on the basis of average results in an experience of a large number of cases of similar type; occasionally it is selected by ascertaining the lowest limits of arterial pulsation. When amputation is made at a low level in order to give the patient a relatively useful limb the flaps not infrequently become gangrenous, and a second and higher amputation is necessary. Moszkowicz¹⁹ originally suggested that the site of amputation should be decided by the absolute lower limit of the reactive flush, a suggestion which has been found to be impracticable. We have already indicated why this test is unreliable; if due precautions are taken the flesh extends eventually to all parts of the living skin. It is clear that if the test is to be of value in selecting the lowest safe site for amputation then some arbitrary time limit for the appearance of the flush must be set that is compatible with the healing of such skin when cut into flaps. At the moment we cannot state what this time is with finality, but we suggest as a working basis that if a bright flush appears in the heel within fifteen seconds of circulatory release then amputation just above the ankle is safe, and if the flush appears in the toes within twenty seconds then amputation of the toes is likely to be successful. Illustrative cases supporting this suggestion are Cases 3 and 6 in the table.

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STUDIES ON THE AETIOLOGY OF BLOOD DISEASES

A PATHOGENIC AGENT IN NORMAL HUMAN BONE MARROW

BY

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In January, 1932, we began researches on the aetiology of pernicious anaemia and acute leukaemia. The work is still incomplete, but since there has been a compulsory break in the course of our investigations we wish to report the results so far obtained. In our experiments we have used rabbits, and we have tested the effect of intrathecal injections of bone marrow and blood obtained from human cases. All experiments made with blood have been completely negative; with bone marrow the results have been striking.

In each experiment with bone marrow a little marrow was ground in saline until a fine emulsion was formed; then into each rabbit 0.3 c.cm. of the emulsion was injected through the occipito-atlantal membrane into the theca. Only rabbits weighing 2,500 to 3,000 grams were used. Cultural tests were made of all inocula. Cerebrospinal fluid and portions of brain were taken post mortem from all animals that had received injections, and these,

too, were tested by cultural methods. We shall place on record only experiments in which all cultural media used in the tests remained perfectly sterile. The injection of bone marrow emulsion was followed by an incubation period of from a few days to three weeks; then, in almost all our experiments, a very characteristic picture developed. As a rule, the first symptom to appear was a paresis of the hind legs. In a few of our experiments, however, the earliest symptoms appeared in the muscles of the neck, spasmodic contractions pulling the head into the most grotesque attitudes. Rabbits in which symptoms appeared first in the muscles of the neck showed abnormal voracity, whereas those of the other type lost appetite and weight. In some rabbits symptoms progressed for a time, and then remained stationary or even disappeared; in others disability progressed steadily until death, which occurred within a few days or weeks of the date of the injection. We give in briefest outline a few typical protocols:

EXPERIMENT I

Test of Bone Marrow from a Case of Acute Leukaemia

Rabbit 252.—January 14th, 1932, injection; January 28th, complete paralysis of hind legs; February 10th, moribund, killed.

EXPERIMENT II

Test of Bone Marrow from a Case of Acute Leukaemia

Rabbit 300.—July 28th, 1932, injection; August 9th, complete paralysis; August 10th, moribund, killed.

Rabbit 301.—July 28th, 1932, injection; no symptoms developed.

Rabbit 302.—July 28th, 1932, injection; August 9th, paralysis of hind legs; August 18th, complete paralysis; September 16th, moribund, killed.

EXPERIMENT III

Test of Marrow from a Case of Pernicious Anaemia

Rabbit 299.—July 26th, 1932, injection; August 19th, paralysis of hind legs; August 29th, dead.

EXPERIMENT IV

Test of Marrow from a Case of Pernicious Anaemia

Rabbit 387.—November 15th, 1932, injection; December 5th, complete paralysis; December 20th, dead.

Rabbit 288.—November 15th, 1932, injection; no symptoms developed.

It is evident that in rabbits we have produced a definite encephalitis by the intrathecal injection of bone marrow taken from cases of pernicious anaemia and acute leukaemia. We have tried to find whether this encephalitis is transmissible from rabbit to rabbit. We have taken bone marrow, brain, and liver from encephalitic animals, and of each of these we have made emulsions which we have injected intrathecally into fresh rabbits in 0.3 c.cm. doses. The following are summarized protocols of the experiments.

EXPERIMENT V

Rabbit 305.—August 29th, 1932, injection of bone marrow from Rabbit 299 (pernicious anaemia); no symptoms developed.

Rabbit 306.—August 29th, 1932, injection of brain of Rabbit 299; September 12th, spasmodic contraction of muscles of the neck, distorted position of head, frequent rotations round the body axis, abnormal voracity; September 15th, moribund, killed.

Rabbit 307.—August 29th, 1932, injection of liver of Rabbit 299; September 1st, complete paralysis; September 3rd, moribund, killed.

EXPERIMENT VI

Rabbit 317.—September 15th, 1932, injection of bone marrow of Rabbit 306, an animal which represented those of the first passage; October 6th, paresis of forelegs; October 15th, moribund, killed.

Rabbit 318.—September 15th, 1932, injection of liver of Rabbit 306; October 6th, paresis of hind legs; October 10th, killed.

Rabbit 319.—September 15th, 1932, injection of brain of Rabbit 306; no symptoms developed.

Thus the encephalitis of Rabbit 299 (pernicious anaemia) was transmitted to Rabbits 306 and 307 by means of emulsions of brain and liver, and the condition was passed on again to Rabbits 317 and 318 by means of emulsions of liver and bone marrow of Rabbit 306.

Passage from Rabbit 307 was attempted, but with negative results. Attempts to transmit from Rabbits 300 and 302 (acute leukaemia) also failed.

Before these results can be discussed with profit it is necessary to give the results of control experiments in which rabbits were injected intrathecally with human bone marrow taken from cases not suffering from disease of the haemopoietic system. The following protocols are representative of all.

EXPERIMENT VIII

Rabbit 381.—November 10th, 1932, injection; November 17th, paralysis of hind legs; December 12th, moribund, killed.

Rabbit 382.—November 10th, 1932, injection; November 17th, paralysis of hind legs; December 5th, moribund, killed.

EXPERIMENT IX

Rabbit 385.—November 14th, 1932, injection; November 21st, complete paralysis; November 22nd, dead.

Rabbit 386.—November 14th, 1932, injection; November 21st, complete paralysis; November 22nd, dead.

EXPERIMENT X

Rabbit 391.—November 21st, 1932, injection; December 1st, complete paralysis; December 3rd, dead.

Rabbit 392.—November 21st, 1932, injection; November 25th, paralysis of hind legs; December 12th, dead.

EXPERIMENT XI

Rabbit 488.—April 28th, 1933, injection; May 6th, paralysis; May 24th, dead.

Rabbit 489.—April 28th, 1933, no symptoms developed.

Thus, in rabbits, the intrathecal injection of normal human bone marrow produced symptoms exactly like those produced by marrow from cases of pernicious anaemia and acute leukaemia. So far encephalitis produced by normal human bone marrow has not been successfully transmitted from one rabbit to another—at least, we cannot say that we are certain that it has been. Certain experiments were suggestive, but the symptoms which developed were not sufficiently pronounced and typical to be accepted with confidence. Twenty rabbits were injected intrathecally with emulsions of bone marrow, liver, or spleen of normal rabbits, but no symptoms of encephalitis appeared. Similar negative results were obtained when bone marrow from guinea-pigs was injected into rabbits.

HISTOLOGICAL FINDINGS

Striking changes were found in the livers of eight rabbits which had been injected with human bone marrow. In paraffin sections stained with haematoxylin and eosin the liver cells resembled plant cells rather than animal cells, the whole organ presenting a honeycomb-like appearance. The picture strongly suggested liver cells packed with glycogen, and Best's stain applied to tissue which had been fixed in a suitable manner showed that massive deposits of glycogen were indeed present. This massive deposition of glycogen was observed only in rabbits which had been injected with human bone marrow and had developed symptoms of encephalitis. These histological findings closely resemble those described by v. Gierke,¹ Schönheimer,² van Crefeld,³ and Beumer and Loeschke⁴ as occurring in a disease of children which is characterized by an enormous enlargement of the liver due to massive deposition of glycogen in the liver cells. In the livers of our rabbits, besides the enlargement of parenchyma cells, we frequently found marked perivascular cellularity. It seems doubtful whether this cellularity is of any patho-

logical significance, since the same histological appearances were sometimes found in the livers of normal rabbits.

Another remarkable histological finding in our rabbits was an extraordinary degree of haemosiderosis of the spleen, detectable by the Prussian-blue method. A long series of control observations demonstrated that normal rabbit spleen is, as a rule, free from haemosiderin. In only one control rabbit was haemosiderin detected, and then the amount was very small. In most of the intrathecally injected rabbits the spleen was blue throughout when stained for iron. The haemosiderin was sometimes observed as early as the second or third day after the injections. In some cases it was still present after five weeks. This histological change in the spleen has nothing to do with the encephalitis. It was observed also in rabbits which had been injected with rabbit organs and had shown no symptoms of encephalitis. Even the intrathecal injection of cerebro-spinal fluid taken from normal rabbits produced a marked haemosiderosis of the spleen in rabbits. This effect is probably only a consequence of a meningeal irritation. If this is the true explanation and if we can accept the view that endothelial cells are altered in such conditions, then here is an interesting proof that the central nervous system has an action upon the reticulo-endothelial system.

In our encephalitis rabbits the blood picture was, as a rule, unchanged. In one animal, Rabbit 302, a marked anaemia was observed seven weeks after the injection (haemoglobin 38 per cent.), but that is all we have found.

DISCUSSION AND SUMMARY

Our experiments have demonstrated that in normal human bone marrow there is an agent which is pathogenic for rabbits. It causes an encephalitis if injected intrathecally into those animals. This agent cannot be one of the common bacteria, since all our sterility tests have proved negative. That the agent is a living virus is an attractive suggestion. The fact that the agent was present in all specimens of normal human bone marrow tested by us demonstrates that generally the agent is non-pathogenic for man, but this does not exclude the possibility that under some special conditions this same agent may become pathogenic for man and cause disease. It will be of importance to determine whether the agent is confined to human bone marrow.

Since our attempts to transmit the encephalitis from rabbit to rabbit through an indefinite series have met with indifferent success, we think that as yet there is insufficient proof that the agent we have been studying is a living thing. Even if the transmissibility of the encephalitis were accepted, several different interpretations would have to be considered. The matter will be more fully discussed in a subsequent paper. Here we only mention the suggestion that possibly a virus normally present in rabbits might be stirred up to produce an encephalitis when human bone marrow is injected intrathecally. We have in mind, of course, the virus of Kling.⁵ We think that this explanation cannot be the true one for the following reasons: (1) the intrathecal injection of bone marrow produces encephalitis with almost complete regularity, whereas the encephalitis virus of Kling is present in the brains of only a limited number of rabbits; (2) bone marrow of rabbits and guinea-pigs never produced encephalitis, although in such cases the non-specific irritation of the brain must be the same as with human bone marrow. It is, of course, possible that in the isolated experiments in which encephalitis was transmitted from one rabbit to another we were really dealing with an agent similar to that of Kling.

Our results seem to be of especial interest with regard to the researches on the aetiology of Hodgkin's disease, which were published by Gordon⁶ during the course of our

investigations. Gordon discovered that when extracts made from glands taken from cases of Hodgkin's disease are injected intracerebrally into rabbits, symptoms of encephalitis are regularly produced. Although Gordon failed to transmit the disease from rabbit to rabbit he believes that his experiments demonstrate the virus nature of Hodgkin's disease. In our rabbits the symptoms have closely resembled those observed by Gordon, but there are differences of a quantitative kind, and there are differences in the incubation period and clinical picture. Whether these differences are due simply to differences in material or in technique can be determined only by further inquiries. We refrain from theoretical discussion until it is settled whether or not encephalitis produced by lymph glands from cases of Hodgkin's disease is identical with that produced by normal human bone marrow.

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A CASE OF ADDISON'S DISEASE

BY

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The advent of suprarenal cortical extracts into the realm of practical therapeutics has aroused new interest in Addison's disease. It is unnecessary here to recapitulate the history of the introduction of this form of substitution therapy or to deal in detail with the results obtained by American observers and by others in this country and on the Continent. References to most of the published work are given by Levy Simpson¹ and G. A. K. von dem Borne.²

EFFECT OF CORTICAL EXTRACT ON SYMPTOMS

While all are agreed upon the dramatic results which follow the administration of cortical extracts in the crises of Addison's disease, there is a considerable difference of opinion among many writers as to the more remote results which can be obtained. It has been held that the administration of cortical extracts is followed by the amelioration of all the several groups of symptoms—muscular, cardiovascular, gastro-intestinal, psychological, and pigmentary. The asthenia, wasting, sense of fatigue, dysphonia (Segre), poor heart action, subnormal temperature, hypersensibility to cold, lowered metabolic rate, anorexia, nausea, retching, vomiting, hiccup, yawning, lethargy, insomnia, photophobia, irritability, negativism, pessimism, involuntary crying and grimacing, abdominal and loin pain, and lowered resistance to infection—all tend to disappear. Pigmentation is said by many but not all observers to follow suit, and the same in regard to the lowered blood pressure and hypoglycaemia.

It seems certain that the blood pressure is raised by the giving of cortical extracts during the acute crises of the disease, when very low readings may be found; but it is by no means clear whether this is a direct or an indirect effect. During stationary phases little effect, if any, may be seen. The symptoms resembling those of hypoglycaemia, associated with a drop in the blood sugar, which occur just before and during a crisis, are relieved by cortical extracts, and normal blood sugar readings are restored. Pigmentation is said to be little or in no way altered by treatment in many cases. Britton, Harrop,

and Weinstein³ held that these three symptoms are quite uninfluenced by the administration of cortex, and it is of interest to recall the fact that these three phenomena were considered to form no part of the symptom-complex determined by adrenalectomy in dogs—a fact perhaps indicating the caution necessary in interpreting the symptoms in Addison's disease in man in terms of the results of animal experiment. On the other hand, later work suggests that the syndrome in animals may be more analogous to that in man than was at first supposed. The signs of impaired renal function, as evidenced by albuminuria and high blood urea, clear up under treatment with cortical extracts; the lowered creatinine excretion and rise in urinary creatine may also be corrected, but no diminution of the raised blood cholesterol is effected.

Despite the initial improvement which has taken place in practically all cases treated with cortical extracts—and cortical extracts are a life-saving remedy in the acute crises of the disease—complete restoration to the normal has never, according to von dem Borne, been attained. Experience at the Mayo Clinic showed that, even though very considerable improvement was temporarily established and life prolonged, death inevitably followed. Other cases, it is true, have been reported in which health has been restored and maintained for many months, even after cessation of treatment, but the ultimate results have not been made known and the underlying pathological condition has remained unrevealed. Apart from the evidence sometimes afforded by radiography (calcification in some tuberculous cases) there is no certain method of discriminating, during life, between those cases (70 to 90 per cent.) in which the lesion is tuberculous and those in which there is said to be a simple atrophy. Levy Simpson, however, holds that all the symptoms of Addison's disease tend to clear up under treatment with cortical extracts given in sufficiently large doses, and that health can be restored and maintained.

PREPARATION OF CORTICAL HORMONE

Failure to obtain better results in man in the past have been due in part to the difficulty in assessing correct dosage. Suprarenal extracts have certainly differed widely in their hormonal content, and it seems doubtful if any very satisfactory means of standardization has yet been devised. W. W. Swingle and J. J. Pfiffner⁴ have defined a dog unit (D.U.), using as criteria "the maintenance of body weight and blood level of non-protein nitrogen (or urea)," and in the experimental animal obtained complete control after adrenalectomy by giving cortical extracts which possessed an adrenalin content of only 1 part in 1,250,000 to 2,500,000; but whether such a method of standardization is applicable for man remains uncertain. The same is probably true of the method of biological assay in rats adopted by N. V. Organon, for the preparation "cortin" (J. W. R. Everse and P. de Fremery; A. A. Adler, J. W. R. Everse, P. de Fremery, and A. Luchs; and M. Tank and A. W. M. Pompen).

The mechanism whereby the internal secretion of the adrenal cortex exerts its normal functions on the body is unknown. It has been suggested that this gap in our knowledge will be filled when it is possible to prepare the cortical hormone in a pure state. It is by no means certain that there may not be more than one cortical hormone. The problem, indeed, may be a more complicated one than would appear at first sight. In man none of the biochemical tests which may be carried out would appear to have proved very satisfactory as a means of evaluating the results of cortical extract therapy. Such tests are useful, though possibly not more useful than a study of the patient's reactions to treatment