurred or threatens, as the immunization takes about four months to develop.

SEROLOGICAL DIAGNOSIS OF WEIL'S DISEASE

BY

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Considerable attention has recently been paid to certain outbreaks and sporadic cases of Weil's disease in the British Isles. As the laboratory diagnosis by serological methods is somewhat specialized, and an early and definite diagnosis is of extreme importance, a description of the procedures may possibly be of use to those wishing to carry out serological tests for this disease.

Agglutination Test

I shall only refer briefly to this test, which has been perfected by Professor Schüffner (1934) and his co-workers in Amsterdam. It consists essentially of allowing varying concentrations of the patient's serum to interact for two hours at 32° C. with an equal volume of a young culture of *L. icterohaemorrhagiae*. After incubation small drops from the various dilutions are placed on a slide and are examined, without a cover-slip, by dark-ground illumination.

One factor which may make the results difficult to read when using living cultures is that lysis of the leptospira occurs in certain dilutions, and the result is consequently masked. In order to overcome this difficulty Professor Schüffner has, for the last two years, been using formalized cultures; with these no lysis occurs, but there is a tendency for acid agglutination to take place unless the strength of the pure formalin in the suspension is reduced to 0.5 per cent.

According to Postmus (1933) a positive agglutination reaction may be expected on the sixth day of disease. The reaction reaches its maximum on the twentieth day, when the titre of the serum may rise to 1 in 100,000. Control sera in Schüffner's hands have not shown positive agglutination even at a 1 in 10 dilution. Residual agglutinins may persist for several years: Postmus has demonstrated their presence 6,066 days after the onset of the disease, and in one of the cases recorded by Fairley (1934) agglutinins were shown to be present twelve and a half years after recovery.

The Adhesion Test

In many ways the results of this test are easier to read than the agglutination reaction, and it can be performed more rapidly. Its history is briefly as follows. A number of observers (Laveran and Mesnil, 1901; Rieckenberg, 1917; Brussin, 1925; and Kritschewski and Tscherikower, 1926) had drawn attention to the fact that particles such as bacteria or blood platelets would become adherent to trypanosomes and spirochaetes in the presence of immune sera. Brown and Davis (1927) applied this as a means of differentiating serological types of leptospira, and proposed the name of the adhesion test.

The questions now arise whether this test is specific and sensitive, and whether the reaction persists for some time after the disappearance of the symptoms of the disease. As regards the specificity of the test, we had no difficulty whatever in differentiating *L. biflexa* from *L. icterohaemorrhagiae* on the one hand, and *L. hebdomadis* on the other. We were also able to prove by means of this reaction that five strains of *L. icteroides* which Noguchi (1925) maintained were the cause of yellow fever and were morphologically and serologically distinct from *L. icterohaemorrhagiae*, were indistinguishable from the

latter. In studying the serology of certain patients in the Andaman Islands suffering from jaundice I found (Brown, 1928) that the serum gave a positive adhesion test with two strains of leptospira, Rachmat and Deli A, both from Sumatra, but not with another Sumatra strain nor with certain Dutch and English strains. It would appear, therefore, that the test is distinctly specific. It does not show any tendency to split up into subtypes types which have been determined by other serological methods—for example, protection tests in animals or agglutination. In other words, the adhesion test is neither more nor less specific than other serological tests.

As regards the length of time that these adhesion antibodies remain after the patient has recovered from the disease, in the sera which Dr. Hamilton Fairley kindly placed at my disposal I have been able to demonstrate adhesion to high titre in serum taken as long as eight and a half years after the disease. The sensitivity of the test is shown in the table, where in each case the total concentration of the serum at which adhesion and agglutination have been observed in various sera is noted. It will here be seen that adhesion disappears approximately at the same dilution as represents the end-point of agglutination.

A	B	1 in 10	1 in 30	1 in 100	1 in 300	1 in 1,000	1 in 3,000	1 in 10,000	1 in 30,000	1 in 100,000	C	D
M	{ Agg Adh	+	+	+	+	+	-	-	-	-	1½ years	1 in 1,000
S	{ Agg Adh	+	+	-	-	-	-	-	-	-	1 year	? 10
CG	{ Agg Adh	+	+	+	+	+	-	-	-	-	2 years	1 in 1,000
G	{ Agg Adh	+	+	+	-	-	-	-	-	-	5½ years	1 in 30
P	{ Agg Adh	+	+	+	+	-	-	-	-	-	8½ years	1 in 100
V	{ Agg Adh	+	+	+	+	+	+	+	+	-	During convalescence	Not done
B	{ Agg Adh	+	+	+	+	+	+	+	+	-	ditto	1 in 30,000

Column A: Code initials of various patients.

Column B: Agg = Agglutination. Adh = Adhesion.

Column C: Interval which elapsed between the onset of the disease and the date of testing the serum.

Column D: Professor Schüffner's record of agglutination titre six months previously.

In this table, column C, is also shown the time which has elapsed between the onset of the disease and the date upon which the serum was tested. It is interesting to note in this connexion that the titres which I obtained for agglutination were almost identical with those found by Professor Schüffner for the first five sera that he examined six months previously (column D). In two cases my results show a slightly higher titre. This is probably due to the fact that I allowed interaction between the serum and formalized culture to take place for twenty-four hours at room temperature, instead of the four to twelve hours employed by Schüffner when using formalized cultures.

Technique of the Adhesion Test

The following reagents are required: (1) the patient's serum; (2) a young broth culture of *L. icterohaemorrhagiae*; (3) a saline suspension of a young culture of *B. coli* or other similar organism; (4) a fivefold dilution in saline of fresh guinea-pig's serum (this is not required if the patient's serum is tested on the same day as the sample is taken).

One volume (about 20 c.mm.) of each of the above reagents is placed in a small agglutination tube and the contents mixed; a control tube contains known normal serum in place of the patient's serum. The tubes are incubated at 37° C. for thirty minutes, and then a small drop is placed on a microscope slide, covered with a coverslip, and examined by

Positive reaction.

Negative reaction.

The adhesion test as applied to leptospira.

dark-field illumination. In the event of a negative reaction the leptospira will be seen swimming freely and totally unimpeded by the presence of bacteria. In a positive reaction the bacteria will be seen to be firmly adherent to the leptospira. Not all the leptospira will be affected in this way, but only a certain proportion, unless the serum is of a very high titre.

At least twenty leptospira should be observed before a negative result is recorded. It may be that one or more bacteria, in a positive reaction, will adhere to the end of the organism, and no amount of movement on the part of the leptospira will dislodge them. Other leptospira will be seen almost completely covered with bacteria, with only the terminal portions still in sight. The reaction is very dramatic, and no difficulty should be experienced in reading the results.

What occasionally happens with a negative serum is that a leptospira may be seen more or less in the centre of a small clump of bacteria. In this case it will be noticed that it is not adherent to the bacteria, but slides in and out of the bacterial clump—a totally different state of affairs from that in which there is true adhesion. When there is a positive reaction with the patient's serum a second test can be made, using dilutions of serum which will give total concentrations up to 1 in 30,000. Owing to the small number of cases of Weil's disease in this country it is impossible to state the exact day of disease upon which the reaction appears, but it certainly is very marked at the end of the second week, and there is little reason to believe that it will not appear as early as the sixth day, as in the agglutination reaction.

The culture medium used in the adhesion test is a modification of that recommended by Fletcher (1927), and is as follows: distilled water, 3 c.cm.; lemco broth, 0.5 c.cm.; inactivated rabbit's serum, 0.25 c.cm. The culture is incubated at 32° C. for six days. It is preferable to use a strain which has lost its pathogenicity. In a series of wild rats examined by Brown and Davis (1927) adhesion antibodies to *L. icterohaemorrhagiae* were found in 36 per cent.; on this occasion no attempt was made to titrate the serum. In the last two wild rats recently examined the adhesion titre was 1 in 3,000 and 1 in 1,000, with agglutination titres of 1 in 3,000 and 1 in 300 respectively. Leptospira were not found in the kidneys of either of these rats.

I should like to express my thanks to Dr. Hamilton Fairley for placing five of the sera investigated at my disposal; to Professor T. R. Elliott and Dr. Wolstencroft for the other two sera; and to Professor Schüffner for his kindness in sending me full details of the technique of his agglutination test; also to Dr. Paul Fildes for his permission to reproduce the figure from the *British Journal of Experimental Pathology*.

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HEREDITY AND MENTAL DEFICIENCY *

BY

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Those members of the medical profession who are not familiar with the science of genetics and its application to human beings may not unreasonably ask why it is not sufficient to collect a large number of pedigrees of mental defectives, and, having analysed them, settle the problem of heredity and mental deficiency once and for all. This paper attempts to explain why this simple procedure does not, and cannot, provide all the necessary information.

Diversity of Views Held To-day

In spite of the numerous researches carried out on this subject, very divergent views are held by different authorities. For example, Professor McNeill¹ recently published a paper in the *British Medical Journal* entitled "Heredity a Minor Factor in Mental Deficiency." As a contrast, a passage may be quoted from the article on mental deficiency by Dr. Rudolf Pintner in the *Handbook of Child Psychology*.² "The great majority of feeble-minded individuals are simply born that way. They occur in families where feeble-mindedness or neuropathic conditions are common. No causal factor can be discovered. There may be secondary or aggravating factors, but not sufficient to cause feeble-mindedness. We therefore say that feeble-mindedness is inherited. The percentage of such inherited cases is put at about 90 by Hollingworth and about 80 by Tredgold. Amentia caused by disease or accident is therefore a relatively minor consideration.

The complete and final proof of a hereditary mechanism is the demonstration of Mendelian ratios. When inheritance is simple and uncomplicated, and when non-genetic influences are not operating effectively, this is an easy task experimentally, and not too difficult in the human subject. If mental deficiency were as simple as this the whole subject would have been settled long ago. Clearly, complexities exist that make genetical analysis much more difficult.

Mental Defectives Cannot be Sharply Separated from the Rest of the Population

The first difficulty is that mental defectives do not form a class that can be sharply distinguished from the rest of the population. The legal criterion is essentially one of

social adaptation. Where precisely that line is drawn does not have any biological meaning. In terms of the closely related, but not identical, intelligence tests, the distribution of mentality is continuous. Where the line is drawn that separates normality from mental deficiency is arbitrary. Whatever criteria are adopted, therefore, a sharp line of division cannot be secured. Dullness and retardation shade off into mental deficiency.

This does not mean that in certain cases mental deficiency cannot be due to a simple genetic difference. The continuity of the distribution may be partly due to the way that intelligence is measured. It is more likely, however, that many defectives, especially those falling into the legally feeble-minded group, do represent the lower end of the ordinary curve of variability, just as individuals occur who differ from their fellows in being very short.

Lewis³ has elaborated this conception, and terms those to whom it applies the "subcultural" group. This group is distinguished from those defectives in whom the mental state is the result of a definitely pathological condition, hereditary or otherwise. If, then, many defectives are to be regarded as extreme variants at the lower end of the curve of distribution of intelligence, it is clear that biometrical researches are required. Large numbers of individuals must be measured, and, in addition, it is not sufficient merely to contrast defectives and normals. Quantitative measurements are necessary, and the problem must be approached in the first instance by studying mentality in general—that is, all grades in the population. Biometrical studies on relatives are capable of yielding much information as to the part played by heredity in determining any characteristics that can be measured.

Mental Deficiency is not a Clinical or Genetic Entity

Another complication is due to the fact that the term covers a number of conditions, that may or may not be clearly distinguishable. Mental defectives have in common an underdeveloped and an ill-developed nervous system, but they show among themselves many differences both of kind and of degree in this state of underdevelopment. These differences are also found in mental defectives in regard to physical characteristics such as stature or size of head. Mental deficiency, then, is neither a clinical nor a genetic entity. It is not a genetic entity because it is found that there are genetic differences between groups selected in different ways. For example, it is generally accepted that those showing gross defect have fewer affected relatives than those with lesser degrees of abnormality. Mongols, too, differ from other defectives in having fewer affected relatives, and in addition the occurrence of mongolism shows a clear association with advanced maternal age.

Clinical and Genetic Entities are not Identical

It is important to recognize that clinical and genetic entities often do not correspond. For example, congenital stationary night-blindness may be inherited as a Mendelian dominant, as a recessive, or may be sex-linked. Clearly, therefore, at least three, and probably more, genetic factors exist, any one of which is capable of producing the same clinical end-result. It can be shown in other cases, such as albinism, that any one of several factors that show the same method of transmission may be responsible for the condition. Sometimes there are slight differences, constant for the family group, that indicate that different factors are at work in different cases. In two families that I had the opportunity of observing there were a number of members suffering from congenital cataract of the same type, and in both

* The substance of a paper read to the Maternity and Child Welfare Section of the Society of Medical Officers of Health, November 30th, 1934.