to the purely degenerative character of the lesions make this term seem preferable to encephalopathy or encephalosis.)

Bacteriology.—Cultures from brain, heart, and right and left lung were all sterile at the end of 72 hours.

Virus Studies.—The following virus studies were negative: (1) Lung tissues were inoculated into twelve mice, one rabbit, and one guinea-pig intranasally by Major T. F. McNair Scott, M.C., U.S.A. (2) Glycerinated lung tissues were inoculated into cotton-rats and were reported as non-effective by Dr. C. H. Andrewes, National Research Institute, Hampstead. (3) Brain tissues were inoculated by Major McNair Scott intracerebrally into six mice, one rabbit, one guinea-pig, and one hamster. The rabbit died with encephalitozoon infection on the fifth day after inoculation.

Discussion

Clinical descriptions of cases of atypical pneumonia often stress the frequency and severity of headaches. Thus out of 87 cases we have observed at one hospital, 41 (47%) complained of headache—14 mild, 6 moderately severe, and 21 severe. The headache is usually frontal or ocular, though it may be generalized in severe cases. It occurs as a rule at the onset of the disease, and is rarely absent in severe cases of atypical pneumonia. Coughing increases the headache considerably.

Excluding the case reported above—the sole fatality in our series—lumbar puncture was performed in only one other case. This was one with severe frontal headache but no signs of meningitis or encephalitis; spinal fluid was normal. We have had under observation another patient who, during a previous attack of atypical pneumonia, had severe headache and positive Babinski, Chaddock, and Oppenheim reflexes. Unfortunately lumbar puncture was not performed. Recovery was complete.

Apparently our patient died of encephalitis rather than of pneumonia, since dyspnoea and rales became less in the last few days and abdominal distension disappeared. Indications of central nervous system involvement appeared abruptly on the sixth day in hospital, when the patient began to complain of severe headache. At the same time the temperature rose and slight neck rigidity was noticed. This encephalitis must be distinguished from a post-infectious encephalitis, which is characterized histologically by patchy areas of demyelination. These were not a prominent feature in this case, in which the brain lesions resembled more closely those of epidemic encephalitis.

If our case of atypical pneumonia was due to a virus it might be conjectured that, as with analogous virus infections, antibodies would develop in the blood of convalescents, and that transfusions of convalescent blood might favourably affect the course of the disease. Flexner and Garon (1943) report three cases in which small transfusions from convalescents were administered. Two of these patients, very ill at the time of transfusion, eventually died; the third, who had had a sustained temperature of about 103°, became afebrile within 24 hours and remained well. Kneeland (personal communication) has informed us of two unpublished cases in which convalescent blood was employed. In one case a husband, three weeks convalescent, gave 500 c.cm. of blood to his wife, who had apparently contracted the infection from him. The patient, who was pregnant and desperately ill, showed marked and rapid improvement. In the other case, receiving only 50 c.cm. of convalescent blood, no apparent benefit was obtained.

Our patient seemed to show a remarkable, though transient, response to the first transfusion; this was manifested by a rapid drop in temperature to normal, and by definite clinical improvement. The second transfusion appeared to have no effect. The third seemed to benefit him somewhat without affecting the temperature. The amounts of blood given on each occasion were small, as circulatory failure was feared from a larger transfusion. Yet the smallest transfusion, the first, appeared to have the greatest effect. The first donor had been the most severely ill of the three and had been longer convalescent than the others. Thus it might be supposed that his serum would have a higher antibody titre than the others'.

Summary and Conclusions

A case of fatal atypical pneumonia with encephalitis is presented, with necropsy findings.

The incidence of encephalitis in atypical pneumonia is discussed.

Convalescent blood transfusion in atypical pneumonia is considered.

Special thanks are due to Lieut.-Col. Yale Kneeland, jun., M.C., U.S.A., for the unpublished data recorded above. We are deeply indebted to Lieut.-Col. John B. Hazard, M.C., U.S.A., for the histopathological descriptions and review of findings in the reported case.

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THE FAILURE OF H 11 TO INHIBIT GROWTH OF TUMOURS IN MICE

BY

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The preparation known as H 11, which is being used in the treatment of cancer, is an extract of urine. It is claimed that the extract contains "anti-growth substances" which have "an inhibitory effect upon cancerous growth." The method of preparation of H 11 and the standardization of the "growth-inhibitory power" of the product by its effect in checking the growth of oat seedlings are described by Thompson and his collaborators (1941). Thompson, Holt, and Forbes Jones (1943) claim that certain fractions of urine have been obtained which stimulate the growth of transplanted tumours. The urine is fractionated by precipitation with copper salts; the precipitate is extracted with dilute hydrochloric acid, which yields a tarry substance partially soluble in sodium hydroxide. The part soluble in alkali is said to accelerate growth, and therefore it would appear that by removal of the "growth-promoting fraction" the "growth-inhibiting properties" of the residue are increased. The improved extract is called 80 K.

It is not our purpose to examine or discuss the general questions of "growth promotion" and "growth inhibition" in relation to the problems of the pathology and treatment of cancer. We wish simply to put on record the results of experiments designed to test whether H 11 or the new improved preparation obtained after precipitation with copper salts does in fact inhibit or affect in any way the growth of cancer cells. Mr. J. H. Thompson of Hosa Research Laboratories has provided generous supplies of H 11 and of his new preparation 80 K; for his kindness we wish to express our indebtedness.

General Remarks on the Testing of "Cancer Cures"

In these laboratories we have gradually worked out routine methods of examining substances supposed to have valuable therapeutic properties for cancer. Our first step is usually a test of the direct action of the substance on cancer cells. For this purpose we use the technique of tissue culture. The substance to be tested is applied in various dilutions to cultures consisting of growths of normal and malignant tissues. The growth of the tissues is measured before and after varying periods of treatment. Control cultures are measured at the same time. From the two sets of measurements the extent to which normal and malignant growth are influenced by the substance can be calculated. Confirmatory tests are made by adding the substance under investigation, in the weakest concentration previously found to arrest malignant growth, to the

media used in preparing the cultures of normal and malignant tissues. Growth of normal tissues in such cultures, and concurrently no growth of the malignant tissues, would indicate a specific inhibitory action upon malignant growth. Treated cultures are examined microscopically for any cellular changes that might have been induced. The estimation of the significance of any changes requires a thorough acquaintance with the morphology of the tissues employed, during both growth and degeneration.

Our second step is to test the effect upon transplantable tumours growing in animals. A convenient method is to take one of the well-known transplantable tumours which have been observed over a period of years and whose growth behaviour is well known. It is important to select a tumour which when grafted gives a high percentage of "takes" and which recedes rarely. Many tumours which are widely used in cancer research are apt to retrogress after some preliminary growth. Such tumours are obviously unsuitable for research in therapeutics. An example of such a tumour is the Brown-Pearce skin carcinoma of the rabbit. The Imperial Cancer Research Fund possesses two transplantable mouse cancers which are eminently suitable for testing chemotherapeutic substances; they are the mammary carcinoma No. 63 and the spindle-cell sarcoma No. 37. Both graft easily, giving 95 to 98% of "takes" in mixed laboratory stock mice; both tumours grow steadily and kill the host in three to four weeks. Retrogressions are not more than about 1%. Injections of the therapeutic substance are made at a site remote from the tumour and are not begun until the graft has become vascularized and the "daughter tumour" is palpable.

Although these two well-known transplantable tumours are suitable for preliminary tests of the "growth-inhibitory" properties of any substance or preparation, we should not regard even a conspicuous retardation of tumour growth as being valid unless confirmation could be obtained on tumours of a homozygous strain of mice grown in the same strain. The grounds for such scepticism are well known and have often been stated in Reports of the Imperial Cancer Research Fund. Briefly the reasons for this extra precaution are as follows:

The tumours 37 and 63 occurred sporadically in two mice of mixed stock more than 30 years ago, and grafting was carried on, again in mixed stocks, until grafts grew readily in a high percentage. These tumours have now been carried through hundreds of generations of mice, but they are still foreign cells so far as the grafted host is concerned. There is in some mice an antagonism to these foreign cells, and in such mice retrogression of grafted tumours occurs. In spontaneous tumours of mice or men the cells of the tumour belong to the body, are a part of the body, and there is no such resistance to their presence in the body. The final test of any therapeutic method in cancer must evidently be made on spontaneous tumours, since these are the strict equivalent of human cancer; the variations which naturally exist among tumours must of course be kept in mind. Spontaneous tumours, even if of the same organ—for example, cancers of the breast—do not all grow at the same rate and behave in the same way. While the majority of mice with such tumours survive a comparatively short time—a matter of weeks—after being noticed clinically, some live for months. There is thus no uniformity in the growth rate of the tumours, and consequently, in order to get reliable therapeutic results of an order short of rapid complete cure, it is necessary to treat large numbers of animals, leaving an equal number of untreated controls. It is probable that some of the good reports and the optimism which seem to accompany many "cancer cures" tried out on human beings have their source in the fact that some cancers do not kill their hosts quickly. The case which naturally survives a long time and which is under a new treatment may be regarded as a proof that the new treatment has been to a certain extent effective. The credit belongs to Nature, not to Art. As a consequence of such apparent success other cases are treated similarly, and this goes on until at last the remedy is abandoned.

We are able to evade these complexities and to detect minor inhibitory effects by the use of homozygous strains of mice. Tumours arising in a homozygous strain grow uniformly when

grafted in mice of the same strain; there is no resistance to their growth, and such tumours are for practical purposes comparable with spontaneous tumours. The strains most commonly used in the laboratories of the Imperial Cancer Research Fund are Strong A, Paris R 111, and C 57 black.

Results of Treatment with the Preparations H 11 and 80 K on Animals bearing Tumours

The following tumours have been used in our tests:

- (1) Strong A carcinoma growing in pure-line Strong A mice; 10 treated and 6 controls.
- (2) Methylcholanthrene C 57 sarcoma growing in C 57 mice; 6 treated and 6 controls.
- (3) Mammary carcinoma 63; 6 treated and 6 controls.
- (4) Spindle-cell sarcoma 37; 6 treated and 6 controls.

Mr. Thompson recommends the injection of 1 c.cm. intra-peritoneally twice daily. Although we cannot believe that intraperitoneal injections are of vital importance in the treatment—and it is unlikely that the recommendation is insisted upon in the treatment of human cases—we have nevertheless followed Mr. Thompson's method in three out of the four tests, but always with due regard for the health of the treated animals. Intraperitoneal injection of 1 c.cm. twice daily becomes an ordeal for a 20-g. mouse; it is the equivalent of 14 lb. of fluid injected daily into the peritoneal cavity of a 10-st. man. As soon as the weight of a mouse began to drop appreciably the subcutaneous route was employed in order to give the animal a chance to recover. It should be noted here that any treatment which depresses the animal's health, or even an intercurrent infection, may retard the growth of a transplanted tumour. Many transplanted tumours cease to grow, or the growth rate falls strikingly, when the host becomes pregnant. This applies particularly to the mammary cancer No. 63. It will be observed from these brief comments that thorough and critical examination of a "cancer cure" demands experience and knowledge of the behaviour of transplantable tumours.

The rigid application of these rules has been relaxed in our tests of H 11. Thus with tumour 37 we "favoured" the remedy by infiltrating the growing margins of the tumour with H 11; while with tumour 63 almost all the rules were broken, as will be shown later. With Strong A carcinoma and C 57 sarcoma we adhered strictly to the rules which we have adopted to exclude a deceptive appearance of a favourable effect.

* In all our experiments with mice the tumours in the treated animals grew steadily and at the same rate as in the controls. We could distinguish no "growth-inhibitory" effects whatever. With the rapidly growing tumour 37 the growth of both treated and untreated tumours was so rapid that the experiment came to an end after 11 days: the tumours had become so large that further treatment was unjustifiable. The Strong A carcinoma grows more slowly, and treatment has been more prolonged—30 days; but again there was no evidence of any checking of growth or of any alteration in the cellular structure of tumours of animals killed during the course of treatment. The C 57 sarcoma was likewise unaffected.

One experiment only need be given in any detail, since it is representative in its negative results. Moreover, it was set up after it had become evident that H 11 does not inhibit the growth of tumours, and was consequently planned to give the treatment every advantage that could be justifiably arranged. In the first place, we were enabled to use the latest improved extract 80 K, which, in Mr. Thompson's words, "has proved particularly potent as an inhibitor to malignant growth." In the second place, instead of grafting the tumour cells in our mixed stock mice, in which the tumour grows very rapidly, we grafted 12 mice of the inbred homozygous strain Paris R 111; the tumour cells grow more slowly in these mice, especially during the ten days following grafting, and regressions are more common. The tumour cells were thus placed in a comparatively uncomfortable environment, and it might be expected that a "growth inhibitor" would have a good chance to show its powers in such circumstances. The rules for testing chemotherapeutic substances were further violated by beginning injections of the extract only four days after grafts had been made—that is, a week before any daughter tumour could be palpated. The "growth inhibitor" was

injected twice daily—mostly intraperitoneally, but subcutaneously when the health of the animals became affected—from March 22 to April 15 inclusive; 25 days in all. The accompanying chart illustrates the growth of the treated and untreated tumours. A glance at it shows that Mr. Thompson's extract has not inhibited the growth of the tumours; there is no difference beyond chance variation between the treated and untreated animals. In this experiment the lack of "growth-inhibitory" power became evident very early; for in spite of injections being started before palpable tumours had appeared and when the amount of living tumour tissue could not have been more than 0.02 c.cm. — the volume of the original inoculum — measurable tumours equal to those in the control mice were present after 8 days of treatment. These continued to grow normally, as the chart shows. The sizes attained by the tumours can be gauged by the weight of tumour in Mouse 1 of the chart on April 15; it was weighed after removal, and equalled 9.9 g.

Paris R 111 strain of inbred mice grafted with mouse carcinoma 63 on March 18, 1943. Mice 1 to 6, inclusive, treated with 80 K. Injections were begun on March 22, and were continued daily to April 15.

The results of tests on tissue cultures were also negative. The tumours used were carcinomas No. 63 and Strong A, and C 57 sarcoma; the normal tissues used for comparison were embryo heart fibroblasts and embryo kidney. In experiments in which H 11, in a final concentration of about 1 to 4, was added to 3-day-old cultures in active growth there was no detectable influence on the normal or malignant cells. When the urine extract was added to the medium immediately the tissues were explanted, it was found that in final dilutions of 1 in 2 normal and malignant cells were killed equally; in dilutions of 1 in 10 growth was equal in normal and malignant cells. No specific inhibition of growth of tumour cells was observed.

From the results of these investigations we conclude that H 11 has no "growth-inhibitory" effects on the tumours used in the experiments.

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OBSERVATIONS ON THE USE OF H 11 IN CARCINOMA

BY

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During the past two years an increasing number of workers have described growth-inhibiting properties of urinary extracts. Turner (1939) indicated an inhibiting effect from an alcoholic fraction of urine, while Bowman and Mottshaw (1941) studied the growth-inhibiting properties of a similar extract on tumour growth. Other workers include Rohdenburg and Nagy (1937) and Murlin *et al.* (1939), who claimed anticarcinogenic properties for such extracts. Thompson *et al.* (1941) published a preliminary account of the preparation of an alcoholic fraction of urine named H 11, and described not only its biological properties and anticarcinogenic action in transplanted animal tumours but also the inhibitory effect on human cancer.

The Present Investigation

An investigation into the effect of H 11 extract on carcinoma was begun at the Kingston County Hospital in Oct., 1940, and it is now thought advisable to issue an interim report, although no conclusions can be drawn, as the cases have not been followed up for a sufficient period.

Of the 51 advanced cases 14 received insufficient treatment, and of the remaining 37 patients 11 are dead and no reply was received from 5. Of the 21 patients known to be alive 4 have been under observation for under one year, 12 for over

one year, and 5 for over eighteen months. All the surviving cases except two received some other form of therapy, and in these two—Nos. 18 and 38—the diagnosis was not confirmed, as biopsy could not be performed.

The series (see Table) contains different types of carcinoma situated in various parts of the body. In most instances the condition was inoperable, and usually the patient was in poor general health, although there was considered to be a sufficiently reasonable expectation of life to justify treatment. This period of time was assessed at six weeks in accordance with the statement made by Thompson that objective signs of improvement do not usually occur until such an interval of treatment has elapsed. Some cases had other treatment, including operation and radium, but with few exceptions they were cases in which the operation or radium therapy was considered to be insufficient to eradicate the local disease.

There is some evidence that in a few cases the rate of growth has been slowed up or inhibited, but in no case confirmed by section has, as yet, any growth disappeared as the result of H 11 therapy only. Further research is essential in order to purify the extract, and a more satisfactory method of dosage must be evolved so as to keep the concentration in the blood at a constant level.

No case of carcinoma should be treated by H 11 alone unless a competent surgeon has advised that it is inoperable and unsuitable for radiotherapy, etc. Treatment must be given for at least six months, and the cost should be carefully considered before advising or starting this form of therapy.

H 11 extract has been supplied for this investigation by the generosity of Standard Laboratories, who have prepared the extract. All the research and experimental work has been carried out at the Hosa Research Laboratories by the Director of Research, Mr. J. H. Thompson, and his staff.