THE INFLUENCE OF HELENINE ON TUBERCULOSIS.¹

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It has long been desired to find a drug capable of influencing the course of phthisis and tuberculous disease generally. Many drugs have been introduced as specifics, but the results of further experience have never substantiated the statements of their original introducers. In the case of helenine, a substance derived from "inula helenium," the statements as to its action in tuberculosis seem to rest on a somewhat better and more scientific basis, and it was with a view of corroborating or confuting the statements made as regards its efficacy that the present research was undertaken.

Before giving my own results it will be as well to give a short epitome of the work already published on the question. In 1833 Valenzuela² stated that he had used helenine with success in cases of tuberculosis, early phthisis, pertussis, etc. The drug was described by him as possessing a yellow colour, of formula $C_{21}H_{33}O_9$, with melting point $72^\circ C.$, and boiling point $140^\circ C.$

In 1885 Baeza³ stated that the drug diminished all the secretions, but especially those of the trachea and larynx. In small doses it prevented the sialagogue and diuretic action of jaborandi. He found, too, that 0.01 gramme, added to 1 litre of urine, prevented putrefaction. In the same year Korab⁴ stated that tubercle bacilli suspended in sterile serum, to which had been added a small quantity of helenine, refused to develop, and the serum was incapable of inducing tuberculosis if injected into animals. He also stated that, if given in food, helenine acted as a preventive to infection by inoculation, and modified favourably already existing disease. The formula of the body employed by him was stated to be C_8H_8O .

In 1887 Marpmann⁵ wrote two important papers dealing with both the chemistry and therapeutic action of helenine. He stated that helenine consisted of two bodies, alantol and alantic acid, both of which were useful in medicine. Their administration caused death of the bacilli in tubercle nodules, and they also acted as expectorants. On man the drug had no injurious action; it was excreted mainly by the lungs, and after prolonged administration to phthisical patients the tubercle bacilli disappeared from the sputum. The excretion of urine and of uric acid was also increased, and it was suggested that the drug might therefore be useful in chronic gout.

It would seem from the above extracts that while all were agreed as to the efficacy of a substance obtained from elecampane root in tuberculosis, there was a considerable discrepancy as to the drug employed. It was very desirable, therefore, to make a preliminary chemical investigation as to the actual substances obtainable from the root. The literature on the subject is somewhat scanty, but we find in Philip's *Materia Medica* the following data: "Crystals may be obtained by distilling or even gently heating the root, and are described chemically as the anhydride of alantic acid ($C_{15}H_{20}O_4$). This is accompanied by a small quantity of helenine (C_8H_8O), also crystalline, and of alantic camphor ($C_{10}H_{16}O$), which in taste and smell suggests peppermint. The anhydride obtained by distilling the root with water is impregnated with alantol, which may be separated as an oily liquid."

At Dr. Brunton's suggestion I communicated with Dr. Schuchardt, of Görlitz, who promised to prepare me substances as follows:

1. Helenine—melting point, $109-110^\circ C.$ Formula, C_8H_8O . White crystalline needles.

¹ Report to the Therapeutic Committee, British Medical Association.

² *El Siglo Medico*, October 28th.

³ *La Cronica Medica*, February 5th.

⁴ *Comptes Rendus*, xcv.

⁵ (1) *Breslau. aerztl. Ztschr.*, No. 5; (2) *Centralbl. f. allgem. Gesundheitspf.*, Bonn, 1887.

2. Alantcamphor ($C_{10}H_{16}O$), a crystalline mass, melting point, $64^\circ C.$

3. Alantic anhydride, crystalline, melting point, $66^\circ C.$

4. Alantol, a yellow liquid.

Difficulties apparently rose, however, in isolating these substances in quantity, and up to the present I have only been able to obtain from him a supply of pure alantic anhydride.

I also made, with the aid of Mr. Ball, F.C.S., of the firm of Burroughs, Wellcome and Co., to whom my best thanks are due, an attempt to isolate some of the above substances. By sublimation we obtained as a first product a camphor-like body, which sublimed in plates, and melted at $60^\circ C.$ to $62^\circ C.$ On raising the temperature somewhat higher a substance sublimed over identical in appearance with the crystals obtained an exposing turpentine to sunlight for a prolonged period. These crystals were needle-shaped, with a melting point of $68^\circ C.$ to $69^\circ C.$

During the sublimation of the first body it was noticed that while this collected in the "head" of the apparatus, another substance collected in the form of crystals in the receiver. We believe that this will prove to correspond with the substance alantic anhydride, and to differ from "helenine" only in the fact that it is a less oxidised body. These substances were all soluble in fats, spirits, ether, chloroform and petroleum ether.

During the distillation of the powdered root with alkaline water, a volatile liquid possessing a very pungent odour passed over in small quantities into the receiver and dissolved in the water. We have not been able to obtain any of this substance in the pure state, or to separate it even by the aid of a freezing mixture. We assume, however, that this corresponds with the alantol of other workers. Lastly, we have extracted a yellow resin from the residue left in the retort.

The only substance we have been able to prepare in large quantities is that which we consider to be alantic anhydride, and it is only by a very laborious process of fractional sublimation that we can obtain this substance of a constant melting point of $66^\circ C.$ The other bodies were, however, isolated in quantities sufficient for laboratory experiments.

My subsequent experiments have been carried out with all of the above substances, and with Schuchardt's "alantsaure anhydride." I first directed my attention to the influence of these substances on the growth of the tubercle bacillus, and of some other micro-organisms in artificial culture, mixing them in various proportions with the nutrient media prior to inoculation with the organism. In this way I have found that any of the crystalline bodies will prevent the growth of the tubercle bacillus if present even in the proportion of 1 in 10,000. I have confirmed this with various nutrient media, but the result is the same whether I use solidified blood serum, agar-agar glycerine mixture, broth with glycerine, broth with solid egg albumen, or a solution of alkali albumen obtained from blood serum (sheep) or from egg albumen. All of the above, without the addition of the elecampane derivative, form excellent media for the cultivation of the tubercle bacillus.

I have further found, with the liquid media containing helenine, that these even if containing large quantities of bacilli in suspension, are incapable of producing tuberculosis or even an enlargement of the nearest lymphatic glands when inoculated into healthy guinea-pigs. Inoculations of normal nutrient media with these cultures also fail to produce any growth. I may therefore conclude that the drug, even in the strength of 1 in 10,000 is fatal to the tubercle bacillus, and my results in this particular fully corroborate those of Korab.

I have incidentally attempted to grow other micro-organisms on the same medicated media, and it may be interesting to state here the results obtained. In every case I employed solid media, to which were added what is probably a mixture of helenine and alantic anhydride. I found that the more luxuriant and rapidly-growing micro-organisms were practically unaffected in their growth by the presence of even 1 part of the drug in 1,000 of the nutrient medium. On the other hand, the streptococci (for example, *st. pyogenes*, *st. erysipclatis*) and some bacilli (for example, *bacillus typhosus*, *bacillus mallei*) refuse to grow on these prepared media.

Having established, then, that helenine and the asso-

ciated bodies have a real action on the tubercle bacillus, I next desired to find out the influence of the drug on the tuberculous process in animals. The lines on which I have worked are as follows:—

1. To administer the drug to guinea-pigs during prolonged periods, and subsequently to inoculate them with tuberculous material, control inoculations being made with normal animals.

2. To prepare the animals by feeding them on helenine; to inoculate these, continuing the feeding with the drug.

3. To inoculate animals with tuberculous matter, and then to commence feeding them with helenine.

Owing to the very slight solubility of all the elecampane derivatives I was obliged to use them in the solid form, and I found it most convenient to administer the dose either in the form of a pill or, better still, as a small tablet, which was kindly made for me by Messrs. Burroughs, Wellcome and Co. These tablets were very compact, disintegrated fairly readily, and ensured accuracy of dose. The preparation used was always either the alantisaure anhydride of Schuchardt or the mixture of helenine with alantic anhydride (of a somewhat higher melting point) prepared by Mr. Ball.

Without entering into details as to the result in each case, I may state that, however great the daily dose of helenine, no ill effect was produced by the drug itself; but in no case am I able to say that the course of the disease following inoculation with virulent material was arrested. That it was considerably retarded, however, I think there can be no doubt. This is shown both by the date of death and also by the condition of the organs as seen under the microscope. In the first series of experiments I used tuberculous sputum for inoculating the animals. Only three animals were used in this case; the control died of acute tuberculosis in 57 days; the other two died, one after 67 days (acute), the other after 93 days (caseation and cicatricial tissue very abundant).

Series 2 was inoculated from the liver of the previous control. The dates of deaths were as follows:

Guinea-pig, 1, control.	Died in 109 days.
Guinea-pig, 2, control.	Died in 161 days. ⁶
Guinea-pig, 3, fed.	Died in 157 days. ⁶
Guinea-pig, 4, fed.	Killed in 136 days.
Guinea-pig, 5, fed.	Died in 179 days.

The last two animals presented nothing but very chronic lesions; the nodules were considerably cicatrised, and the lymphatic glands were much enlarged and fibrous. Very few bacilli were found in the tissues of any of the "protected" animals.

Series 3 consisted of ten animals, three being controls, and seven protected by feeding with helenine.

Guinea-pig, 1, control.	Died on the 48th day.
Guinea-pig, 2, control.	Died on the 54th day.
Guinea-pig, 3, control.	Died on the 43rd day.
Guinea-pig, 4, fed.	Died on the 62nd day.
Guinea-pig, 5, fed.	Died on the 81st day.
Guinea-pig, 6, fed.	Died on the 29th day. ⁷
Guinea-pig, 7, fed.	Died on the 89th day.
Guinea-pig, 8, fed.	Died on the 70th day.
Guinea-pig, 9, fed.	Killed on the 121st day.
Guinea-pig, 10, fed.	Died on the 135th day.

This series seems to show that the animals derived a considerable amount of protection from the helenine feeding. They were inoculated from human sputum, and the death of Guinea-pig No. 6 was probably due to accidental infection. The lesions in the six protected animals exhibited appearances identical in character with those observed in the previous series, the microscopic appearance of the lungs showing none but old cicatrised tubercles with very few bacilli; in fact, a careful search was necessary to discover their existence in several cases.

Series 4 consisted of five "protected" animals and three controls. They were inoculated from a first culture on serum obtained from one of the controls of the last series. They received daily doses of the drug in pill form, and some of the powdered root was mixed with their food. The atmosphere of

the room in which they were kept was also impregnated with eucalyptus oil. The result was as follows:

Guinea-pig, 1, control.	Died in 48 days.
Guinea-pig, 2, control.	Died in 59 days.
Guinea-pig, 3, control.	Died in 44 days.
Guinea-pig, 4, control.	Died in 89 days.
Guinea-pig, 5, control.	Died in 106 days.
Guinea-pig, 6, control.	Died in 99 days.
Guinea-pig, 7, control.	Died in 135 days.
Guinea-pig, 8, control.	Died in 120 days.

The result is thus not markedly better than in the previous series.

Series 5. Six animals were inoculated in this series. Instead of feeding them with the drug, I administered it dissolved in olive oil and injected it deeply into the subscapular fossae. At first I had considerable difficulty from the fact that the injections produced local suppuration. I attribute this to the fact that the syringe used was difficult to sterilise, and thus allowed of the introduction of micro-organisms which produced the suppuration. Since I have adopted the syringe recently described by me in the *BRITISH MEDICAL JOURNAL*, I have had no difficulty from this cause. The oil is easily absorbed, and the animals remain perfectly well under the treatment. Results were as follows:

Guinea-pig, 1, control.	Died in 54 days.
Guinea-pig, 2, control.	Died in 36 days.
Guinea-pig, 3, control.	Died in 70 days.
Guinea-pig, 4, injected.	Died in 76 days.
Guinea-pig, 5, injected.	Died in 42 days.
Guinea-pig, 6, injected.	Died in 99 days.

These results were not entirely satisfactory, so it occurred to me to use, instead of a very virulent bacillus, one which had been attenuated somewhat by cultivation on glycerine agar-agar, but which was still capable of producing death in a normal animal. Here I met with the difficulty that it is very easy to render the tubercle bacillus so weak that it is incapable of producing anything but hardness of the lymphatic glands nearest the seat of inoculation. However, by using the same tube for the whole series I was able to obtain trustworthy results.

Series 6. Eight guinea-pigs, 4 of which were controls.

Guinea-pig, 1, control.	Died in 93 days.
Guinea-pig, 2, control.	Died in 108 days.
Guinea-pig, 3, control.	Died in 130 days.
Guinea-pig, 4, control.	Still living.
Guinea-pig, 5, injected.	Still living.
Guinea-pig, 6, injected.	Still living.
Guinea-pig, 7, injected.	Died in 142 days. ⁸
Guinea-pig, 8, injected.	Still living.

It is now more than six months since these were inoculated. The animals now living presented soon after inoculation an enlargement of the lymphatic glands of the groin, with in one case local suppuration. This has disappeared, and the animals are now apparently quite healthy. I am repeating these experiments, which will, I think, far more nearly represent the kind of infection which takes place in the human subject than do those in which inoculation is made with a virus capable of setting up an acute miliary tuberculosis. The results as far as they go certainly tend to show that helenine has a real protective action against the disease.

I may add that nearly all the animals used in the above experiments were bred by myself, and kept under observation in the country for some time before use. I was thus able to choose animals which were in every way healthy, and which corresponded fairly nearly in age. This would seem to be a great consideration in experiments on a disease, the duration of which depends so much on individual receptivity.

As regards the use of helenine in the human subject, I fear I have little to say. The great drawback to its extensive trial lies in the cost of the preparation. Dr. V. D. Harris kindly made use of it in some cases under his care at Victoria Park, but he employed only small doses (6 gr. per diem),⁹ and I maintain that it will be necessary to use considerable quantities in order to obtain any appreciable effects. I have myself four cases now under observation, but they have not

⁶ With an acute pleuritic effusion.

⁷ Large numbers of micrococci were found in the effusions of this animal, but on isolation and cultivation these proved non-pathogenic to guinea-pigs.

Caught sputum septicæmia from another animal.

⁹ *St. Bartholomew's Hospital Reports*, 1890: "The Antiseptic Treatment of Phthisis."

been taking the drug for a sufficient length of time to allow me to eliminate the effect of change of weather, etc. It will be better for me to defer any account of these till I have more definite information.

I have brought these results before the Association at this time in the hope that some others who have more ample opportunities than myself may be perhaps induced to make trial of helenine in early phthisis. I do not think I am justified in saying that any one of the constituents of eclecpane root possesses greater value than the mixed product; probably this would meet all the requirements of clinical experiment.

The above research was carried out under the direction and at the cost of the Therapeutic Committee of this Association. I owe my very best thanks to Dr. Lauder Brunton, F.R.S., who has throughout given me very valuable suggestions and help, and in whose laboratory at St. Bartholomew's Hospital the experiments were mostly carried out.

THE PATHOLOGY OF TUBERCULOUS MENINGITIS WITH REFERENCE TO ITS TREATMENT BY TAPPING THE SUBARACHNOID SPACE OF THE SPINAL CORD.

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In view of the recent procedure of tapping the subarachnoid space of the spinal cord in cases of tuberculous meningitis, the pathology of the disease derives quite a new importance. The prospect of relief by surgical interference in these cases depends on the relation which increased intraventricular pressure bears to other pathological changes and to the symptoms, and whether, from the condition found after death, there seems a fair chance of our being able to relieve this pressure by tapping the subarachnoid space of the cord.

In trying to answer these questions I have used my notes of fourteen cases of meningitis observed during the last few years, and I have prepared a table showing the symptoms and corresponding pathological changes present in ten of the cases. In one case I did not make the *post-mortem* examination, but as I observed the case during life and carefully examined the brain after death, I have included it among the ten. Nearly all the cases are tuberculous, only three are non-tuberculous; these I have added for comparison. For most valuable information which will aid us in this inquiry we are indebted to Dr. Gee's thorough observations on the pathology of the disease, made many years ago.

First, then, we must consider the pathological effects of the increased intraventricular effusion, and we must satisfactorily answer the question as to the patency or otherwise of the communication between the fourth ventricle and the subarachnoid space. Pathologically we find that together with the excess of fluid in the ventricles there is marked softening of the white matter in and around them. Is this softening due to the pressure of the excess of fluid or not? In considering this question we may, I think, exclude the theory that it is merely due to soaking of the fluid into the brain tissue after death. The fact that we find the whole of the corpus callosum so often softened when its anterior part must lie above the ventricles, even in the horizontal position of the body, seems to me to disprove this theory.¹ And, further, the observations of Dr. Bastian have shown that this softened tissue has a diminished specific gravity, and contains pathological cells. Putting on one side this theory then, we have to consider whether the softening is due to the pressure of excessive intraventricular fluid, or to inflammatory changes in the brain tissue.

That the tuberculous disease penetrates much farther into

¹ In one case of tuberculous meningitis in which I made the *post-mortem* examination, the only part softened was the corpus callosum; the parts at the base and posterior portion of the brain were not at all softened.

the brain than might be supposed we know from an observation made by Dr. Gee. He mentions a case where the capillary vessels throughout one hemisphere were everywhere studded by miliary tubercles. They remained after the softened cerebral tissue had been washed away. If, then, tubercles and granulation cells, as described by Dr. Bastian, are found in this softened tissue, and we remember the intimate relation existing between the vessels of the pia mater and the brain, I think we shall be right in considering that in this softening we have some inflammatory action and not a mere pressure effect. Dr. Gee thinks inflammation of the lining membrane of the ventricle is enough to cause serous effusion into the brain tissue, the softening being rendered complete, as he expresses it, "by the malnutrition which ensues in consequence of the more or less arrested capillary circulation."

But in all the cases of tuberculous meningitis which I have examined there has not been the slightest increase in vascularity in the softened tissue, which seems to me to show that inflammatory changes are not sufficiently marked in themselves to cause the softening, but that the pressure of the excessive fluid has much to do with it. In the chronic condition of excess of fluid—chronic hydrocephalus—it is true we have expansion and thinning rather than softening; but the gradual effect of the pressure in these cases may, I think, explain the difference, just as we have impaired mental power rather than coma.

Then there is Dr. Bastian's theory of thrombosis in the veins of Galen as a cause of this softening. I have often carefully examined for this thrombosis and have never found it. Dr. Gee says: "The blood may be made to flow from the veins of Galen backwards into the straight veins with perfect ease, and the plexuses are often quite pale and nearly empty."

If, then, the excess of fluid causes softening around, one would expect tapping the subarachnoid space to relieve the symptoms, supposing the ventricles still communicate with it. In making *post-mortem* examinations in cases of tuberculous meningitis, I have again and again tried to find out the condition of the openings in the pia mater closing in the fourth ventricle, but invariably have I found it so torn in removal of the brain that my observations were of no value. The foramen of Magendie is not the only opening that must be blocked in order to shut off the subarachnoid space from the ventricles. There are two others, one on each side, behind the upper roots of the glosso-pharyngeal nerve in the pouch-like extension of the membrane beneath the flocculus.

In only three out of ten cases is there a note that the membrane closing in the ventricle was thickened, but I should say not so altered as to block all the openings.

Dr. Gee says: "I have several times examined the cerebro-spinal opening *in situ*, and have always found the membrane about it perfectly natural."

Again, on this point, which is a pathological one, we have distinct clinical evidence. In one of the four cases in which the subarachnoid space was tapped, reported by Dr. Wynter, the immediate change in the condition of the pupils was so marked as to lead me to think the pressure in the ventricles must have been relieved by tapping the subarachnoid space of the cord.

There are, however, some cases which seem especially to favour the view that the excess of fluid is a mere obstructive accumulation. I refer to cases where we find the ventricles very distended with fluid, and very little evidence of meningitis, cases which gave rise to the old name of acute hydrocephalus. The fluid is quite clear, and its excess is the one thing we notice *post mortem*; but with so little lymph (and in these cases we find rather opacity of membrane than lymph), it is quite unlikely the foramen of Magendie and other openings into the fourth ventricle are blocked, but rather that there is an inflammatory change in the ependyma of the ventricles (hardly recognisable except by the microscope), which has caused the excess of intraventricular fluid; and it is this inflammatory condition of the pia mater forming the choroid plexus that is doubtless the cause of the excess of fluid in all cases. It may be clear, as in a pleurisy with effusion, but often it is turbid and sometimes distinctly reddened—conditions which show its inflammatory origin.

Now we come to a very important part of the subject, and