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INVESTIGATIONS ON THE PREVENTION OI NUISANCES ARISING FROM FLIES ANI PUTREFACTION[1].

BY F. W. FOREMAN, M.A., F.I.C.
AND G. S. GRAHAM-SMITH, M.D.

(With Plates I—V, four Text-figures and three Charts.)

CONTENTS.

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Introduction[1].

In the first part of this paper we summarize the series of preliminary experiments and observations which led us to consider that coal-tar creosote oil, alone or combined with other reagents, would prove of great use for a variety of purposes, including the prevention of putrefaction in exposed bodies, the deodorization of putrefying carcases, the destruction of fly maggots in animal refuse and manure, and the prevention of nuisances caused by flies.

In Part II we record the results of our investigations in regard to certain phenomena, such as the production of gas and odours, the exudation of fluid and chemical changes in the tissues, which precede or accompany the disintegration of the principal constituents of the

[1] These investigations were aided by a grant from the Local Government Board.

body under various conditions. The importance of these phenomena and the chief factors in their causation are discussed, and the means at our disposal for estimating their effects. The mode of entry into the tissues of putrefactive organisms, and their relationship to the changes which occur in carcases, the effects of treatment of the skin and of injection of various reagents into the blood vessels are considered. Finally we summarize our views on the series of changes which occur in exposed bodies, and suggest methods by which the nuisances arising from them can be mitigated.

In Part III we consider the actions of various coal-tar oils and their constituents on maggots, and the results of treating the carcases of small and moderate sized animals exposed in the open.

In Part IV we record the results of the use of creosote oil mixtures, either in experiments devised to simulate as closely as possible conditions likely to be encountered in Public Health practice, or in warfare.

To those who are concerned with the practical application of methods for dealing with the nuisances caused by flies and putrefaction Parts III and IV of this paper may be of some value. We trust that those who are specially interested in the processes of putrefaction and the problems in connection with them will find in Part II data, which will suggest useful lines of research.

Our investigations were undertaken with the express purpose of discovering easy and practicable means for mitigating the various nuisances arising from exposed animal matter, and in pursuing this object we were compelled for lack of time to abandon, often at a most interesting stage, several series of experiments designed to throw further light on the more striking phenomena observed in decomposing carcases. Those observations and experiments which illustrate these striking phenomena, or indicate the difficulties which have to be surmounted in dealing with them, or explain the reasons, which led us to suggest the use of certain methods have been quoted at some length. Other experiments which, though of considerable scientific interest, seemed to be less intimately associated with the practical aspects of the question we have not described. The latter experiments deal with the actions of dilute acids and other reagents on bacteria, and the chemical substances in the tissues which certain species attack.

Part I. Preliminary Investigations.

The Destruction of Adult Flies.

In the early summer of 1915 we carried out a series of careful experiments in the hope of finding some method of destroying adult flies with the aid of such reagents as might be safely used in houses or by troops in the field. No satisfactory substance at once cheap, easily obtained, non-poisonous and attractive to flies was found, and we soon came to realize that even if such a substance was found its use would have little effect, except as a temporary measure, in diminishing the fly nuisance. Suitable screens together with the use of repellents would probably be more efficient than food or vapours, poisonous to flies, in keeping dwellings free from them. Since house flies seem to have a restricted range of flight and their numbers are determined by the amount of attractive material available as food for the larvae the attack should be made on the larvae, which are confined as in a trap in their breeding places, instead of allowing the adults to emerge and then attempting to destroy them. By efficient treatment of the breeding places we might hope to diminish the numbers in succeeding generations to a material extent, while the destruction of a small proportion of the adults could have little or no effect in this respect, for though fewer eggs would be deposited the competition amongst the larvae would be decreased, and the numbers emerging in the next generation would not be affected. No traps or reagents could act as efficiently as winter in destroying the adults, yet if food is available for the larvae and other conditions are favourable the swarms of adults are as numerous as ever during the succeeding year.

Owing to these considerations our attention was directed mainly to means for destroying maggots in carcases and so preventing the swarms of those species of flies whose larvae feed on such materials, but before passing to this aspect of the subject it may be of interest to quote some of our preliminary experiments with the adults.

Flies are susceptible to poisons absorbed (1) from the alimentary canal and (2) as vapours through the respiratory apparatus.

(1) *Poisons absorbed from the alimentary canal.*

The habits of flies render it impossible to observe with accuracy the effects of reagents taken by the mouth, if experiments are conducted with many individuals at a time. Some may feed well; others feed

badly or not at all; some vomit the material they have swallowed; others retain it; members of some species are more susceptible than those of others. We used various common species of flies and each experiment was conducted with a single, apparently healthy individual, so that we could ascertain the extent to which it fed, note the effects after various intervals of time, and watch the symptoms for hours without the possibility of confusion, certain to occur when several individuals are confined in one cage. Whenever any symptoms of toxic action were noticed the experiment was repeated. Our experience has shown that prolonged observations are essential since on the one hand flies which appear to be dead often recover after remaining motionless for hours, and on the other hand flies, which are apparently not affected for some time, die subsequently.

In Table I we have classified as far as possible the drugs used according to their chemical relationship.

TABLE I.

Showing the effects of various reagents on flies when taken by the mouth.

Acids.	
Sulphuric acid 1 %	Slack some hours later.
Ortho-phosphoric acid 1 % ...	No effect for 24 hours.
Meta-phosphoric acid 1 % ...	„ „
Carbolic acid (pure phenol) 1 % ...	Ill in 30 minutes. Recovered later.
Tannic acid	No effect in 24 hours.
Bases.	
Soda N/10	No effect in 24 hours.
Aniline saturated aqueous solution (about 3 %) + equal volume of syrup	The fly becomes ill at once. It turns on to its back, buzzes violently for a few seconds, and then lies still with occasional quivering of the legs and wings. This phase lasts about 20 minutes. If sufficient is taken death invariably follows. Probably the symptoms are due partially to effects on the respiratory system. Numerous experiments were made.
Aniline hydrochloride 2 % ...	Effects similar to those of aniline.
Monomethylaniline + 1 drop HCl	Appeared to be dead in 10 minutes; recovered in two hours.
„ „ (watery extract) (only slightly soluble)	No effect.
Toluidine Ortho- + 1 drop HCl ...	Effects similar to those of aniline.
„ Para- + „ „ ...	No effect.
„ „ (aqueous extract)	„ „
Zylidine (25 % in water emulsified with a trace of bile)	Dead in three minutes.

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Zylidine (1 % with sugar) ...	*C. erythrocephala* no effect. *L. caesar* ill, but re covered.
Nicotine 1 % (Commercial) ...	Flies fall over on their sides while drinking and ceas to move in a few seconds, except for twitchings.
„ 1 % on sugar	No effect.
„ 1 % sprayed on liver ...	Soon became ill, but recovered.
Hydroxylamine hydrochloride 2 %	Little, if any, effect.
Metaphenylene diamine 1 % ...	No effect.

Salts.

Strontium chloride 1 %	One fly defaecated 16 times in 45 minutes, an became very weak. Others suffered from dia rhoea, and appeared to be dead in 30 minutes.
Barium chloride 1 %	No effect within a few hours, but the flies die within 24 hours.
Barium sulphide (emulsion in water)	Died in five hours.
Copper chloride 0·2 %	No effect in 24 hours.
Manganese chloride	Flies dislike it, and it appears to possess som toxicity.
„ „ 1 % in syrup	Died in three hours.
Zinc chloride 1 %	Flies fed well, but soon vomited. Otherwise un affected.
Sodium nitrite 2·5 %	Soon became ill, and died within 24 hours.
Sodium hyposulphite	No effect.
Ferric chloride 1·5 % in 5 % alcohol	Died within 24 hours.
Potassium cyanide 1 %	No effect.
Tartar emetic (strong solution) ...	No effect in 40 minutes, died in 24 hours.

Miscellaneous.

Naphthalene, sugar and water ...	Very ill in 30 minutes, but recovered.
Iodine in Potassium iodide (dilute)	No effect.
Liver of sulphur	„
Saponin 1 %	„
Formalin 2 % Aniline 5 % Bile 0·12 %	Very ill in a few minutes, and appeared to be dea in 15 minutes.
Aniline 5 % Phenol 2 % Bile 0·5 %	Very ill in a few minutes, and appeared to be dea in 15 minutes.

This table exemplifies that substances toxic to animals are not necessarily toxic to flies.

Finding aniline to possess toxic properties we persevered to some extent with experiments on adult flies with bases of the aniline family to see if toxicity could be associated in any way with chemical constitution. Orthotoluidine apparently possesses the same toxicity as aniline, but paratoluidine, in the form of its soluble hydrochloride, seems to be non-toxic. The transposition of the CH_3 group into the

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para position appears to eliminate the toxicity. We had no metatoluidine at the time. The toxicity was not increased by introducing the CH_3 group into the NH_2 group of the aniline. Monomethylaniline for example was not more toxic.

(2) *Poisons absorbed as vapours through the respiratory passages.*

Many volatile organic substances appear to affect the fly through its respiratory passages, especially when they are brought into contact with the exterior of the insect. Some of these, such as ether and chloroform in moderate doses, produce temporary anaesthesia, while others produce effects which are fatal. The rapidity with which effects are produced in an enclosed space appears to depend upon the rate of evaporation. When the fluid comes into actual contact with the fly, the effect may be almost instantaneous. This is especially the case with those fluids which are capable of spreading, and entering the spiracles. We found that certain volatile organic bases of the aromatic series, such as aniline, pyridine, and such substances as crude naphtha, coal-tar oils, terpenes and volatile organic acids produced rapidly fatal effects, if brought into contact with the flies, even in minute doses.

THE DESTRUCTION OF EGGS AND LARVAE.

For reasons given in the preceding section we next turned our attention to means for destroying maggots especially in carcases.

We were so impressed with the toxic action of aniline upon flies that we decided to try its effect upon eggs, larvae and pupae, since it was cheap and easily obtainable in large quantities.

Some of our preliminary experiments, of which three are quoted, showed that very dilute emulsions of aniline rapidly killed eggs and maggots.

(1) Masses of fresh blow-fly eggs were placed on two pieces of liver and the pieces were then sprayed with 1 % nicotine solution and saturated aniline (3 %) water respectively. In the first case the maggots hatched and devoured the liver. In the second the eggs failed to hatch.

(2) 24 full grown maggots were placed in a small quantity of bran moistened with a little aniline water. All were dead, brown and soft in a few hours.

(3) No flies emerged in seven weeks from a mixed lot of fly pupae sprayed with a little aqueous solution of aniline. Controls moistened with the same quantity of water emerged in a normal manner.

After many similar experiments, which gave practically identical results, we proceeded to treat the carcases of small animals exposed in the open. It soon became evident that treatment with an emulsion of aniline, or a solution of one of its salts, would kill any maggots already present on the carcase, and prevent the hatching of any eggs that might be deposited within several days of the treatment, but to accomplish this the whole surface of the body, including the natural orifices, had to be thoroughly wetted with the solution.

After numerous experiments on the bodies of such animals as guinea-pigs, rats and rabbits we thought the efficiency of the solution might be enhanced by an increase of the strength of the aniline, and the inclusion of a substance which possessed a toxicity of a different character. To increase the proportion of the aniline over 3 % it became necessary to make up the fluid in the form of an emulsion. This we accomplished by the addition of 2 % soft soap or 0·5 % ox bile[1]. The latter substance has the advantage of causing the fluid to spread quickly and evenly over the skin and among the hair roots, and facilitates penetration. As a second toxic agent we chose carbolic acid, which differs from aniline in being acid in character and at the same time does not form a stable salt of aniline in such a solution. We hoped in this way to increase the efficiency of the solution. We compared solutions of the soluble salts of aniline, such as the hydrochloride and acetate, with 5 % emulsions of aniline and found that though these salts were toxic they did not give such good results as the free aniline. As however penetration into the skin of the carcase would be assisted by a soluble salt we arranged that our fluid should contain one part of the aniline in the form of aniline acetate and four in the free state to exercise its undoubted repellent action on flies.

Finally a fluid of the following composition, which we have termed "Solution A," was prepared:

Solution A.

Aniline	50 c.c.
Glacial acetic acid	6·6 c.c.
Phenol	5 grms.
Bile	5 c.c.
Soft soap	20 grms.
Water up to	1000 c.c.

[1] We believe that ox bile might be used with considerable advantage for the preparation of insecticides and fungicides intended for application to rough surfaces.

In this solution the acetic acid is added in order to convert one-fifth of the aniline into the form of its acetate, the soft soap to produce an emulsion and the bile to assist diffusion and penetration.

Before treating carcases with this fluid we exposed them until maggots were present, and in many cases waited until the maggots were full grown, especially in the natural openings of the body. Then sufficient only of the fluid was sprinkled over the surface to completely wet the coat after roughly rubbing it in with the fingers. Finally a little of the fluid was poured into the mouth, nostrils, eyes, anus and genitalia. In some cases the carcases were opened and the thoracic and abdominal organs exposed. When this was done some of the fluid was poured into the thoracic and abdominal cavities. A guinea-pig weighing 400–500 grms. received about 100 c.c. It should be noted here that the smaller the body the greater is the ratio of surface to weight, and consequently the amount of fluid required for the treatment of a small carcase is greater per unit of weight than for a larger one. The nature of the coat makes an appreciable difference in the quantity required, the woolly coat of the rabbit absorbing much more than the short coat of the guinea-pig. The presence of bile in the fluid so facilitates the spread and penetration of the fluid that the amount necessary to thoroughly wet the coat and skin is greatly reduced, and the differences due to coats of varying character minimised.

Experiments with solutions containing aniline.

It seems unnecessary to quote in detail the very numerous experiments we carried out and our reasons for undertaking them, and therefore we have been content to summarise briefly the steps involved in the evolution of "Solution A." We feel, however, that it is desirable to describe a few experiments exemplifying our methods of observation, and the results obtained with "Solution A," and modifications of it.

The carcases were laid on the ground, in some cases exposed in the open, in others protected from the sun and rain in such a manner that the flies had free access to them. Each carcase was thoroughly examined every day, in some cases for a period of six weeks, and the results recorded. In each series of experiments an untreated carcase was included as a control, and examined daily with the others.

On 9 July an unopened carcase of a guinea-pig, not previously exposed to flies, was treated with an emulsion containing aniline 5 % and soft soap 5 %. It was examined daily till 14 August, and neither eggs nor larvae were seen on it at any time. On this date the carcase was opened and found to be moderately decomposed. When

re-examined on 3 Sept., it was found to be much decomposed, and many maggots were present under the skin. *Note.* The carcase remained free from eggs and maggots for five weeks, but when untreated surfaces were exposed eggs were deposited and maggots developed.

Similar results were obtained with a rabbit treated in the same way at the same time.

Controls were reduced to skeletons within a week.

Aniline in conjunction with other reagents.

The experiments quoted below were carried out synchronously on the carcases of guinea-pigs, which had been opened and exposed for 48 hours, and contained innumerable maggots.

I. 100 c.c. of the following solution was applied. Aniline 5 c.c., nitrobenzene 1 c.c., bile 0·25 c.c., water up to 100 c.c. By the next day all the maggots were dead. Two masses of eggs were deposited on the second day. No maggots were found until the 16th day, when a few were present in the mouth. By the 21st day the body was much decomposed.

II. 70 c.c. of the following solution was applied. Aniline 5 c.c., formalin (40 %) 2 c.c., bile 0·2 c.c., water up to 100 c.c. After 24 hours some of the maggots were still alive, although many were dead. On the 2nd day a few maggots were still alive in the mouth. On the 21st day the remains of the carcase were much decomposed, but no maggots or eggs were seen.

III. 70 c.c. of an aqueous solution of aniline acetate corresponding to 5 % aniline were applied. By the next day all the maggots were dead, and none were seen subsequently on the carcase. By the 21st day the carcase was somewhat decomposed but not to the same extent as I or II.

IV. 70 c.c. of a 4 % solution of acetic acid were applied as a control to III. The maggots were unaffected, and the carcase was completely eaten on the 5th day.

V. 100 c.c. of the following mixture was applied. Aniline 5 c.c., glacial acetic acid 0·66 c.c., bile 0·5 c.c., phenol 0·5 c.c., water up to 100 c.c. Next day all the maggots were dead. Some eggs were deposited on the 9th day. On the 21st day no maggots were seen and the carcase was much decomposed. On the 28th day many maggots were found in the coat between the skin and the ground.

VI. On the same day the carcase of another guinea-pig in the same condition was treated with 100 c.c. of "Solution A," which only differs from the above in containing 2 % soft soap. Next day all the maggots were dead. On the 10th day a batch of eggs were laid. No maggots hatched up to the 28th day and the body was hard and appeared mummified. Later some maggots were found between the skin and the ground, and some of these subsequently attacked the carcase.

We found in various experiments that 0·5—2·0 % phenol together with 0·5 % bile had little, or no, effect upon maggots feeding in a carcase. Nevertheless other experiments led us to believe that when present with aniline in a mixture the toxicity of the fluid and its antiseptic properties were increased.

Thinking that it might be desirable to ascertain the extent to which the aniline penetrated into the tissues after application to the skin in the usual manner we carried out the following experiment. An unopened carcase of a guinea-pig with maggots in the mouth and on the coat was treated with 100 c.c. of "Solution A" (without soft soap), and left on the ground for three days. The skin was then carefully removed in order to avoid contamination of the underlying tissues, and the (*a*) skin, (*b*) lungs and stomach, (*c*) abdominal organs, (*d*) the remainder of the carcase, and (*e*) the soil underlying and immediately surrounding the carcase to a depth of three inches were separately placed in flasks with water, and distilled with steam. Three successive fractions in each case were titrated with bromine water standardised against pure aniline hydrochloride. The following results were obtained:

(*a*)	Skin	0·347 grms.
(*b*)	Lungs and stomach ...	0·039 ,,
(*c*)	Abdominal organs	0·082 ,,
(*d*)	Remainder of carcase ...	0·420 ,,
(*e*)	Soil	1·049 ,,
		1·937 ,,

In spite of the fact that only two-fifths of the aniline was accounted for the experiment demonstrates that some absorption into the tissues takes place. Some was probably lost by evaporation and some by diffusion into the surrounding soil, and doubtless more would have been obtained if the tissues had been ground with sand.

The experiments we have quoted show the usual results obtained by treatment with such a fluid as "Solution A" during at least three weeks, but we must emphasize that to obtain such results a thorough treatment is necessary since the maggots may gain entrance at an untreated place. Further the atmospheric conditions seem to influence the results. Hot and sunny weather tends to mummify the bodies, and rain to letch away the soluble constituents of the fluid and to hasten putrefaction. Given favourable conditions the fluid was efficient, even when diluted with twice its volume of water. Sometimes maggots present in the cavities were killed before they could reach the surface, in which case they soon turned brown. Those not instantly rendered immobile came out, but invariably died either on the carcase or in its vicinity, though in some cases death was delayed for hours.

At this stage we had achieved a considerable measure of success in the purpose, the killing of maggots in carcases, for which the

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experiments had been devised, but throughout the progress of this part of the work we felt that much more could be done in mitigating the nuisances arising from flies and putrefaction if means could be devised for arresting, or at least materially diminishing, putrefactive changes, and for repelling flies from decaying carcases. Several competent observers, who closely followed our work, expressed the same opinion. From this point onwards our attention was directed to the study of putrefaction with this aim in view, and we have published the foregoing pages mainly to illustrate the stages through which our researches passed until we found in the creosote oil mixture, we have called "Solution C," a fluid which combines together with the capacity for destroying maggots other useful functions of great importance in preventing nuisances arising from exposed carcases. Nevertheless "Solution A" might be of use under exceptional circumstances when it was desired to prevent flies from breeding in carcases without appreciably checking the progress of putrefaction. Here we should like to point out that the burial of carcases containing eggs or maggots does not prevent the subsequent emergence of the flies, for the maggots continue to develop and when full fed make their way towards the surface of the ground where they pupate (Graham-Smith, 1916, p. 503). It is therefore advisable to treat all carcases with some maggot-destroying fluid before burial.

Early Attempts to check Putrefaction.

"Solution A," which contains two antiseptic agents in phenol and aniline, when applied to the skin appeared to check putrefaction to a slight extent, and we proceeded to determine whether the addition to it of larger quantities of phenol and other antiseptics would check putrefaction still further. We therefore experimented with the following variations of "Solution A," which we called "Solutions B 1 and B 2."

"Solution B 1" consists of "Solution A" with 1 % of crude carbolic acid added. This solution showed no advantage over "Solution A" in several experiments carried out with carcases of puppies, rabbits, etc.

"Solution B 2" consists of "Solution A" with from 1 to 3 % of bone oil added.

The carcases of three guinea-pigs with the abdominal organs exposed and containing numerous maggots were each treated with 100 c.c. of "Solution B 2" containing 1, 2 and 3 % respectively of bone oil. In each case nearly all the maggots were killed with great rapidity. During a month's observation neither eggs nor

maggots were seen on these bodies. The bodies were protected from the rain, and on all their exposed surfaces a crust formed below which putrefaction proceeded. With such small bodies we found difficulty in comparing the odours from different carcases, but we believe that bone oil tends to diminish the putrefactive stench. We also observed in these and in several similar experiments that flies were less inclined to visit these carcases than others.

Bone oil contains various organic bases including aniline, pyridine and its homologues as well as various nitriles, hydrocarbons, etc. It therefore contains several substances which exercise repellent action on flies and are toxic to maggots.

A number of experiments with other disinfectants indicated that putrefaction could not be checked to any material extent by the application of aqueous solutions to the skin. This is hardly surprising since rain water or water arising by capillarity from the ground dilutes the fluid and letches away both the disinfectant and those products of bacterial activity which tend to check putrefaction. As we will show later the presence of water and a favourable temperature are the most potent factors in accelerating putrefactive processes. We entertain little doubt that on the one hand any agent which softens the skin aids the maggots in gaining entrance into the body, and on the other hand any agent which hardens the skin tends to prevent their entrance and retards the liquefaction of the superficial tissues, a change which seems to be correlated with the admission of water and air.

Internal combined with external treatment.

Seeing that surface applications with aqueous solutions were of little value in retarding putrefaction, we injected disinfectants both into the serous cavities and into the blood vessels in the hope of evenly distributing the antiseptics throughout the tissues and thus checking the action of putrefactive bacteria. One series of experiments may be quoted:

Four rabbits, treated 24 hours after death, were exposed in the open. The weather became wet and unfavourable.

I. Weight 7 lbs. 4 c.c. crude carbolic acid containing aniline 2 % and bile 1 % were injected into the thoracic cavity and 4 c.c. into the abdominal cavity. 3 c.c. of "Solution B 1" were injected subcutaneously at different situations, and the surface was treated as usual with 300 c.c. of "Solution B 1." By the 5th day the body was becoming distended with gas. By the 8th the hair was coming off everywhere, and numerous eggs were present on the back. On the 9th day thousands of small maggots were present under the fur. On the 12th day the body was much decomposed, and on the 21st day horribly putrid.

II. Weight 5 lbs. The abdominal cavity was opened. Proportionately to its weight this carcase was treated in the same manner as I. On the 8th day the abdominal wound was green, the hair was coming off and there was some odour. On the 9th day thousands of small maggots were present under the hair of the back. On the 21st day putrefaction was less advanced than in I, but a horrible stench arose when the body was disturbed.

III. Weight 5 lbs. The blood vessels were injected through the carotid artery with 3 c.c. of crude carbolic acid containing aniline 2 % and bile 1 %. The surface treatment was the same as for II. On the 8th day the skin was green. On the 12th day the under surface was very putrid, and there were many small maggots present. By the 21st day the maggots were very numerous.

IV. Weight 5 lbs. The blood vessels were injected through the carotid artery with 50 c.c. of 5 % formalin, and the surface treated in the same manner as I. On the 7th day a few maggots were present. By the 12th day numerous maggots of all sizes were working actively. On the 16th day the body was much decomposed. On the 21st day it was so putrid that the legs came off at the slightest touch and the stench was horrible.

These experiments illustrate the effects of rain in decreasing the efficiency of aqueous solutions applied to the surface in checking the development of maggots.

We concluded from many such experiments that satisfactory results could not be obtained from injections of disinfectants, if means were not taken to cut off the access of water to the carcase from all sources, rain, dew, humid atmosphere, soil saturated with water, and water rising by capillarity from an unsaturated soil. In seeking the best way of achieving this purpose we conceived the idea of applying over the whole surface of the carcase a film of some insoluble oily substance. As a natural consequence of this decision it occurred to us that the oil should be made a vehicle for applying agents dissolved in it possessing fly deterrent, maggot destroying, deodorising and antiseptic properties. Now substances identical with, or similar in nature to, those we have already shown to possess some of these properties are present in coal-tar distillates, which constitute the cheapest substances obtainable possessing the desired oily character. We were influenced in our selection by the following important considerations: the fluid should have (*a*) a high percentage of disinfectants, (*b*) a low rate of evaporation in order to ensure that the film should remain operative as long as possible, (*c*) a high flash point so as to avoid danger from fire, (*d*) no unpleasant smell, and (*e*) no undesirable poisonous properties. After some preliminary experiments with several coal-tar products we concluded that creosote oils would be the most suitable for our purpose.

Creosote oils from different sources show very considerable variations in composition, depending upon the kind of tar, the method of preparation, and the extent to which they are contaminated with other products. In the best managed works the creosote oil is uniform in character, while "in some works every residue which cannot be used for any other purpose finds its way into the creosote oil well."

The following varieties of creosote oil can be distinguished:

Coal tar creosote oil "London make"...	4— 7 % tar acids
,, ,, "Country make"	14—18 % ,,
Blast furnace tar creosote oil	20—35 % ,,
Water gas tar creosote oil	practically no tar acids.

Though the blast furnace tar creosote oil contains the highest percentage of tar acids it appears to be unsuitable in other respects, being a thin liquid lighter than water which evaporates faster than the coal-tar creosote oils, and lacks oilyness. We chose therefore a trustworthy country make which we found on analysis to contain tar acids 13·85 %, bases 3·94 %, the remainder consisting of oily hydrocarbons, traces of water, etc.

Treatment with creosote oil has enabled us not only to suggest methods of practical value, but also to study putrefactive processes in a manner previously impossible. During the progress of our earlier experiments we felt the need of satisfactory standards when attempting to compare the results obtained by various methods. Written descriptions of the appearances noted are necessarily vague and lacking in precision and the element of personal bias, which cannot be eliminated, leads to the introduction of considerable errors. Moreover the manifestations of putrefaction vary both in degree and kind in carcases kept under similar conditions, and to a far greater extent in carcases kept under different conditions. We therefore decided to devote some attention to the study of the more important phenomena which accompany putrefaction. These researches were carried on partly concurrently with attempts to find practical methods for dealing with decomposing carcases, and partly after these methods had been devised and tested in the field.

For descriptive purposes we felt it would be best to separate the more scientific from the more practical aspects of the work, and we decided to deal first with the former in Part II. By this arrangement we hope the procedures adopted and the conclusions arrived at in Parts III and IV will be rendered more easily intelligible.

Those, however, who desire to follow the more practical part of the work in the order in which it was carried out may prefer to pass immediately to Parts III and IV in which it is described.

Conclusions.

1. Attacks on the adult fly are not likely to produce appreciable effects on the numbers in succeeding generations.
2. In order to diminish the fly nuisance the eggs and larvae should be destroyed in the breeding places, where they are confined as in traps.
3. Watery emulsions or solutions of larvicides, if properly employed, kill eggs and larvae in carcases, but soon lose their efficiency in the presence of water.
4. Larvicides of an oily nature retain their potency for long periods and are, therefore, the most suitable agents to employ.

Part II. Investigations on Putrefaction.

In order to obtain some knowledge of the early stages of putrefaction and the factors which influence them we made careful observations on the bodies of small animals. Some of these were kept indoors in a dry atmosphere at temperatures ranging between 60 and 80° F., others in a moist atmosphere at 26·5° C., others were placed outside on the ground covered to protect them from sun and rain, others on the ground without protection, and others exposed and frequently wetted. Daily observations were made and notes taken, and some of the carcases were dissected at different stages. All were dissected when it was thought that the observations had been carried on sufficiently long. Most of these experiments were carried out in the summer and early autumn.

External Manifestations of Putrefaction.

The first signs of putrefaction usually noticed in the carcases of small animals are distension of the abdomen and protrusion of the anus. The body soon assumes a cylindrical shape owing to the extension of the gas throughout the subcutaneous tissues. The period during which distension remains at its maximum depends upon external conditions, principally temperature and moisture.

During the period of distension a clear yellowish fluid exudes and raises the hair and superficial epidermis from the deeper layers of the skin over an area on the left side, approximately corresponding to the cardiac end of the stomach. On removing the hair and superficial epidermis over this area the follicles appear as pits in the smooth, moist, deeper layer of the skin. From the patch described the process usually extends over the abdomen and later over the whole area of the skin, resulting in the loosening of the hair everywhere. The distension gradually subsides and concurrently large quantities of reddish fluid escape from the body, and the odours of putrefaction become more evident. The source and nature of these odours will be discussed later (p. 156). After this the skin desquamates and the soft tissues of the carcase gradually liquefy, if sufficient moisture is present.

In the early stages of distension the skin especially over the abdominal area exhibits a bluish green discoloration. It has been suggested that this discoloration is due to the action of sulphuretted hydrogen, one of the earliest gases to be evolved, upon the iron-containing pigments in the blood. The sulphuretted hydrogen probably arises from free cystine. We may point out that many organisms produce green colonies on blood agar plates when carbohydrates are present. Ruediger (1906) suggests that this is caused by the action of organic acids produced from the carbohydrates on the red corpuscles or haemoglobin. The discoloration rapidly extends over the whole surface of the carcase, and throughout this stage numerous organisms are found in the green tissues.

The influence of maggots on carcases.

Fly maggots, besides eating up the carcases, may in the meantime exert great influence in other ways. Young maggots seem to be able to live on the surface of the skin upon the excluded fluid, and may reach a large size apparently without any other food. When maggots gain entrance into the carcase they assist in the distribution of the bacteria and ferments in the muscular tissues. If, as we believe, the skin is more important than the alimentary canal as a source of putrefactive organisms this action of the maggots would tend to hasten putrefaction in the muscles. On the other hand the opening up of the carcase tends to introduce aerobic conditions. We have made no attempt to investigate the extent to which the presence of maggots influences putrefaction.

Internal Appearances.

The stomach and the liver are the first organs to show decided changes. A red-purple patch of discoloration soon appears over the cardiac end of the stomach, and this area becomes so soft that the organ ruptures upon the slightest manipulation. Eventually in many cases this area of the stomach wall becomes completely dissolved. A corresponding patch appears on the parietal peritoneum and the colour extends through the abdominal wall. The fluid which exudes from the body first escapes from this area.

The liver soon becomes soft, and at an early stage the formation of gas results in a honeycomb condition, which is sometimes confined to the superficial lobes. At this stage the organ floats in water, although much of the gas is discharged into the peritoneal cavity. Finally the gas escapes leaving the liver thin and flat. The spleen and kidneys become soft, but their substance exhibits no evidence of gas formation. The intestinal walls seldom rupture, until a late stage in decomposition. Changes occur slowly in the thoracic organs. The muscles exhibit their characteristic colour and consistency long after visible changes have occurred in the other organs. The muscles retain the red-pink colour if anaerobic conditions prevail, but become gray when exposed to the air, possibly through oxidative changes. They also lose their firm consistency, first becoming soft and easily detached from the bones and later diffluent. The change in consistency becomes evident earlier in the softer muscles, such as the psoas.

When a carcase which has reached the point of maximum distension with gas is opened large quantities escape from the peritoneal cavity. Gas is usually found in the intestines, the degree of distension probably depending upon the kind and amount of food eaten some hours previous to death. Separate large pockets of gas occur in the retroperitoneal tissues, and gas is found between the layers of muscle and in the subcutaneous tissues, producing subcutaneous emphysema.

In most carcases extensive oedema of the subcutaneous and other tissues occurs. The conditions found on the dissection under water of the body of a guinea-pig which had reached this stage are given below.

When a perforation was made in the skin of the shoulder about 10 c.c. of gas escaped. The gas was evidently in the interstices of the subcutaneous tissue, since more could be forced out by pressure. About 40–50 c.c. came out of the peritoneum, and the whole intestine was considerably distended with gas. Its mucous membrane seemed to be destroyed in most places. Much gas was present in the pleural cavities.

After opening the peritoneal cavity gas was still present in the subcutaneous and retroperitoneal tissues. It was also present in the intermuscular connective tissue of the limbs. No gas was found in the kidney which sank in water. The liver was soft and less bulky than normal, and showed large numbers of small gas vesicles. It floated in water. After the intestines and stomach had been removed and all the larger and smaller cavities containing gas opened the carcase still floated in water.

GAS PRODUCTION IN THE CARCASES OF SMALL ANIMALS.

Method of estimation.

The animals were killed and the carcases, supported on glass pedestals about two inches in height, so as to keep the bodies above any fluids which might drain from them, were placed head downwards in wide mouthed bottles (*A*) fitted with air-tight rubber bungs, pierced for delivery tubes. The bottles were arranged in a constant-level water bath (*B*) kept at a uniform temperature of 26·5° C. by means of a regulator. A delivery tube (*C*) connected each of these bottles with another bottle (*D*) of 800 c.c. capacity acting as a receiver. The receiver was fitted with a rubber cork with three perforations. The delivery tube (*C*), which was cut off level with the bottom of the cork, passed through one. Through another a tube (*E*) passed and was prolonged for one inch below the bottom of the cork. The exposed end of this tube was fitted with a glass tap, and beyond this with a piece of rubber tubing (*F*), by means of which it could be attached to a gas measuring burette. The extension of the tube below the cork was for the purpose of leaving a gas cushion, so as to ensure against water being sucked backwards through the delivery tube into the bottle (*A*), when the atmospheric pressure rose. The third opening admitted a siphon (*G*) passing from the bottom of the receiver and connected to an aspirator (*H*). Twelve such systems were connected with the same aspirator, thus reducing as far as possible the absorption of gases by the water, and rendering the conditions comparable. After all the connections had been made and sufficient time had elapsed for a temperature equilibrium to be established the glass tap was opened, and the aspirator raised so as to permit the water in each receiver to rise to the lower ends of the tubes (*E*). The taps were then closed and the aspirator lowered until the level of the water in it was three inches lower than in the receivers. The tube (*E*) was now connected with the measuring burette and the level of the water in each receiver exactly adjusted. The systems were finally tested during some hours for leakage by observing whether

the water kept at the same level in the receivers. It will be observed that there was a slight negative pressure in the receivers until they became half full of gas.

Every day the measuring burette filled with water was connected with the tube (*E*) the bulb lowered and the gas withdrawn until the water had risen to its original level, and the collected gas measured. Owing to the great volumes of gas produced during the 3rd, 4th and 5th

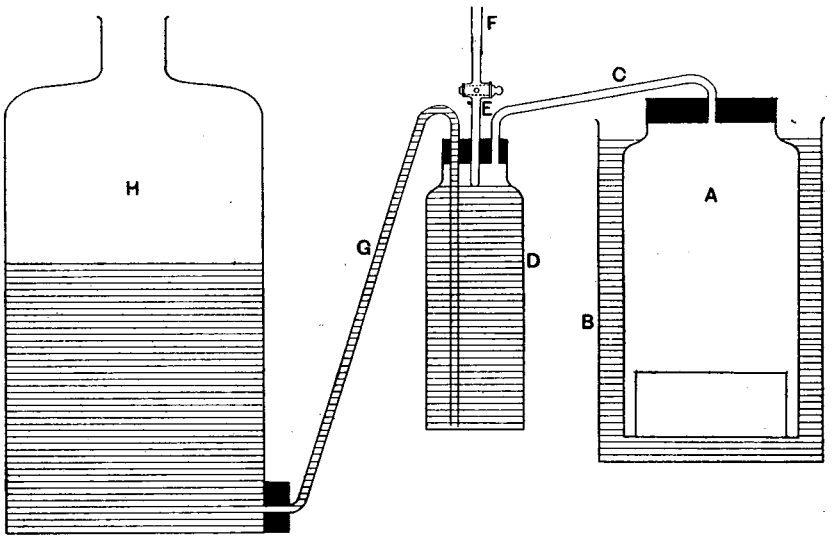

Fig. 1. Diagram of apparatus for collecting and measuring the gas evolved from small carcases.

A. Bottle containing the carcase. *B*. Constant-level water bath. *C*. Delivery tube passing to the receiver *D*. *E*. Tube through which the gas passes into the measuring burette. *F*. Rubber tube for attaching the gas measuring burette. *G*. Siphon passing to the aspirator *H*.

Twelve such systems were attached to the aspirator, but only one is shown in the diagram.

days two measurements had to be made on these days. The volumes measured were not corrected for temperature and pressure as the systems were under the same conditions and the experiments were all comparable. Each series of observations were continued for three weeks.

At first the conditions in the bottle (*A*) were aerobic, but became anaerobic when all the air had been expelled. The atmosphere was probably saturated with water vapour.

We fully realise that the conditions in these bottles differed widely from those prevailing outside.

When a small carcase is exposed outside in summer time its temperature is higher by day than by night. In the day time the bacteria which prefer a high temperature tend to increase, and during the night those which prefer lower temperatures. Profound changes result in relation to such conditions as the proportions of various species of bacteria present at different times and their products, symbiotic relationships, the consequences of bacterial antagonism, etc., and affect the order, rate and degree of putrefactive processes. The drying of the surface in warm weather, the action of sunlight, the aerobic conditions prevailing at any rate externally and absorption by the ground of exuded fluids containing products of bacterial action, which if allowed to accumulate would check bacterial growth, exert influences difficult to estimate. In large carcases these factors probably play a less important part.

While the method we have described fails to simulate natural conditions with precision it affords a very stringent test of the action of antiseptics, since all the circumstances are very favourable for rapid putrefaction.

Results of experiments for estimating the gas produced in small carcases.

Primarily our experiments were designed to show the rate of gas formation in small carcases, untreated or treated in various ways, and in some of the more important organs. We carried out three series of experiments and quote the results of two in detail.

In series I, quoted in Table II, an intact carcase (*A*) was compared with one in which the abdominal cavity had been opened (*B*), with one from which the blood had been drained by opening the large vessels of the neck (*C*), with one from which the stomach and intestines had been removed (*D*), with one from which the liver, stomach and intestines had been removed (*E*) and one from which these organs and the skin had been removed (*F*). When removing the organs the greatest care was taken to avoid gross contamination with their contents by employing double ligatures and dividing the tissues between them with a cautery. Also in the case of the body from which the skin was removed precautions were taken to avoid undue contamination by removing as much as possible of the hair by plucking, and singeing off the remainder before any incision was made. The liver, stomach and intestines (*G*) removed with as little disturbance as possible were placed in a bottle, a stomach and intestines (*H*) in another, and a stomach and intestines (*I*) opened in several places in a third.

TABLE II.

Showing the volumes of gas produced daily from the carcases of small animals. (Series I.)

Days	Atmospheric pressure in mm.	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
1	733	52·3	39·3	136·1	41·6	35·8	16·6	39·4	41·6	106·1
2	736	47·7	6·7	413·1	539·6	48·0	228·4	121·0	49·1	104·9
3	747	63·4	537·5	477·5	811·9	405·1	531·8	97·0	66·9	53·9
4	746	172·7	511·4	421·9	375·6	212·8	238·9	26·9	66·5	23·7
6	755	458·5	186·0	357·0	176·8	249·9	216·5	13·2	54·5	0
7	758	451·8	115·0	471·5	123·4	144·4	114·0	19·1	33·6	0
8	752	220·9	75·1	120·3	60·7	69·1	60·7	92·8	26·2	0
9	747	238·1	95·4	163·7	113·0	80·1	72·0	49·4	30·0	0
10	763	105·1	27·7	25·0	45·7	20·1	18·8	16·5	7·5	0
11	766	238·7	47·0	78·4	67·2	45·2	30·3	32·5	14·9	0
12	764	210·1	48·3	42·5	47·6	36·9	33·2	34·0	9·1	0
13	769	197·3	31·1	22·3	33·5	19·4	19·2	25·7	0	0
14	769	142·6	49·8	41·6	46·5	40·2	24·3	46·5	9·2	0
15	755	142·0	47·6	46·9	38·7	36·9	33·1	55·7	8·2	0
16	766	41·5	20·2	0	0	0	0	37·2	0	0
17	774	40·0	25·3	1·9	9·4	5·6	0	38·1	0	0
18	773	68·6	49·6	15·8	19·1	20·8	9·9	25·3	0	0
19	767	65·4	58·0	32·9	33·1	26·6	35·4	20·5	0	0
20	769	33·1	31·4	11·2	10·1	9·4	8·2	3·6	0	0
21	758	69·2	65·8	32·7	32·3	27·2	25·6	14·7	0	0
22	770	12·2	22·7	0	0	0	0	0	0	0
Total gas produced		3071·2	2090·9	3112·3	2625·8	1533·5	1716·9	809·2	417·3	288·6
Weight of body in grms.		348	331	311	252	321	172	98	100	107
Gas per grm.		8·82	6·32	10·02	7·91	4·78	9·98	8·26	4·17	2·69

Another series (II) of experiments were carried out in order to ascertain what differences in gas production, if any, would be brought about by treatment with antiseptics. An intact carcase (*J*), one in which the abdominal cavity had been opened (*K*), one from which the blood had been allowed to drain (*L*) and one from which the skin, liver, stomach and intestines had been removed (*M*) acted as controls. With these were compared a carcase treated externally with 10 c.c. of creosote oil (*N*) and one similarly treated with an equivalent quantity of 5 % aqueous emulsion of cresols in 1 % soft soap (*O*), a carcase injected through the carotid artery with 5·75 c.c. of creosote oil (*P*), and one injected with an equivalent volume of the cresols' emulsion (*Q*) and carcases with the abdominal cavity opened and the surfaces of the abdominal organs and skin treated. The skin of one (*R*) was treated with 12·6 c.c. of creosote oil and the peritoneal surfaces with 5·2 c.c.,

and the other (*S*) was treated with equivalent volumes of the cresols' emulsion. A liver (*T*) alone was placed in a bottle. The results of these experiments are given in Table III.

TABLE III.

Showing the volumes of gas produced daily from the bodies of small animals. (Series II.)

Days	Atmospheric pressure in mm.	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*
1	750	56·0	66·0	54·2	15·4	67·3	82·6	107·5	92·7	75·3	66·8	2·2
2	758	410·4	92·9	207·6	259·1	139·6	88·5	101·8	102·5	65·5	58·9	60·9
3	767	382·9	235·7	862·7	388·3	503·0	202·2	200·7	437·4	227·7	85·2	92·3
4	766	169·8	687·4	493·1	190·8	303·4	422·0	657·2	569·7	398·8	367·0	27·9
5	765	109·4	394·0	310·2	91·9	157·0	267·6	366·9	282·1	168·0	242·0	2·2
6	765	109·1	245·9	231·7	57·7	102·0	107·2	252·5	170·3	100·5	197·9	0
7	759	105·5	179·3	173·8	43·3	74·3	80·0	177·3	149·5	81·7	204·1	0
8	755	84·8	96·3	102·2	32·3	54·0	96·2	161·5	116·8	71·6	161·8	0
9	750	73·0	94·7	101·5	34·1	57·8	78·5	166·5	142·6	75·4	136·2	0
10	746	55·2	93·6	92·1	36·5	42·9	52·8	130·7	91·4	69·1	109·1	0
11	751	39·4	47·9	58·4	27·6	32·2	31·6	92·9	60·4	54·5	67·9	0
12	746	44·9	69·8	72·5	33·2	42·2	49·5	158·7	99·9	83·7	86·5	0
13	745	37·4	56·7	63·5	22·8	32·9	32·5	120·0	70·5	57·1	60·8	0
14	744	37·0	46·7	44·6	20·2	23·5	23·9	76·4	60·0	43·4	56·1	0
15	746	32·2	46·9	44·0	19·1	24·0	24·6	100·4	64·8	50·0	55·5	0
16	757	18·2	34·4	29·2	4·0	4·3	6·5	50·9	30·3	18·9	31·2	0
17	760	13·6	13·1	16·1	0	−5·1	−3·1	29·7	17·2	6·5	21·5	0
18	757	21·1	19·3	28·2	18·6	24·1	33·7	61·8	46·7	35·7	44·2	0
19	757	20·1	15·6	32·1	9·5	9·4	16·6	58·6	49·9	25·6	37·4	0
20	753	20·6	15·1	31·5	4·0	8·0	9·0	51·6	43·4	19·3	35·8	0
21	761	16·9	12·4	40·2	5·8	8·2	0	48·5	37·2	16·7	34·0	0
Total gas produced		1857·5	2563·7	3089·4	1314·2	1710·1	1705·5	3172·1	2735·3	1745·0	2160·1	185·5
Weight of body in grms		272	305	295	147	284	202	382	316	387	380	17
Gas per grm.		6·83	8·4	10·47	8·93	6·02	8·44	8·30	8·66	4·51	5·69	10·91

It will be seen that gas was produced under all the circumstances investigated and from all the organs. Great quantities were evolved during the first week, and in most cases considerable quantities continued to be evolved during the next two weeks.

Further analysis of the figures given in Tables II and III revealed some interesting facts, the importance of which can be estimated only in conjunction with the results of the examination of the carcases at end of three weeks. We proceed therefore to describe the conditions found on examining the bodies used in the second series of experiments.

Dissections of bodies used in the second gas experiment.

The carcases were removed from the bottles and examined according to a definite procedure. The following notes summarize the results of these examinations. In each case some of the fluid, which had drained from the body, and a portion of the thigh muscle were reserved for chemical analysis.

J. Intact carcase. Original weight 272 grms. 86 c.c. of red fluid had exuded. *Hair* loose everywhere. *Skin* gray and pliable. *Lungs* shrivelled; float in water. *Liver* hardly recognizable, lying like a piece of crumpled wash leather on the stomach; floats in water; sections show it to consist of a honeycomb of connective tissue. *Intestines* dark greenish-brown; rupture very easily; impossible to separate the coils. *Muscles* very soft, and pink in colour. *Smell* unbearable. *Remarks.* The carcase was supported with the head *upwards* on stones in such a manner that none of the fluid which exuded remained in contact with it. In consequence the carcase was much better preserved than other untreated bodies in the series as they were not altogether removed from the influence of the fluids by the glass supports on which they rested.

K. Abdominal cavity opened. Weight 305 grms. At least 50 c.c. of clear red fluid had exuded. *Hair* very loose everywhere. *Remarks.* The body had collapsed and part of it was immersed in the fluid. Those portions of the carcase which were immersed were semi-solid and unrecognizable, while in the remainder the muscles were just recognizable. The general condition of the carcase was such that it fell to pieces. *Smell* intolerable, with a suggestion of acetamide.

L. Bled. Weight 295 grms. At least 30 c.c. of fluid had exuded. *Hair* very loose everywhere. *Skin* very putrid. *Lungs* and *heart* completely disintegrated. *Liver* flat as in *J.* *Stomach* and *large intestine* unrecognizable. *Small intestine* recognizable and not easily torn. *Muscles* disintegrated. *Smell* intolerable, with a suggestion of acetamide. *Remarks.* This carcase was in a very advanced stage of decomposition.

M. Skin, liver, stomach and intestines removed. Original weight of body 265 grms. Portions removed weighed 118 grms. Remains placed in bottle weighed 147 grms. At least 20 c.c. of fluid had exuded. *Remarks.* The carcase was in such a condition that it could be stirred easily with a glass rod, only the bones, fibrous tissue and muscle aponeuroses being recognizable. *Smell* intolerable.

N. Skin treated with creosote oil. Weight 284 grms. 53 c.c. of red, almost odourless fluid had exuded. *Hair* slightly loose over the back and abdomen, but elsewhere nearly as firmly attached as in life. *Heart* and *lungs* soft. *Liver* moderately soft, flat and honeycombed. *Kidney* soft. *Intestines* shrunken, tough and contain no gas. *Muscles* resemble fresh muscle in appearance, colour and consistency. Some small gas pockets in the retroperitoneal tissues. *Remarks.* There was very little appearance of decomposition in this carcase.

O. Skin treated with cresols' emulsion. Weight 202 grms. 45 c.c. of brown fluid had exuded. *Hair* very loose everywhere. *Skin* soft, moist and greenish

gray. *Heart* and *lungs* soft but recognizable. *Liver* very soft, flat and honeycombed. *Kidney* soft but recognizable. *Intestines* full of gas bubbles, but retain their shape. *Muscles* very soft and putrid looking. Pockets of gas in retroperitoneal tissues. *Smell* intolerable. *Remarks.* The carcase had a very putrid appearance.

P. Injected with creosote oil. Weight 382 grms. *Smell* intolerable. *Remarks.* The carcase was in such a condition that it could be stirred with a glass rod. Except *Q* the worst specimen in the series.

Q. Injected with cresols' emulsion. Weight 316 grms. About 46 c.c. of fluid had exuded. *Remarks.* The body was in the same condition as the last.

R. Skin and peritoneal surfaces treated with creosote oil. Weight 387 grms. 60 c.c. of fluid had exuded. *Hair* firmly attached everywhere. *Skin* tough and leathery. *Heart* and *lungs* shrunken and tough. *Liver* flat, and floats in water. *Kidney* shrunken but not soft. *Intestines* shrunken and tough. *Muscles* pale pink and resembling fresh muscle in shape, size and consistency. *Smell* none, except that of reagent. Some gas pockets in the retroperitoneal tissue. *Remarks.* This carcase was extraordinarily well preserved.

S. Skin and peritoneal surfaces treated with cresols' emulsion. 45 c.c. of fluid had exuded. *Hair* loose everywhere. *Skin* very soft and detached from the underlying muscles by gas. *Heart* and *lungs* soft but recognizable. *Liver* very soft, flat and honeycombed. *Kidney* soft, but recognizable. *Intestines* contain gas bubbles and are moderately well preserved. *Muscles* very soft and strip easily from the bones. *Smell* horrible. *Remarks.* This carcase is a little better preserved than *J.*

According to these examinations the carcases may be classified into well preserved and putrid.

TABLE IV.

			Gas per grm.
Well preserved	*R.*	Skin and peritoneal surfaces treated with creosote oil...	4·51 c.c.
	N.	Skin treated with creosote oil	6·02 ,,
Putrid	*S.*	Skin and peritoneal surfaces treated with 5 % cresols ...	5·69 ,,
	J.	Intact carcase	7·82 ,,
Very putrid	*K.*	Abdominal cavity opened	7·36 ,,
	L.	Bled	10·24 ,,
	M.	Skin and abdominal organs removed	9·45 ,,
	O.	Skin treated with 5 % cresols	8·44 ,,
	P.	Injected with creosote oil	8·3 ,,
	Q.	Injected with 5 % cresols	8·66 ,,

Results of experiments on gas production. Untreated carcases.

In Table V we have given the total quantity of gas produced per unit weight in three weeks in three series of experiments, and the proportion produced during the first week.

TABLE V.

Showing the total quantity of gas produced by organs and treated and untreated bodies per unit weight in three weeks, and the proportion produced in the first week.

	c.c. of gas per grm.				Percentage of the total gas given off during the first 7 days			
	Series I	II	III*	Mean	I	II	III*	Mean
Organs.								
Liver	—	11·28	13·0	12·14	—	100	100	100
Liver, stomach + intestines	8·26	—	8·04	8·15	50·6	—	100	75·3
Intestines opened... ...	2·75	—	6·55	4·65	98·2	—	70·8	84·5
Stomach + intestines ...	4·17	—	4·3	4·23	81·1	—	66·3	73·7
Untreated carcases.								
Bled	10·02	10·47	—	10·24	83·4	75·5	—	79·4
Stomach + intestines removed	10·42	—	—	10·42	81·1	—	—	81·1
Skin and organs removed	9·98	8·93	—	9·45	91·9	79·5	—	85·7
Intact	8·82	6·83	—	7·82	47·8	72·5	—	60·1
Abdomen opened	6·32	8·4	—	7·36	70·4	74·2	—	72·3
Liver, stomach, intestines removed...	6·12	6·88	—	6·5	80·5	—	75·9	78·2
Treated carcases.								
Injected "5 % cresols" ...	—	8·66	—	—	—	65·9	—	—
„ creosote oil ...	—	8·3	—	—	—	58·8	—	—
Skin "5 % cresols" ...	—	8·44	—	—	—	73·3	—	—
„ creosote oil	—	6·02	—	—	—	78·7	—	—
„ + peritoneum "5 % cresols"	—	5·69	—	—	—	56·6	—	—
„ „ creosote oil	—	4·51	—	—	—	64·0	—	—

* The experiments of series III have not been quoted elsewhere.

It will be noticed that in nearly all instances in which two experiments were carried out with the same materials the results were similar. The exceptions include the experiments with the intestines opened in regard to the total quantity of gas produced, and with the liver, stomach and intestines in regard to the rate of production. Differences in the nature and amount of food consumed before death would be sufficient to account for these discrepancies. The other notable exception is the difference in the rates of production exhibited by the intact carcases. The conditions in these two experiments, though apparently similar, differed fundamentally. In the experiment in series I the carcase had dropped to the bottom of the bottle, and was immersed in the fluid which exuded from it, while in the experiment of series II it was supported clear of the fluid.

Considering first the abdominal organs it will be seen that the liver shows the highest gas production (12·14 c.c.) per unit weight, and that

the whole of the gas is produced during the first five days of the experiment. The stomach and intestines yielded much smaller quantities (4·24 c.c.). The liver, stomach and intestines, removed with as little disturbance as possible, gave twice as much gas (8·15 c.c.) as the stomach and intestines alone. The gas produced from the liver in this experiment would only account for about one-quarter of the increase. From this experiment and from others to be given later we are of opinion that the apposition of these organs results in direct communication of organisms and ferments, which conduces to greater gas formation.

If we turn to the untreated carcases all became putrid and yielded large quantities of gas per unit weight, the largest being given by those from which the most blood escaped during the manipulations. Possibly this result was due to the removal of a part of the bactericidal substances, which are present in the blood. The removal of the liver together with the intestines resulted in a decrease in the evolution of gas.

Origin of the gas.

Before considering the results of treatment we may with advantage discuss the origin of the gas, which is produced at various times. In the liver relatively large quantities of carbohydrates are accumulated. Apart from the liver, the blood and tissues as well as the contents of the alimentary canal contain carbohydrates. Glycogen, which is present as a reserve food material in the organs, especially in the liver and muscles, is rapidly hydrolysed to dextrose in the body after death. The liver of such an animal as the guinea-pig becomes glycogen free in a very short time. Many species of intestinal organisms produce gas very rapidly from carbohydrates in cultures, and as we will show later such organisms are in a position to attack the carbohydrates found in the body.

Carbohydrate material would also become available from the gluco-proteins, the prosthetic group of which is easily split off by hydrolysis, and from the nucleic acids, depending upon the rate of action of the autolytic ferments.

The figures given in Tables II and III indicate that the daily production of gas is largest on the 2nd, 3rd, 4th and 5th days, when control observations show only slight visible evidence of muscle degeneration. We therefore consider that in the early stages the gas arises mainly as the result of the action of organisms on the carbohydrate material. The fact that the liver yields the whole of its gas within four days supports this view. The early production of sulphuretted hydrogen

indicates however that other constituents of the body, such as cystine, may be attacked to some extent in the first few days.

We consider that the gas evolved during the later periods is due chiefly to the action of organisms upon the degeneration products of the principal nitrogenous constituents.

An early high yield of gas may bear little, or no, relation to putrefaction as evidenced by dissection and chemical analysis (p. 152) at the end of three weeks, but a total high yield indicates considerable changes.

The results of experiments on gas production. Treated carcases.

Now turning to the treated carcases we find that all those treated with the cresols' emulsion were much decomposed at the end of three weeks, and, with the exception of one in which the peritoneal surfaces were treated, yielded large total volumes of gas.

The application of the antiseptic to the peritoneal surfaces may affect gas production in two ways, by interrupting the direct passage of organisms through the intestinal walls to the surrounding organs and by destroying organisms subjected to its influence, or possibly by partly inhibiting their zymogenic functions.

Injection into the blood vessels of small quantities of 5 % cresols' emulsion or of creosote oil gave poor results perhaps partly owing to the accumulation of these fluids in the larger vessels and partly to the fixing of the phenolic constituents by albuminous substances. Larger injections might have given better results (p. 202). On the other hand treatment of the skin with creosote oil and especially application to the peritoneal surfaces combined with skin treatment gave excellent results with small total yields of gas, 6·02 c.c. and 4·51 c.c. per unit weight respectively. The former procedure, while not interfering with the carbohydrate-fermenting intestinal organisms, prevents the invasion of putrefactive organisms from the skin. We find, as might be expected, a high rate of early gas formation. Subsequent examination reveals the intestines to be tough, indicating that little putrefaction has occurred, and dry owing to the draining away of the fluids. The treatment of the peritoneal surfaces with creosote oil acts more efficiently than treatment with 5 % cresols' emulsion, and, combined with the checking of the invasion from the skin, gives astonishingly good results.

These experiments together with those quoted in Part III lead us to think that the putrefactive bacteria invade the tissues mainly from the exterior, and that the bacteria responsible for early carbohydrate

fermentation are mainly intestinal in origin. The sodden area of skin through which the fluid first exudes probably constitutes the chief early portal of entry of the organisms present in the skin.

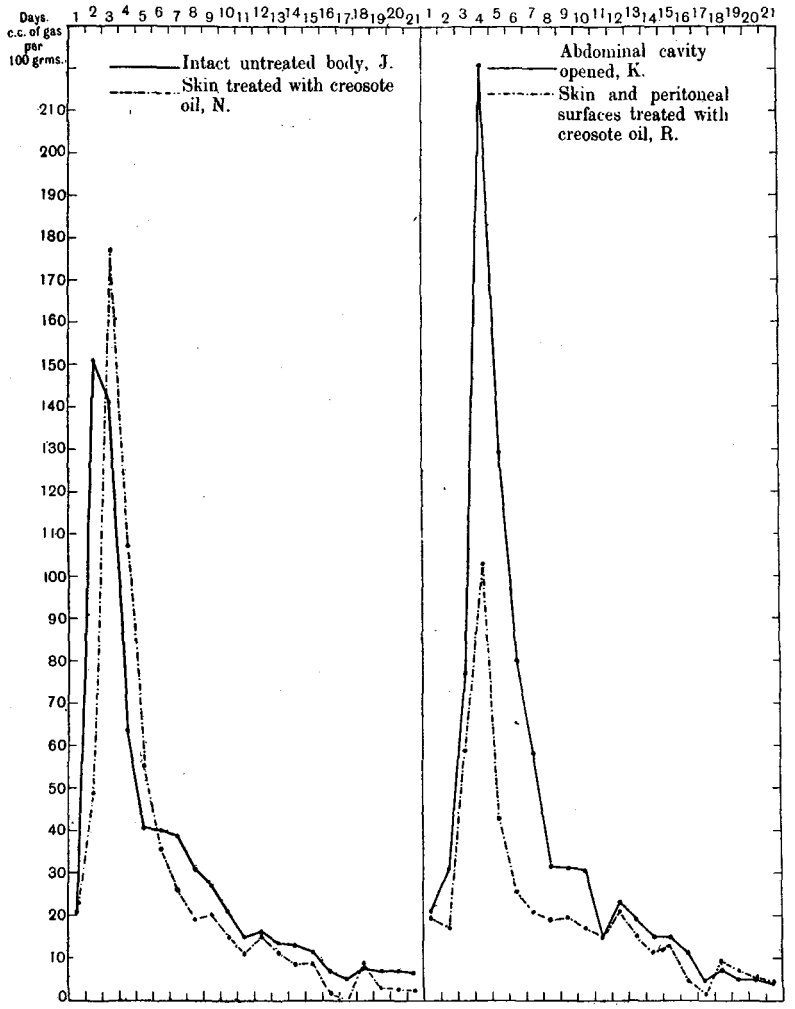

Chart 1. Showing daily production of gas per 100 grms. in the intact carcase (*J*) compared with the carcase treated externally with creosote oil (*N*), and in the carcase with the abdominal cavity opened (*K*) compared with the carcase with the skin and peritoneal surfaces treated with creosote oil (*R*).

In Chart I it will be seen that the effect of the skin treatment with creosote oil upon the initial voluminous gas formation is negligible,

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but the skin-treated body gave smaller daily yields of gas in the later stages. On the other hand treatment of the skin and peritoneal surfaces with creosote oil resulted in a great decrease in the initial gas production, which confirms our view that the organisms responsible for this early gas production emanate from the intestines. Again the treated body gave smaller daily yields of gas in the later stages.

The Distribution of Intestinal Bacteria in the Organs during Life and after Death.

Our views in regard to the distribution of intestinal organisms in the tissues are based on certain carefully conducted experiments in which the opportunities for accidental contamination were reduced as far as possible. The method adopted for removing and crushing portions of organs, under sterile conditions, were those devised and used by Cobbett and Graham-Smith (1910, p. 6) in their investigation of Grouse Disease. They found that bacteria of intestinal origin were rarely present in the livers and other organs of grouse unless the coeca have been injured by the presence of large numbers of worms, *T. pergracilis*. Our experiments on the organs of such animals as guinea-pigs, dogs, rats and pigeons show that various organisms occur constantly in cultures from the lungs, but are seldom found in cultures from the other organs, with the exception of the mesenteric lymph glands. In these *B. coli* and other bacteria sometimes occur. In cultures from the liver bacteria of intestinal origin as well as others are occasionally found. Cultures on agar from the livers of 16 out of 29 healthy guinea-pigs remained sterile, 6 showed one or two colonies of *B. subtilis*, 5 one or two colonies of cocci, and 1 two colonies and 1 several colonies of *B. coli*. Cultures from the livers of 5 dogs remained sterile. Cultures from the livers of 6 out of 8 rats remained sterile, 1 yielded two colonies of *B. subtilis* and 1 several colonies of *B. coli*. Cultures from the livers of 10 out of 11 pigeons remained sterile, while the other showed two colonies of *B. coli*. In spite of the fact that cultures on agar made with moderate quantities of liver tissue generally yield negative results we think that intestinal bacteria not infrequently gain entrance into the liver during life, and that positive results would be more frequently obtained if larger masses of the tissue and special media were used in cultivation. This view is borne out by the fact that large portions of liver tissue taken out of the body with every precaution and placed in sterile vessels rarely remain sterile, while portions of other organs, such as the kidney and spleen, usually do remain sterile.

Soon after death intestinal bacteria are found in considerable numbers in the liver and other organs. Cultures from the livers of guinea-pigs, rats and pigeons, made 24 hours after death, almost invariably produced numerous colonies of coli-like organisms.

According to Harden (1901) *B. coli* and allied organisms produce carbon-dioxide, hydrogen and nitrogen in varying quantities from glucose in the presence of peptone.

CHEMICAL CHANGES IN THE MUSCLES AND ORGANS.

As putrefaction proceeds the muscles lose their characteristic colour becoming pale pink, and later gray. They also lose their firm consistency, first becoming soft and easily detached from the bones, and later diffluent. The organs exhibit similar changes but at a more rapid rate than the muscles. Each of the various stages through which the muscles and organs pass is more or less recognizable with experience, but without a special nomenclature is difficult to describe in such a manner as to convey an intelligible picture to the reader.

It was found practically impossible to compare the disinfecting powers of different disinfectants with any degree of certainty by means of such methods. The possibilities of error were very great, and for purposes of comparison the results were lacking in precision. The elaboration of a new method, involving if possible definite figure comparisons, was therefore very desirable. We have obtained such figures by a method which enables us to estimate the amount of certain products present at any stage of the putrefaction.

The method is based upon the idea we entertained that the activity of putrefactive organisms depends upon the rapidity with which proteins and other complex organic substances present in the animal body are broken down into simpler substances, the latter constituting the real food of the organisms. Proteins for example are broken down into amino acids by means of proteolytic enzymes found in the organs and tissues of a body after death, and it can be easily shown that under suitable conditions putrefactive organisms destroy amino acids obtained in a tryptic digest with great rapidity. The ultimate nitrogenous products are ammonia or substituted ammonias of the volatile type. It is very difficult to conceive that organisms can break up the protein molecule without the assistance of proteolytic ferments, and we are inclined to the view that the organisms produce their own proteolytic ferments when they destroy enzyme-free protein. The more rapid

production of these bases in a tryptic digest may be accounted for if this view is correct. It follows from this that the true test of the power of a disinfectant would be determined by ascertaining to what extent it could prevent organisms from decomposing the amino acids of a tryptic digest. This would be best determined by estimating the proportion of amino acids to volatile bases in a proteolytic digest after incubation for a sufficient time. The enzymes responsible for the autolytic changes in the dead body of an animal are extremely resistant to many substances which kill the bacteria in cultures. In a carcase treated with a strong germicide the proteolytic enzymes of autolysis continue to act and produce amino acids, consequently the amino acids accumulate and chemical examination reveals a high ratio of amino acids to bases. Such a condition may be regarded as proof of the relative absence of bacterial action.

Changes due to autolytic enzymes.

In order to throw some light upon the relative rate of change in the various organs brought about by autolytic ferments after death, weighed portions of organs of a freshly killed dog were finely ground with sand in a mortar and triturated with water until three times as much water had been added. The liquids were then strained through muslin, and portions withdrawn, boiled to destroy their colour and filtered. A 10 c.c. portion of each filtrate was diluted, neutralised to phenol-phthalein, treated with neutral formaldehyde and titrated with N/10 soda according to the method of Sörensen. In order to inhibit bacterial action 1·5 % of toluene was added to the extracts, and they were incubated at 37° C. After certain periods portions were withdrawn, boiled, filtered and treated as before. The pancreas was diluted five times with water and the portions were not boiled before titrating. The following results were obtained:

TABLE VI.

Results of formyl titration in c.c. N/10 soda to neutralise.

	Immediate	1·75 hours	18 hours	24 hours	43 hours
Pancreas	1·2	3·7	9·6	10·7	10·7

	Immediate	17 hours	65 hours
Spleen	0·6	2·4	6·0
Liver	0·8	2·2	3·4
Kidney	0·6	1·8	2·9
Muscle	0·3	0·3	0·6*

* We are in doubt as to this figure, since shortly afterwards the culture was found to be contaminated with bacteria.

Except in muscle, a considerable development of substances containing the amino group in their constitution occurred. As a corresponding increase in acidity accompanied the increase in formyl titration the change may be attributed chiefly to the production of amino acids, the final products of the proteolytic ferments. Any ammonia which may be present is included in this titration, and it should be noted that relatively more ammonia in proportion to amino acids is produced in these digests than is produced by the action of trypsin on a protein. We attribute this higher proportion of ammonia chiefly to the action of the enzymes concerned with the breakdown of nucleic acid in the nucleoproteins and nucleo-histones, which occur in such organs as the pancreas and lymphatic structures. This process results in the production of purine bases, guanine and adenine, either free or in the combined state as nucleosides. These in their turn are deaminised by the deaminases with the production of free ammonia. In the pancreatic digest the fermentation was very considerable and reached its maximum within 24 hours when incubated at 37° C. In another experiment four and a half to five days' incubation at 18° C. were required to reach the same titration value.

We have already noticed how slowly the muscles exhibit putrefactive changes. This fact is due probably to the lack of production of suitable conditions for putrefactive bacteria owing to the exceedingly slow rate of proteolytic change.

In order to ascertain the influence of aerobic and anaerobic conditions the following experiments were carried out. 100 grms. of the pancreas of a bullock, killed at 11·30 a.m., were ground up with sand at 3·0 p.m., and 250 c.c. of water, boiled for ten minutes to expel the dissolved oxygen and then cooled, added. The liquid was then lightly shaken, strained through muslin and 2 % of chloroform added. 5 c.c. portions were formyl titrated immediately, giving readings of 0·5 c.c. N/10 soda, and 10 c.c. portions distributed in wide mouthed test tubes arranged for anaerobic cultivation as described elsewhere (p. 143). A tube *A* with a cotton wool plug was kept as a control, and through the others various gases were passed for 3 minutes, and then the inlet tubes were sealed. The side tubes were filled with boiled, cooled water. Air was passed through tube *B*, oxygen through tube *C*, nitrogen through tube *D*, hydrogen through tube *E*, carbon dioxide through tube *F* and hydrogen sulphide through tube *G*. These tubes were incubated at 37° C., and daily a 5 c.c. portion from one of each series was formyl titrated.

TABLE VII.

Showing the influence of aerobic and anaerobic conditions on autolytic changes in the pancreas. The results are given in c.c. N/10 soda.

		18·5 hours	42 hours	66 hours
A.	Control	1·9	3·0	3·35
B.	Air	1·9	—	3·6
C.	Oxygen	2·35	3·8	3·9
D.	Nitrogen	1·8	3·05	3·6
E.	Hydrogen	1·95	2·6	3·5
F.	Carbon dioxide ...	1·45	2·65	3·45
G.	Hydrogen sulphide	0·7	0·8	1·0

Oxygen increases the initial rate of production of substances responding to formyl titration. Within three days the contents of most of the other tubes had reached about the same level, somewhat below the level reached by the oxygen tube on the second day.

In a few hours the liquid in the hydrogen sulphide tube assumed a green tint, like that developed in the skin of a carcase after a few days' exposure, and it was found impossible to obtain a satisfactory titration without removing the hydrogen sulphide. For this purpose 5 c.c. of the contents of the tube were dried in a vacuum desiccator over potash, and then emulsified in water and titrated. It was found that the rate of action had been greatly retarded. A 5 c.c. portion after 66 hours' incubation was dried in vacuo and then emulsified with water and 2 % chloroform added. This fluid was incubated for 50 hours at 37° C. and titrated. It gave a reading of 1·35 c.c. N/10 soda. The presence of hydrogen sulphide to this extent therefore seems to inhibit enzyme action considerably. Judging by the reduction in titration to phenol-phthalein after drying there was not more than 0·112 % of hydrogen sulphide present.

Possibly the hydrogen sulphide produced by organisms in carcases has some inhibiting influence upon the action of autolytic enzymes.

The action of certain putrefactive organisms in pure culture have been studied to some extent, but the results obtained are of little value in elucidating the problems involved in putrefaction under natural conditions, since the consequences of enzyme action, of symbiotic association and of other important factors are not taken into consideration.

We desired to study the chemistry of putrefactive changes under conditions approximating as nearly as possible to those which occur in nature in order to throw some light on the phenomena we observed, and to help us in our selection of practical means for overcoming the nuisances occasioned by decomposing bodies. For this purpose two series of experiments were made in the following manner.

Methods of estimating chemical changes in cultures.

Series I. Four freshly killed guinea-pigs weighing 1502 grms. were skinned. The bladders and contents were removed, and the carcases

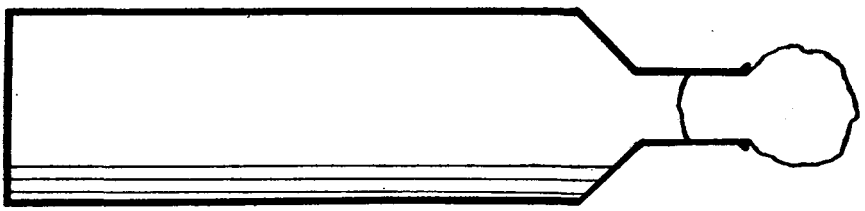

Fig. 2. Apparatus for aerobic cultivation.

and skins passed separately through a mincing machine. The whole of the mince so obtained was passed a second time through the mincing machine, and ground in a mortar. The mince was then thoroughly extracted with successive additions of water, all the fluid strained through muslin and finally passed through flannel by means of a press. Lastly the volume was made up to 1600 c.c. Then the fluid was divided into two equal parts to be cultivated aerobically and anaerobically. 25 c.c. portions were placed in flat medicine bottles loosely plugged with cotton wool (Fig. 2) for aerobic cultures, and 25 c.c. portions in large test tubes for anaerobic cultivation (Fig. 3). The test tubes were provided with air tight bungs pierced with two holes, one of which admitted a tube, passing to the bottom of the test tube, and the other a delivery tube, passing under N/10 hydrochloric acid contained in a second test tube open to the air. Before cultivation hydrogen was passed for 3 minutes through the tube first mentioned, which was sealed off after all the air had been displaced. By this means we permitted gases produced to escape, and provided against any loss of volatile bases.

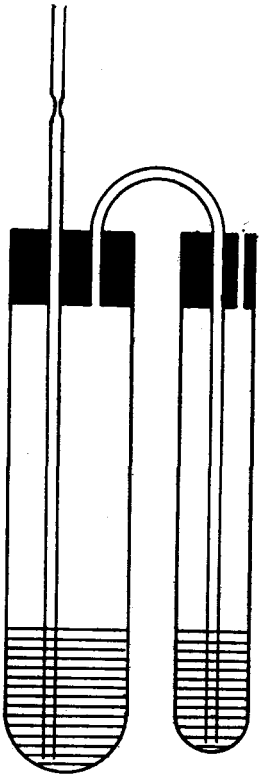

Fig. 3. Apparatus for anaerobic cultivation.

Series II. Since it is generally held that putrefactive organisms gain entrance from

the intestines we decided to make a comparable series of experiments with carcases from which the whole intestinal tract from the oesophagus to the anus, together with the liver, had been removed between ligatures, so as to reduce as far as possible infection from its contents. The material, which consisted of the skins and bodies of six animals, weighing 1500 grms. was treated as previously described and the volume of the fluid made up to 1600 c.c. Both aerobic and anaerobic cultures were made.

Both series of cultures, aerobic and anaerobic, were cultivated at 27° C. and the aerobic bottles were placed on their sides to expose the greatest surface to the air, and were turned over daily.

The nitrogen present in 10 c.c. portions of the fluid first obtained in each series was estimated by Kjeldahl's method.

Series I. 10 c.c. contained 0·0587 grm. nitrogen corresponding to 0·9175 grm. of protein in 25 c.c. of fluid, calculated on the basis of 16 % nitrogen in protein.

Series II. 10 c.c. contained 0·0533 grm. corresponding to 0·832 grm. protein in 25 c.c. of fluid.

25 c.c. of the fluid from each series was taken immediately, 2 c.c. of water added, and made up to 250 c.c. with 97 % alcohol, and was allowed to stand for 24 hours by which means all the proteins, albumoses and peptones were completely precipitated. The precipitate, including the suspended matter, was filtered on tared paper, washed three or four times with 86 % alcohol and dried to constant weight at 100° C. The filtrate containing about 86 % alcohol holds in solution the amino acids and bases. To 100 c.c. of the filtrate excess of cold, saturated baryta solution was added (about 40 c.c.) and the volatile bases distilled over in vacuo at 40° C. into standard acid. The distillate was diluted to 500 c.c. with water and titrated with N/10 soda to methyl orange. The residue in the flask usually measuring about 10 c.c. was acidified whilst warm with hydrochloric acid, filtered and washed with water. To the filtrate, exactly neutralised to phenol-phthalein with soda, neutral formaldehyde was added and the amino acids titrated with N/10 soda according to Sörensen's method.

Every day aerobic and anaerobic cultures of both series were treated in the way just described. The apparatus used is shown in Fig. 4.

The organic acids volatile in steam were estimated in some cases by removing the volatile bases in the way described, acidulating the residue in the flask with dilute sulphuric acid, and distilling with steam and titrating with N/10 soda to phenol-phthalein.

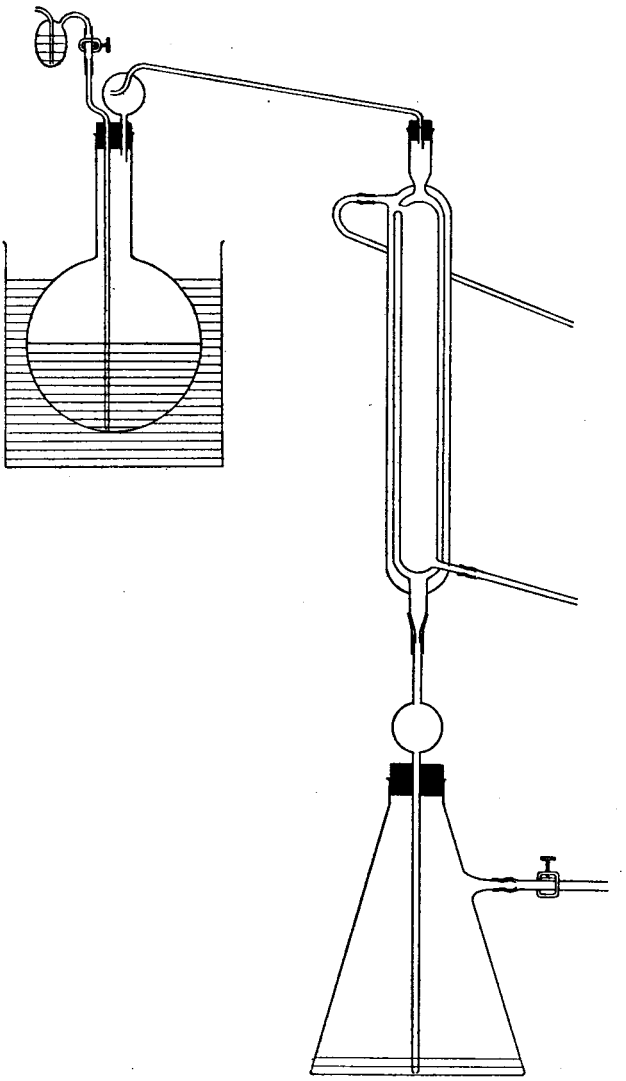

Fig. 4. Apparatus for the estimation of volatile bases.

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TABLE VIII.

Showing the results of the analyses of the cultures. Intestines included.

(Series I.)

	Aerobic				Anaerobic			
	in two-fifths of culture				in two-fifths of culture			
Days	Grms. dry matter insol. in 86% alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids	Grms. dry matter insol. in 86% alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
Orig.	0·9856	2·3	1·75	1·3 : 1	0·9856	2·3	1·75	1·3 : 1
1	0·9220	4·05	1·4	2·9 : 1	0·8310	4·7	3·0	1·6 : 1
2	0·7619	12·85	1·15	11·2 : 1	0·7764	6·2	3·7	1·7 : 1
3	0·6461	18·35	1·3	14·1 : 1	0·7495	8·4	4·15	2·0 : 1
4	0·5857	21·4	1·1	19·4 : 1	0·6259	14·45	4·25	3·4 : 1
5	0·5554	23·9	0·85	28·1 : 1	0·5010	21·0	3·7	5·6 : 1
6	0·5240	25·6	0·5	—	0·5075	—	4·75	—
7	0·5288	25·1	0·35	—	0·4335	22·45	4·3	5·2 : 1
8	0·5244	—	—	—	0·4442	—	—	—
9	0·5186	24·1	0·3	—	0·3905	25·9	4·6	5·2 : 1
10	0·5225	—	—	—	0·3737	—	—	—
11	0·5220	24·35	0·4	—	0·3689	25·5	4·8	5·4 : 1
12	0·5231	—	—	—	0·3309	27·7	—	—
13	0·4915	24·15	0·2	—	0·3165	28·15	4·0	7·0 : 1
14	0·4957	—	—	—	0·3166	—	—	—
15	0·4900	—	—	—	0·3922	—	—	—
16	0·4627	18·8	0·0	—	0·2587	—	—	—
17	0·4712	19·85	0·0	—	0·2614	—	—	—
18	0·4876	—	—	—	0·2857	—	—	—
19	0·4817	—	—	—	0·2781	—	—	—
20	0·4655	—	—	—	0·2580	—	—	—
21	0·4457	15·15	0·0	—	—	—	—	—
22	0·5036	—	—	—	0·2747	—	—	—
23	0·4621	12·1	—	—	0·2508	30·95	—	—
24	0·4622	—	—	—	0·2385	—	—	—

The larger quantity of dry matter in series I is probably accounted for by the inclusion of intestinal contents. In the aerobic cultures of both series only a slight fall in dry matter occurs on the first day, but subsequently the fall is rapid till a more or less constant level, approximately one half of the original, is reached on the sixth day. The disappearance of dry matter is greatest on the second day. This reduction in weight of dry matter is less than the increase in bases represents when calculated into terms of protein at 16 % nitrogen. Since arginine is said to be absent from the products of autolysis the high yield of bases relative to dry matter consumed may be due partly to the rapid action of arginase splitting off urea from the arginine molecule, and the subsequent rapid deamination of the urea.

TABLE IX.

Showing the results of the analyses of the cultures. Intestines excluded.
(Series II.)

	Aerobic				Anaerobic			
	in two-fifths of culture				in two-fifths of culture			
Days	Grms. dry matter insol. in 86 % alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids	Grms. dry matter insol. in 86 % alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
Orig.	0·6802	0·7	1·25	0·56 : 1	0·6802	0·7	1·25	0·56 : 1
1	0·6535	3·4	0·65	5·2 : 1	0·6353	2·1	1·4	1·5 : 1
2	0·5340	7·55	1·2	6·3 : 1	0·5930	5·85	1·5	3·9 : 1
3	0·4574	10·9	0·85	12·8 : 1	0·5529	7·31	1·45	5·0 : 1
4	0·3766	14·15	1·05	13·5 : 1	0·5143	9·3	1·5	6·2 : 1
5	0·3484	15·85	1·0	15·85 : 1	0·4459	13·9	1·25	11·1 : 1
6	0·3262	15·85	1·0	15·85 : 1	0·4063	14·85	1·25	11·9 : 1
7	0·3336	15·8	0·9	17·6 : 1	0·3379	17·25	1·35	12·8 : 1
8	0·3261	14·95	1·05	14·2 : 1	0·3516	—	—	—
9	0·3117	—	—	—	0·3718	15·25	1·25	12·1 : 1
10	0·3281	16·3	0·3	—	0·2837	—	—	—
11	0·3245	16·1	0·15	—	0·2553	20·75	1·55	13·4 : 1
12	0·2935	17·1	0·0	—	0·2572	—	—	—
13	0·3195	—	—	—	0·2942	—	—	—
14	0·2897	—	—	—	0·2741	20·25	1·2	16·8 : 1
15	0·3263	—	—	—	0·2580	—	—	—
16	0·3108	13·65	0·1	—	0·2496	21·5	1·3	16·5 : 1
17	0·2838	13·4	0·0	—	0·2343	—	—	—
18	0·3010	12·4	0·0	—	0·2086	22·75	1·5	16·1 : 1
19	0·2850	12·75	0·05	—	—	—	—	—

In the anaerobic cultures the reduction of dry matter continued and had not reached a constant level at the end of three weeks. By this time about 75 % in series I and 66 % in series II had disappeared. In the anaerobic cultures of series I the highest reduction is on the first day, though the production of bases was only slightly greater than in the aerobic series. On this day the non-nitrogenous constituents, probably the carbohydrates in the intestinal contents, were attacked, as the bases correspond to only 5·4 % nitrogen in the dry matter which has disappeared. In the aerobic cultures of series II, in which the intestinal contents were absent, this phenomenon was not so evident.

In the aerobic and anaerobic cultures of both series the base production corresponded roughly with the disappearance of dry matter. If, however, the amount of nitrogen present in the products be calculated each day as percentage of nitrogen in the corresponding dry matter lost considerable daily variation is shown. On the second and third days in the anaerobic cultures of series I and II a very high figure is

obtained. This suggests the decomposition of bodies such as creatin and urea, which contain high percentages of nitrogen. On the sixth day in both series a very low percentage in the dry matter lost is seen. Such variations might be expected when the complexity of the culture medium is taken into consideration. An estimation of the disappearance of dry matter alone would fail to bring out these points.

In the aerobic cultures of both series the substances which respond to the formyl titration show little change for the first week. In both cases a rapid fall occurs afterwards, and after a fortnight little, if any, of these substances remain. The volatile bases begin to decrease when no amino acids are left. This decrease cannot be accounted for by volatilization during incubation. Under the conditions of these experiments putrefaction seemed to cease about this time. A very great number of factors may be concerned in this phenomenon, but we should like to point out that the activity of the aerobic bacteria especially may depend on the presence of preformed products of autolysis. The disappearance of the bases suggests that when the amino acids have been used organisms seek another source for their nitrogen requirements[1].

In the anaerobic cultures of both series on the other hand putrefaction proceeded throughout the whole period of observation as evidenced by the decrease in dry matter and the increase in bases. In series I the formyl titration increased till the third day and subsequently remained nearly constant, but in series II it remained practically constant throughout, but at a much lower level. Presumably the greater formyl titration in series I is due to the inclusion of the intestinal contents. We have not determined to which of the amino acids the phenomenon of constancy may be attributed, but we have confirmed some of our figures by van Slyke's nitrous acid method.

In order to eliminate the non-spore-bearing organisms 25 c.c. portions of the original fluid used in series II were heated to 80° C. for 20 minutes, and incubated aerobically and anaerobically at 27° C. for 21 days.

TABLE X.

		In two-fifths of culture		
		Dry matter insol. in 86% alcohol	Volatile bases. c.c. N/10 acid neutralised	Formyl titration N/10 soda
Aerobic	...	0·3288	5·8	0·0
Anaerobic	...	0·1537	24·95	1·7

[1] It is interesting to consider these results in relation to the losses of nitrogen which occur in farmyard manure under aerobic conditions.

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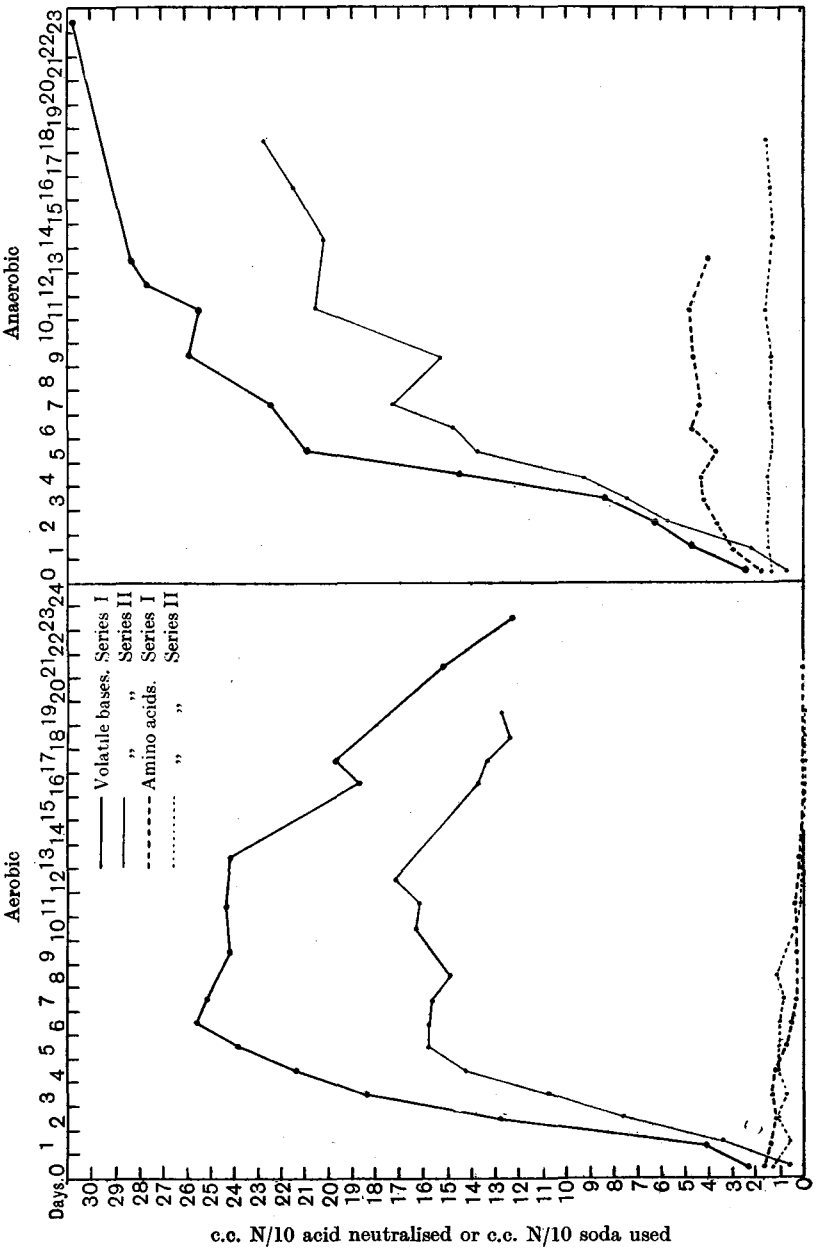

Chart 2. Showing the quantities of volatile bases and amino acids present daily in the aerobic and anaerobic cultures of series I and II.

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In the aerobic culture the disappearance of bases is very evident. Possibly the aerobic spore-bearing organisms are responsible for this disappearance. An anaerobic culture of series II incubated for 21 days would have given similar figures to the culture incubated anaerobically after heating. The inference is that in both the changes have been brought about by anaerobic spore-bearing organisms.

In a preliminary experiment with cultures of series II the following figures for organic acids volatile in steam were obtained, after deducting 5·25 c.c. N/10 soda (due to the presence of carbonate in the soda and baryta used), the figure found in a "blank" experiment.

TABLE XI.

Organic acids volatile in steam in the cultures of series II.

Incubation period Days	Aerobic cultures c.c. N/10 soda required	Anaerobic cultures c.c. N/10 soda required
Original	7·75	7·75
1	7·0	2·05
2	10·25	7·25
3	—	6·6
4	23·85	31·65
5	7·45	10·85
6	2·2	17·80
7	1·0	10·65
8	15·1	—
9	0·35	17·75
10	—	—
11	17·65	—
12	2·45	29·95
13	4·7	—
14	23·25	—
15	—	17·85
16	8·75	—
17	—	—
18	—	—
19	—	—
20	1·25	19·55
Cult. originally heated to 80° C. 21st day	20·1	23·05

Chart 3 and Table XI show that there are great daily variations in the quantities of these acids present indicating that there are several phases in the breakdown of the materials in the cultures. More determinations would be required before the figures could be satisfactorily interpreted. We have been unable to investigate this matter further.

The following quotation from Rettger (1906, p. 81) illustrates some of the difficulties associated with the investigation of putrefaction. "When the obligate anaerobes were cultivated along with other bacteria decidedly different results were obtained and here again my observations support those of Bienstock. The rate and nature of decomposition depend on the particular kinds of organisms with which the anaerobes were mixed. The *B. coli communis* and *B. lactis aerogenes* actually

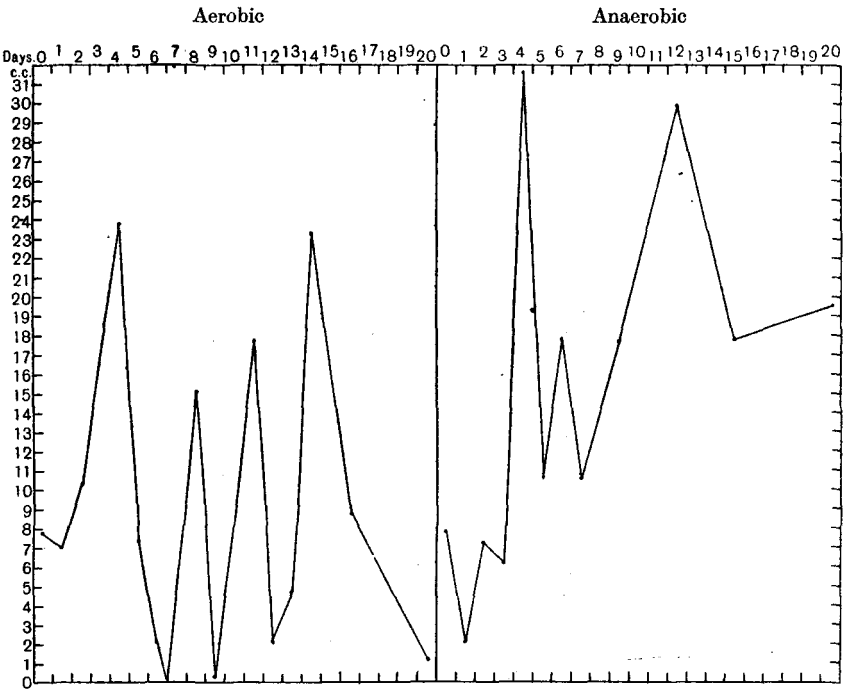

Chart 3. Showing the quantities of N/10 soda neutralised by organic acids volatile in steam in aerobic and anaerobic cultures of series II.

checked the rate of decomposition in egg-meat mixture and hence caused a decided decrease in the amount of certain products in the given length of time....In fact this antagonism of the colon bacillus to the anaerobe was most noticeable at all times and in every experiment. On the other hand the action of *Proteus vulgaris* was most favourable to the rapid disintegrative action of the anaerobes."

In such experiments as we have quoted slight errors from various causes are unavoidable. It is difficult for example to obtain absolute uniformity in the composition of the fluid placed in each culture tube,

various amounts of solid matter stick to the sides of the vessels above the fluid during incubation, etc.

Methods of estimating the chemical changes in fluids draining from carcases.

Passing from experiments with finely divided animal remains we may consider the results obtained by applying the same methods to carcases.

In order to determine the changes exhibited daily by the fluid draining from a carcase the body of a guinea-pig, which had died from natural causes, was placed in a large bottle with a loosely fitting cork, and kept at 37° C. On the second day 52·5 c.c. of fluid, almost free from sediment, had drained from the body and was pipetted out of the bottle. The fluid was filtered and 5 c.c., mixed with 22 c.c. of water, placed in a flask and made up to 250 c.c. with 97 % alcohol. Every subsequent day the fluid was removed from the bottle and measured, and a portion treated in the way described. The alcohol extracts were analysed in the manner described on p. 144.

TABLE XII.

Showing the changes exhibited daily in fluid draining from a carcase.

			In two-fifths of sample		
Days	c.c. of fluid collected	Grms. dry matter insol. in 86% alcohol in 5 c.c.	Volatile bases. c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
2	52·5	0·207	5·3	2·3	2·3 : 1
3	21·0	0·2194	8·6	2·6	3·3 : 1
4	23·0	0·2156	13·0	2·3	5·6 : 1
5	9·0	0·1866	16·05	2·2	7·3 : 1
6	4·5	—	—	—	—
7	5·0	—	—	—	—
8	5·0	0·1117	22·3	2·3	9·8 : 1
9	6·0	0·0905	—	—	—
10	6·0	0·084	26·2	2·8	9·3 : 1
11	6·0	0·066	—	—	—
12	5·5	0·0712	29·9	2·3	12·9 : 1
13	2·5	—	—	—	—
14	4·25	0·0656	—	—	—
15	5·5	—	—	—	—
16	1·5	—	—	—	—
17	3·0	—	—	—	—
18, 19	5·0	—	—	—	—
20, 21	5·0	—	34·9	2·75	12·7 : 1
	170·25				

The original weight of the animal was 404·5 grms., and the remains of the carcase on the 21st day weighed 198 grms., and after complete maceration, removal of fat and drying the bones weighed 19·25 grms.

We see from this experiment that an analysis of the fluid draining from the body yields evidence of progressive putrefactive changes, similar in all respects to those occurring in the anaerobic cultures of series II. The experiment also shows that the products of putrefaction are continually draining away from the carcase. This occurs in the exposed body.

We may next quote the results of our analyses of the fluids which drained from the carcases used in the second gas experiment (p. 130), and compare them with the results of the analyses of portions of muscle. In each case a muscle dissected from the thigh was taken, and the larger masses of connective tissue removed. The remainder was weighed, ground to a paste with sand, triturated with a little water, and the fluid made up to a definite volume with 97 % alcohol. In each case sufficient water was used to bring the final concentration of alcohol to 86 %.

TABLE XIII.

Analyses of the fluids taken from the bottles on the 21st day.

Guinea-pig (p. 130)	Original weight of carcase	c.c. of fluid collected	Grms. dry matter insol. in 86 % alcohol in 5 c.c.	In two-fifths of sample: Volatile bases. c.c. N/10 acid neutralised *	In two-fifths of sample: Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
J	272	86	0·021	17·3	1·7	10·1 : 1
K	305	50	0·148	26·4	2·85	9·2 : 1
M	147	20†	0·164	26·0	2·47	10·5 : 1
L	295	30†	0·114	25·9	1·87	13·9 : 1
O	202	45	0·058	16·7	2·1	8·0 : 1
Q	316	46	0·082	26·2	2·55	10·3 : 1
S	380	60	0·133	17·1	2·5	6·8 : 1
R	387	45	0·092	6·2	5·6	1·1 : 1
N	284	53	0·203	8·3	5·7	1·4 : 1
T	17	5·5	0·127	20·3	3·1	6·5 : 1

* To make certain that the bases or other constituents of creosote oil were not influencing these figures 10 c.c. of creosote oil were treated with 53 c.c. of water (*Note.* Guinea-pig *N* treated with 10 c.c. of creosote oil gave 53 c.c. of fluid) and shaken occasionally during 26 days. The creosote oil was then filtered off and the filtrate again filtered. 40 c.c. of the clear filtrate was analysed in the same way as the body fluid. The volatile bases, presumably ammonia, neutralised 1·0 c.c. N/10 acid and the filtrate gave no formyl titration. As the fluid which exuded from the carcases was filtered and the equivalent of 2 c.c. analysed the presence of creosote oil could not influence the formyl titration, and could not increase the reading for volatile bases by more than 0·05 c.c. N/10 acid.

† Owing to the condition of these carcases (p. 132) it was impossible to be certain that all the fluid had been collected. It should be noted that in *L* and *M* blood escaped before the carcase was placed in the bottle.

While the figures for the volatile bases give an excellent indication of the condition of the carcase the figures in the ratio column place the carcases in nearly the same order as that indicated by the dissections (p. 133), and have the great advantage over descriptions of showing the degree of putrefaction in numerical terms. On referring to the tables of the anaerobic cultures of series I and II it will be seen that putrefaction in carcases treated with creosote oil has only advanced to a very slight extent, equivalent to the stage reached on the first day in the cultures. The much higher content of amino acids in the fluids in these carcases shows that the activity of putrefactive organisms has been reduced to such an extent as to be negligible from the practical point of view. Even the bases found in them may be due largely to ammonia produced by autolytic enzymes and not to bacterial action.

In the carcases with the skins treated with "5 % cresols' emulsion" putrefaction had not advanced so far as in the untreated carcases, but in the injected one very little inhibition of putrefaction had occurred. Owing to an accident no figures for the carcase injected with creosote oil were obtained, but it seemed as much decomposed as that injected with cresols' emulsion.

We believe that this method of determining the ratio of volatile bases to the amino acids will be found to constitute a reliable means of ascertaining the extent to which putrefaction has advanced.

The analyses of the muscles yielded results differing only in degree from the results obtained in the fluids as may be seen by reference to Table XIV.

TABLE XIV.

Analyses of muscles.

Guinea-pig	Weight of muscle taken	Grms. dry matter insol. in 86 % alcohol per grm. muscle	Volatile bases per grm. muscle N/10 acid neutralised	Amino acids per grm. muscle = N/10 soda	Volatile bases + amino acids per grm. muscle	Ratio of volatile bases to amino acids
J	2·260	0·2157*	10·29	1·60	11·89	6·42 : 1
K	1·718	0·1522	10·77	1·31	12·08	8·22 : 1
O	2·833	0·1374	7·94	0·97	8·91	8·18 : 1
S	3·505	0·1514	8·10	1·07	9·17	7·57 : 1
N	2·779	0·2346	2·87	0·72	3·59	3·94 : 1
R	5·819	0·1921	3·22	1·89	5·11	1·70 : 1
Control fresh	3·522	0·2939	0·35	0·21	0·56	1·66 : 1
Control 24 hours at 22° C.	3·511	0·2774	0·39	0·36	0·75	1·10 : 1

* In this case less of the connective tissue was removed from the muscle before grinding than in the others.

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In this table analyses of muscles from a freshly killed guinea-pig and from a body which had been kept for 24 hours at 22° C. have been inserted for the sake of comparison.

Table XIV places the carcases (except *J*) in the same order as Table XIII, but shows that putrefaction had advanced further in the carcase with its skin treated with creosote oil (*N*) than in the one in which the skin and peritoneal surfaces had been treated (*R*). This apparent discrepancy may perhaps be explicable in the following way.

The fluid which exudes, charged with the products of changes occurring in the body, after passing through the skin treated with the strong antiseptic, creosote oil, remains in the bottle without undergoing further changes. The greater part of the fluid is exuded in the first few days, and later, perhaps owing to the action of organisms derived from the untreated intestines, some of the amino acids in the muscles are converted into bases, causing the differences noticed between analyses of the fluids and muscles on the 21st day.

We have then several methods of ascertaining the degree of putrefaction, and for the sake of comparison we have compiled Table XV which shows at a glance the values attached to each method.

TABLE XV.

		Result of dissection	Total gas per grm. in c.c.	Ratio in fluid	Ratio in muscle
R	Skin and peritoneal surfaces treated with creosote oil	well preserved	4·51	1·1 : 1	1·70 : 1
N	Skin treated with creosote oil ...	„ „	6·02	1·4 : 1	3·94 : 1
S	Skin and peritoneal surfaces treated with "5 % cresols"	putrid	5·69	6·8 : 1	7·57 : 1
J	Intact carcase	„	7·82	10·1 : 1	6·42 : 1
K	Abdominal cavity opened	very putrid	7·36	9·2 : 1	8·22 : 1
L	Bled	„	10·24	13·9 : 1	—
M	Skin and abdominal organs removed	„	9·45	10·5 : 1	—
O	Skin treated with "5 % cresols" ...	„	8·44	8·0 : 1	8·18 : 1
P	Injected with creosote oil	„	8·3	—	—
Q	Injected with "5 % cresols" ...	„	8·66	10·3 : 1	—

The necessity for applying ourselves to the more practical aspects of the problem prevented us from developing the experiments described in the last two sections sufficiently to work out some of the more important considerations which they suggest. By slight modifications in the procedures and the form of the apparatus it should be possible to ascertain the precise effects of the draining away of the body fluids, the importance of the skin and various organs, the effects of the food eaten before death, the actions of the enzymes and of different groups

of organisms, alone and in combination, on the various constituents of the body, the conditions which govern their actions, the nature, origin, rate of production and significance of the gaseous products and the effects of antiseptics, applied in various ways.

We need hardly point out how important might be the effects of an accurate knowledge of the factors which govern putrefaction. Changes of a putrefactive nature in the intestine probably exert a great influence on health, and their presence and extent could be ascertained. The benefits to those engaged in work connected with medicine, sanitation, animal nutrition, meat preservation and allied problems would be immense.

Putrefactive Odours.

The stench from a decaying carcase is a combination of odours, and varies in character at different times. At various stages the predominance of one constituent over others can be distinguished. In the earlier stages hydrogen sulphide can be recognized, later such bodies as methylmercaptan, still later, when liquefaction is in progress, the amines. The stenches change in character with changes in the environment. Some of the odoriferous substances are more soluble than others, and the action of water is liable to mask certain odours, and causes others to predominate. A rancid odour due to organic acids of the butyric type can be detected in some cases.

We have found it impossible to give an intelligible description of the odours arising in the later stages of putrefaction. We can, however, obtain some conception of the odours by dividing them into their main groups, namely those arising from organic bases, from organic acids and from sulphur compounds.

The presence in a putrefying fluid of odours arising from organic bases can be demonstrated by adding alkalis to fix the free organic acids, and of those from organic acids by adding sufficient mineral acid to combine with the basic constituents.

A perfect deodorant should contain chemical substances capable of eliminating all the constituents, which go to make up the stench. Deodorants of a purely acid nature can fix only the bases, while setting free the organic acids responsible for the rancid odours. In like manner basic deodorants fix organic acids and set free the bases. On the other hand some deodorants, such as oxidising agents, may destroy substances giving rise to odours and not merely fix them. Chemical action resulting in such destruction may be facilitated by the deodorant containing

solvents for constituents of the odour. Some deodorants only dissolve certain noxious substances and hold them in solution for longer or shorter periods according to their rate of evaporation.

The period during which a deodorant remains operative depends to a large extent on its rate of evaporation, degree of solubility in water, and its power of stopping putrefactive changes in the substances with which it is in contact. In estimating the action of a deodorant it must be remembered that some substances used for this purpose affect the nasal mucous membrane.

By the time we commenced to investigate the stenches arising from putrefying material we had been greatly impressed by the deodorising properties of creosote oil and similar bodies. Nevertheless we decided to compare the results obtained with agents of different types reputed to be efficient, as well as with others, which from their chemical nature might prove of value. We experimented with oxidising agents of varying power and with acids and bases, alone and in combination. Powerful oxidising agents like bleaching powder and potassium permanganate are efficient for a short period, when applied in such quantities as can be used in practice, but since putrefying materials contain large quantities of reducing substances their action is evanescent. Weaker oxidising agents such as potassium bichromate are not so efficient at first, but not being so easily reduced they exert an action for a longer time. Potassium bichromate alone removes many of the odours, except those of a rancid type. The addition of milk of lime removes these also. As potassium bichromate acts more powerfully as an oxidising agent in the presence of acid we have tested this combination, which includes the additional effect of the acids on the basic substances responsible for some odours.

We quote in detail a few only of our experiments for the purpose of illustrating the effects of some of the deodorising substances we have mentioned.

In the first series of experiments we placed portions, weighing approximately 5 grms., of disintegrating intestine from a rabbit in an advanced stage of decomposition in small beakers and treated them with 5 c.c. of the various solutions or emulsions. Observers ignorant of the contents and treatment checked our results. The type of stench differed from that of a putrefying carcase being considerably less basic in character.

The odours in the beakers numbered 2, 3, 4, 5, 6, 8 and 10 did not differ materially from the control No. 1 at any time. In those numbered 7, 9, 11 the stench was obliterated at first, but was as bad as in the

TABLE XVI.

Showing the effects of various reagents on putrefactive odours.

	Reagent added to material	15 mins.	60 mins.	24 hrs.	48 hrs.	72 hrs.	9 days
1.	Water (control)	—	+ + +	+ + +	+ + +	+ + +	+ + +
2.	Mercuric chloride 0·104 %, hydrochloric acid 0·208 % + aniline blue 0·04 %	+	+	+ + +	+ + +	+ + +	+ + +
3.	Mercuric chloride 0·104 %, hydrochloric acid 0·208 % ...	+ +	+ +	+ + +	+ + +	+ + +	+ + +
4.	Aniline blue 0·04 %	+	+ + +	+ + +	+ + +	+ + +	+ + +
5.	Hydrochloric acid 1 %	+	+ + +	+ + +	+ + +	+ + +	+ + +
6.	Soda 0·5 %	—	—	+ +	+ +	+ + +	+ +
7.	Bleaching powder 1 %	*	0	+	+ +	+ +	+ + +
8.	„ „ 0·1 % ...	+	+ +	+ +	+ +	+ + +	+ + +
9.	„ „ 1 % + boric acid 0·5 %	0	0	*	+ +	+	+ +
10.	Bleaching powder 0·1 % + boric acid 0·5 %	+	+ +	+ +	+ + +	+ + +	+
11.	Potassium permanganate 1 %	0	*	+ +	+ + +	+ + +	+ +
12.	„ bichromate 1 % ...	+	*	0?	*	—	—
13.	„ „ 1 % + boric acid 1 %	—	—	*	*	0?	0
14.	Potassium bichromate 1 % + hydrochloric acid 1 % ...	—	—	*	*	0?	0
15.	Crude carbolic acid 1 % ...	+ +	+ +	*	*	0?	0
16.	Phenol 1 %	+	+ + +	+ + +	+	+ +	+
17.	Creosote oil 5 %	+	*	0	0	0	0

0 indicates no odour beyond that of the reagent, * slight smell, + very distinct putrescent odour, + + more marked, + + + almost intolerable stench, — not tested.

control within 24–48 hours. In No. 16 there was some diminution in the stench after 48 hours. In No. 15 the stench diminished in 24 hours and ultimately disappeared. In Nos. 12, 13, 14, 17 a great reduction in stench was very soon apparent, and complete and permanent deodorisation occurred after 24 hours.

In the second series of experiments the much decomposed carcases of guinea-pigs were placed in bottles, and 50 c.c. of the reagent added. In one set the bottles were corked and in the other open.

TABLE XVII.

Showing the effects of certain reagents on putrefactive odours.

	Corked			Open		
Reagent	17 hrs.	48 hrs.	8 days	17 hrs.	48 hrs.	8 days
Bleaching powder 2 %	0	+	+ + +	+	+ +	+
Bleaching powder + boric acid 1 % ...	+	+ + +	+ + +	+ +	+ +	+
Potassium bichromate 2 %	0	*	*	0	*	*
Potassium bichromate 2 % + aniline blue 0·1 %	0	*	*	0	*	*
Crude carbolic acid 2 %	0	0	*	0	0	+

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In the 3rd, 4th and 5th series of experiments a mince made from beef and kept for two, three and eight days respectively at 37° C. was employed. In all the experiments the stench was horrible, but its character was different in each experiment. In each case 5 grms. of the mince was used, and 5 drops of the reagent run over it, and the beakers carefully examined within an hour.

TABLE XVIII.

Showing the effects of various reagents on putrefactive odours.

Reagent	Experiment 3	Experiment 4	Experiment 5
Water	+ + +	+ + +	+ + +
"Cresols' emulsion 5 %" ...	+ + +	+ + +	+ + +
Creosote oil	*	0	0
Creosote oil + bone oil 3 % ...	0	0	0

It was noticed in the course of these experiments that the addition of water robbed the stench of some of its constituents, and that the addition of an equivalent quantity of 5 % cresols' emulsion acted in the same manner and approximately to the same extent.

Taking into consideration the differences in the materials to be deodorised the results agree with those of series I. The bleaching powder produces a temporary effect, the potassium bichromate a beneficial effect for a considerable time, and crude carbolic (2 %), while efficient at first, permits of the development of odour later. Again creosote oil and its emulsions give the most satisfactory results.

Finding that emulsions of certain crude tar oils yielded excellent results we next proceeded to examine the deodorising properties of undiluted coal-tar oils, and of products obtained by their distillation. The latter part of the work was carried out in order to determine the actions of the groups of constituents, and of some component parts of the groups. For example the first fraction of creosote oil, distilling at 170–220° C. and representing 28 % of the oil, was separated into its three principal constituents, (*a*) the tar acids or phenolic bodies, (*b*) the bases, and (*c*) the hydrocarbons. The groups (*a*) and (*c*) were subdivided by fractional distillation. In the group (*b*) an "insoluble" oil was separated by treating the acid extract with soda and a "water soluble" part by further extraction of the fluid with ether.

The experiments were carried out in the same manner as those just quoted, using 10 grms. of a mixture of equal parts of meat and intestinal contents from a decaying body. 10 drops of the reagent were run over the surface of the material to be treated.

TABLE XIX.

Showing the effects of tar oils and certain of their constituents on putrefactive odours.

	10 mins.	45 mins.	20 hrs.
Crude tar oils.			
Crude carbolic acid	+	0	0
"Middle oil"	*	0	0
Creosote oil	*	0	0
"Heavy oil"	*	+	0
Anthracene oil	+ +	*	0
Fractions of creosote oil.			
170—220° C.	*	0	+
220—240° C.	*	0	+
Residue	*	0	*
Fractions of the 3 groups of constituents from the fraction 170—220° C. of creosote oil.			
Phenolic bodies, complete mixture of	*	+ +	+
Fraction 77—191° C.	0	0	0
,, 191—200° C.	0	+	*
,, 200—210° C.	*	+	+
Residue	+	+	*
Bases—"water soluble fraction"	0	0	0
"water insoluble fraction"	0	0	0
Hydrocarbons.—Complete mixture	0	0	0
Fraction 80—170° C.	0	0	0
,, 170—180° C.	0	0	*
,, 180—190° C.	0	0	0
,, 190—200° C.	0	0	0
,, 200—210° C.	0	0	0
,, 210—225° C.	0	*	+
,, 225—240° C.	0	*	0
Residue	0	*	*
"Calcium cresolate"*	+ +	+ +	+
Water extract of creosote oil†	+ + +	+ + +	+
Control	+ + +	+ + +	+ + +

* Extracted from creosote oil by milk of lime; equivalent to 0·91 % calculated as cresols.

† Equivalent to 0·62 % cresols.

We also carried out experiments with substances obtained by fractional distillation of crude carbolic and "middle oils." In the former the fractions which came off up to 187° C. were the more efficient, and in the latter those which came off up to 210° C.

All the crude tar oils produced complete deodorisation in 20 hours, but the "heavy oil" and the anthracene oil took longest to produce this result. We have recorded in Table XIX the impressions of several

observers, who were ignorant of the objects of these experiments. Each of the substances used has its own characteristic odour, which tends to distract attention from the odours arising from the material to be tested. Also some of these characteristic odours seem to affect the nasal mucous membrane to a slight extent for shorter or longer periods. Bearing these facts in mind the observers smelt the beakers with the very greatest care, and if at any time an odour other than that of the reagent was noticed, the fact was recorded. Some of the reagents possess distinctly unpleasant odours and are therefore unsuitable for practical use, but this is not the case with creosote oil, which has a not unpleasant odour. The first fractions of creosote oil undoubtedly contain small quantities of the substances, such as thiophene, which impart the characteristic smell to crude carbolic acid and "middle oils," but their rate of evaporation is retarded by mixture with the other constituents, and hence they do not make their presence evident in whole creosote oil. Consequently the deodorising properties of creosote oil can be more easily determined than those of other coal-tar oils. Taking into consideration its power of stopping putrefaction, of killing maggots, its deodorising properties and pleasant smell we regard creosote oil as the most suitable reagent to employ as a deodorant[1].

We were obliged to relinquish our investigations on the stenches arising from decaying bodies and the methods of dealing with them after we had reached the conclusions just recorded. It is evident however that further research on this very important subject could be undertaken with great advantage.

FLUIDS EXUDING FROM CARCASES.

Mention has been made of the fluid which exudes from the carcase, first appearing in an area on the left side of the body in a small animal. Some experiments were undertaken with the object of ascertaining the origin of the fluid, the factors which determine the locality in which it first appears, and the influence of antiseptic reagents on its production.

The carcases of five guinea-pigs were kept in a dry atmosphere at a temperature of 60° F. in order to eliminate the influence of moisture, thus giving a better opportunity of observing accurately the sequence of events. One carcase was laid on its ventral surface, one on its right

[1] As phenol and its homologues are extremely weak acids, weaker even than carbonic acid, the bases in creosote oil although present in small quantities relative to the phenolic bodies are available to exercise their influence upon the acid constituents of stenches when creosote oil is used as a deodorant.

side, one on its left side, one on its dorsal surface and one was suspended by its head. In all cases the fluid first appeared in a small area situated on the left side below the ribs. The direction of spread depended to some extent on gravity, but in all cases the superficial epidermis over nearly the whole body was loose and moist by the 7th day. On the 8th day the carcases were dissected under water. All were in the same condition as the body described on p. 126. Thinking that the digestive or autolytic ferments were in some way concerned with this phenomenon we carried out the following series of experiments. Immediately after death the body was opened by a median incision, organs excised with precautions to avoid contamination as far as possible, and the body sewn up by a double layer of sutures, one through the abdominal muscles and the other through the skin. That this procedure was efficient in preventing gas from escaping is shown by the fact that many of the carcases became greatly distended with gas.

TABLE XX.

Showing the influence of the abdominal organs on the exudation of fluid.

1.	Stomach alone removed	Fluid appeared in the usual place on 5th day
2.	Stomach ligatured at both ends, but left *in situ*	,, ,, ,,
3.	Stomach and intestines removed ...	,, ,, ,,
4.	Stomach, intestines and mesenteric vessels ligatured, and left *in situ*...	,, ,, ,,
5.	Liver only removed...	,, ,, ,,
6.	Liver vessels ligatured; left *in situ*	,, ,, ,,
7.	Organs cut with scissors, and contents of abdominal cavity stirred with a rod	,, ,, ,,
8.	Duodenum and pancreas only removed	,, ,, ,,
9.	Liver and intestines removed, and stomach placed in pelvis	,, over pelvic region
10.	Stomach and intestines removed, and liver placed in pelvis	,, ,, ,, ,,
11.	Stomach and intestines removed, and pancreas and duodenum placed in pocket under skin of right shoulder	,, over inserted pancreas

These experiments seem to indicate that the removal of one of the larger abdominal organs has little effect on the place or time of the appearance of the fluid, but the complete removal of the liver, stomach and intestines and the replacement of one of these organs in an abnormal situation causes the patch to appear first in that situation.

We suggest that the phenomenon is due to the action of the ferments present in the liver, stomach, intestines and pancreas and that in an

intact body the place at which the action on the abdominal wall is first produced is due in guinea-pigs to the anatomical disposition of the omentum, which partially protects certain portions of the abdominal wall, and partially guides the autolytic fluids exuding from the organs mentioned so that they mix and exert their greatest action on the unprotected area of abdominal wall lying against the cardiac end of the stomach. The action of the ferments allows the fluid to pass through the muscle layers, and so acts on the skin that the hairs become loosened in their follicles, and together with the superficial epithelium are detached from the underlying skin by the exuding fluid.

In order to determine whether the action on the skin was due to moisture rather than to ferment action two further experiments were undertaken. The body of a freshly killed guinea-pig was taken and a testis was inserted under the skin of the right side of the neck, a kidney in the middle line of the back and a piece of small intestine, ligatured at the ends, under the skin of the right thigh. In the same situations in the body of another guinea-pig 1 c.c. of colon contents, small intestine contents and stomach contents were inserted. In the former body the hair began to come off first over the piece of small intestine, a day before the usual patch on the side was noticed. No unusual softening of the skin occurred over the inserted organs. In the second body the skin began to soften first over the spot covering the contents of the small intestine, and on the same day the usual patch was noticed on the left side. No unusual softening of the skin occurred over the areas covering the contents of the stomach or colon.

These experiments seem to show that the contents of the small intestine, presumably due to the enzymes in them, exert a specific action. Other experiments having a bearing on this subject are quoted later.

THE EFFECTS OF INJECTION OF CREOSOTE OIL INTO THE BLOOD VESSELS.

We have already shown (p. 132) that injections of small quantities of antiseptics into the blood vessels, without treatment of the skin, have little effect in stopping putrefaction, when the atmosphere is moist. The following experiments were carried out in order to ascertain the effects of injection through the carotid artery of varying quantities of creosote oil, when the carcases were kept in a dry atmosphere at a temperature of 60° F.

TABLE XXI.

Showing the effects of the injection of creosote oil into the blood vessels.

	Weight of animal grms.	Quantity injected c.c.	c.c. injected per 100 grms.	Other treatment	Remarks
A	383	13·6	3·54	None	5th day usual patch. No gas
B	426	10·0	2·36	,,	,, ,, ,, ,,
C	491	5·8	1·18	,,	,, ,, ,, Distended
D	468	5·5	1·18	Tube in peritoneum	,, ,, ,, ,,
E	530	6·3	1·18	Abdomen opened	,, ,, ,, Little gas
F	508	6·0	1·18	Tube in coecum	,, ,, ,, Slightly distended
G	473	5·6	1·18	Skin over abdomen treated with 7 c.c. creosote oil	,, ,, ,, ,, ,,
H	458	None	None	Skin treated with 23 c.c. creosote oil	,, ,, ,, ,, ,,
I	340	,,	,,	Skin treated with 25 c.c. olive oil	4th day Distended
J	—	,,	,,	None	5th day skin dissolved over stomach

In all cases the skin became moist and fluid appeared in the usual spot on the left side, and in all gas developed except in the first two, which received the largest injections of creosote oil.

On the 20th day the carcases of these animals were examined for the presence of bacteria in the following way. As much as possible of the hair was removed, and the remainder singed off. The body was then fastened on a board, and the under surface wetted with lysol solution to prevent particles from flying about. Then the ventral surface was seared with a cautery and the abdominal and thoracic walls opened with sterile instruments. Next portions of the liver, lung and thigh muscles were removed with sterile instruments, ground between sterile ground glass plates, and cultures made on agar and in broth. Portions of the contents of the stomach and large intestine were also cultivated.

TABLE XXII.

Showing the results of cultures from the organs of creosote oil injected bodies.

	Lungs	Liver	Muscle	Stomach	Coecum
A	0	0	0	spore-bearing bacilli	spore-bearing bacilli
B	0	0	colon-like bacilli	,,	,, ,,
C	few colon-like	0	,,	,,	,, + colon-like
E	0	0	,,	,,	,, ,,
G	0	0	,,	,,	,,
H	colon-like	colon-like	,,	,, + colon-like	,, + colon-like
I	,,	,,	,,	,, ,,	,, ,,
J	spore-bearers	,, + spore-bearers	,, + spore bearers	,,	,, ,,

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These results show that, according to the method adopted, organisms could not be demonstrated by culture methods in the livers of the injected carcases 20 days after treatment, and that in most cases they could not be demonstrated in the lungs. On the other hand only the largest injections prevented the penetration of intestinal organisms into the muscles. Organisms of the intestinal type were plentiful in the organs when the skin only was treated. It is worthy of note that spore-bearing bacilli were found only in the organs and muscles of the untreated control, *J*. From the stomach contents of all spore-bearing bacilli were cultivated in large numbers, and from the coecal contents both spore-bearing bacilli and colon-like organisms.

Experiment *A* indicates that fluid exudes from a carcase in which the organs and muscles are sterile.

On dissection the condition of the body treated with olive oil was distinctly better than that of the control, showing that treatment of the skin with an oil possessing little, or no, antiseptic properties has some effect in retarding putrefaction under the conditions of this experiment.

INJECTIONS OF CERTAIN CONSTITUENTS OF CREOSOTE OIL INTO THE BLOOD VESSELS.

A further series of experiments were carried out under the conditions mentioned in the last section, a dry atmosphere and a temperature of 80° F., in order to compare the results of injection of certain of the constituents of creosote oil with the oil itself.

Without the aid of chemical analyses it is difficult to arrange the results of this series of experiments in their exact order. It is clearly evident, however, that each group of substances separated from creosote oil exerts a very great influence in checking putrefactive changes under the conditions of this experiment. The best results were obtained by the injection of phenolic mixtures of 100 % strength, which not only preserved the bodies to an extraordinary degree but inhibited the formation of gas. Bearing in mind our remarks on the stenches from decaying bodies it is interesting to note that a slight rancid smell was noticed when the carcase marked (*d*) was dissected.

The organs of specimens (*e*) and (*f*) were examined for the presence of bacteria by the method described in the last section. The cultures from the lungs, liver and muscle of (*e*) remained sterile, but those from the liver and muscle of (*f*) contained colon-like organisms. In both cases organisms were present in the contents of the stomach and coecum.

TABLE XXIII.

Showing the results of injections of certain constituents of creosote oil into the blood vessels.

	Weight of body grms.	Material injected	Quantity injected c.c.	Distension day	Fluid exuded, L. side day	Dissection	Date of dissection day
a	605	creosote oil	7·0	7	9	good preservation	14
b	493	fraction to 317° C.[1]	5·9	6	8	,, ,,	23
c	433	,, ,,	2·4	6	6	,, ,,	23
d	575	phenolic mixt. (100 %)[2]	7·0	never	14	very good preservation	23
e	505	phenolic mixt. (100 %)[3]	6·3	,,	5	(see p. 165)	
f	299	,, ,, ,, [3]	1·2	,,	5	,, ,,	
g	464	"watersol." bases(100 %)[4]	5·9	13	13	good preservation	27
h	485	hydrocarbons and bases[5]	5·9	5	6	slightly decomposed	23
i	780	hydrocarbons[6]	7·1	7	9	good preservation	23
j	477	"residue"[7]	5·9	5	8	slightly decomposed	23
k	451	Control	0	7	9	Organs represented by a dark brown deposit. Smell very bad	

[1] Amounted to 81 % of the creosote oil, and contained 17·1 % of phenolic bodies.

[2] Separated from the fraction of creosote oil distilling between 170—220° C.

[3] Complete mixture of phenolic bodies separated from fraction of creosote oil distilling up to 317° C.

[4] See p. 159.

[5] Remainder of fraction 317° C. of creosote oil after taking out the phenolic bodies, consisting of hydrocarbons and bases.

[6] Hydrocarbons only from fraction 170—220° C.

[7] Residue from distillation of creosote oil up to 317° C., amounting to 19 % of the original oil.

In all cases including (*e*) fluid exuded from the body confirming our hypothesis that the phenomenon is independent of bacterial activity. Smears from the exuded fluids showed numerous organisms in specimens (*b*), (*c*), (*h*), (*i*) and (*j*), few in (*a*) and (*g*) and very few in (*d*) and (*e*).

Injections of Various Reagents into the Blood Vessels.

Having shown that even under the optimum conditions for putrefaction, a moist, almost oxygen free atmosphere and a temperature of 26·5° C., treatment of the skin with creosote oil inhibits putrefaction to a very great extent, the opportunity was offered of ascertaining under these conditions the effects on the tissues of injections into the blood vessels of reagents possessing very different properties. We believe the fluid which exudes results from cytolysis and enzyme activity,

and as it has been asserted that the actions of enzymes are stopped by acids and alkalis exceeding 0·1 (N) our first injections were calculated to produce this strength in the blood. Since the exudation of fluid was not influenced by this procedure we injected in some experiments sufficient to produce a strength of 0·1 (N) in the whole of the water in the carcase, calculating the water as 65 % of the total weight. The bodies of freshly killed guinea-pigs were used. In each case the skin and natural orifices were treated with creosote oil and the reagent was then injected through the carotid artery. The experiments were conducted in the apparatus previously described (p. 127), but the gas produced was not collected. The quantity of fluid which exuded was noted daily, and finally the carcases were carefully dissected, some at the end of five days, and others after 10 and 16 days.

TABLE XXIV.

Showing the results of the injections into the blood vessels of various reagents in carcases with the skin treated with creosote oil.

	Weight of body grms.	Skin treatment, c.c. of creosote oil per 100 grms.	c.c. per 100 grms. injected	Reagent		Fluid exuded per 100 grms.	Date of dissection day	Remarks
A	471	4·66	1·22	(N) Hydrochloric acid	3·65 %	16·3	5	moderate preservation
B	284	3·52	2·11	N/2 Sulphuric acid	2·45 %	19·7	12	very decomposed
C	410	3·54	2·13	1·7 (N) Chromic acid	10·0 %	18·0	12	moderate preservation
D	705*	4·68	1·22	N/10 Arsenic acid	0·71 %	7·1	5	very good preservation
E	371*	3·50	1·31	N/5 „ „	1·42 %	12·4	16	poor preservation
F	312*	3·52	2·6	N/5 „ „	1·42 %	7·7	16	good preservation
G	396*	3·50	2·6	N/5 Arsenious acid	0·96 %	7·1	16	very good preservation
H	417*	4·6	1·22	(N) Orthophosphoric acid	4·9 %	11·5	5	moderate preservation
I	479*	3·55	2·44	2·5 (N) „ „	12·25 %	23·4	16	„ „
J	556	4·67	1·22	(N) Monopotassium phosphate	13·6 %	24·4	5	„ „
K	444	4·66	1·23	(N) Formic acid	4·6 %	18·2	5	complete maceration
L	355	3·50	1·23	4 (N) „ „	18·4 %	25·3	16	„ „
M	483	3·54	1·9	4 (N) „ „	18·0 %	23·3	12	well preserved
N	555	4·66	1·22	(N) Lactic acid	9·0 %	17·3	5	very decomposed
O	499	4·60	1·21	(N) Soda	4·0 %	16·6	5	moderate preservation
P	387	3·50	1·22	3 (N) Ammonia	5·1 %	25·8	10	complete maceration
Q	368	3·53	1·87	10·6 (N) Ammonia	18·0 %	23·3	11	moderate preservation
R	490	4·63	1·22	2·5 (N) Sodium fluoride	10·5 %	18·7	5	„ „
S	370	3·50	1·22	(N) Sodium nitrite	6·9 %	11·9	10	very good preservation
T	373	3·50	0	Liver painted with strong mercuric chloride		24·1	16	poorly preserved

* Orthophosphoric, arsenic and arsenious acids were considered as dibasic in making up the solutions, and their molecular weights taken as representing two equivalents.

Dissections of bodies used in this experiment.

The bodies were supported on glass pedestals head downwards resting on the shoulder.

A. Injected with hydrochloric acid. Dissected 5th day. 77 c.c. of brownish fluid had exuded. No smell. *Liver* gas bubbles throughout. *Stomach* soft, full of gas. *Kidney* soft. *Spleen* very soft. *Abdominal wall* on left side dark and emphysematous. *Muscles* pink and slightly soft. *Remarks.* A little smell of rancid type. The quantity of acid injected was evidently insufficient to prevent marked activity of the gas-forming organisms, nor did it seem to have interfered with autolysis or the exudation of fluid.

B. Injected with sulphuric acid. Dissected on the 12th day. 56 c.c. of fluid had exuded. *Remarks.* None of the internal organs or muscles distinguishable. Remains alkaline to litmus.

C. Injected with chromic acid. Dissected 12th day. 74 c.c. of fluid had exuded. Tissues everywhere dark green in colour, probably through the reduction of the chromic acid. *Liver* not soft, full of gas bubbles. *Kidney* not soft. Cardiac end of *stomach* wall dissolved. *Muscles* moderately good. *Smell* very little, if any. *Remarks.* The carcase was moderately well preserved. Alkaline to litmus. Neither gas formation nor exudation of fluid inhibited.

D. Injected with arsenic acid. Dissected 5th day. 50 c.c. of red fluid had exuded. *Lungs* red, oedematous but well preserved. *Liver* pale, normal in size; no gas bubbles; sinks in water; oedematous. *Kidney* looks normal, but soft and oedematous. *Spleen* soft. *Stomach* wall very soft. *Abdominal wall* normal in appearance and consistency. *Muscles* excellent in appearance, but oedematous. *Remarks.* The fluid exuded very slowly, and the total amount was small, but all the organs were oedematous. The carcase was very well preserved.

E. Injected with arsenic acid. Dissected on the 16th day. 46 c.c. of clear, red fluid, containing some sediment, exuded. *Lung* very oedematous, and fluid in thorax. *Liver* much disintegrated, numerous bubbles. *Muscles* soft and disintegrated. *Subcutaneous tissues* oedematous, especially in the dependent parts. *Remarks.* This carcase was not nearly so well preserved as *F*, *G* or *S*.

F. Injected with arsenic acid. Dissected on the 16th day. 24 c.c. of clear, red fluid, containing some sediment. *Thorax* some red fluid. *Lungs* oedematous, but normal looking. *Liver* gas bubbles. *Muscles* of hind leg dry and pale. Collections of gas in the retroperitoneal tissues. *Remarks.* This carcase was not so well preserved as *G*.

G. Injected with arsenious acid. Dissected 16th day. 28 c.c. of reddish-brown fluid exuded very slowly. No smell. *Hair* loose over the usual patch and the abdomen, but not elsewhere. *Thoracic organs* normal in appearance, but oedematous; soft clot in heart. *Liver* yellow, normal in size and consistency; no gas bubbles; sinks in water. *Kidney* normal in appearance. *Intestines* very well preserved, and almost normal in consistency. Mesentery oedematous, and abdominal walls stained black over the intestines. *Muscles* of hind legs and psoas exceedingly well preserved; not oedematous; muscles of dependent parts oedematous. No

collections of gas in the carcase, and at no time was distension observed. *Remarks.* This carcase was very excellently preserved.

Note. *F* and *G* were treated with the same amount of arsenic the former in the form of arsenic and the latter in the form of a solution of arsenious oxide.

H. Injected with orthophosphoric acid. Dissected on the 5th day. 48 c.c. of red-brown fluid exuded. *Thoracic organs* normal in appearance and consistency. *Liver* pale, soft, normal in size; numerous bubbles. *Kidney* very dark, soft, but normal in size. *Spleen* dark and soft. *Stomach walls* disintegrated where in contact with liver. *Abdominal walls*, except at usual left patch, normal in appearance. *Muscles* almost normal in appearance and consistency; psoas very soft and oedematous. Subcutaneous emphysema. *Remarks.* Moderately well preserved. Gas present throughout the tissues.

I. Injected with orthophosphoric acid. Dissected on the 16th day. 112 c.c. of red-brown fluid had exuded. *Liver* full of gas bubbles. *Stomach* tough, and *intestines* well preserved. *Muscles* pink, slightly soft, but not much decomposed. Gas in all the tissues. *Remarks.* Moderately well preserved.

J. Injected with monopotassium phosphate. Dissected on the 5th day. 136 c.c. of chocolate coloured fluid had exuded. *Thoracic organs* very soft. *Liver* pale, soft, full of gas bubbles. *Kidney* soft. *Spleen* soft. *Stomach wall* emphysematous. *Intestines* contain much gas. *Muscles* normal in appearance and consistency. *Abdominal wall* very soft. *Remarks.* Moderately well preserved.

K. Injected with formic acid. Dissected on the 5th day. 80 c.c. opaque, dirty-looking fluid had exuded. *Lungs* and diaphragm disintegrated. *Liver* and *kidney* very much disintegrated. *Stomach walls* dissolved. *Muscles* diffluent, and macerated from the bones. No gas collections found anywhere. Probably decomposition has advanced so far that the gas has been set free. *Remarks.* The carcase is very much macerated.

L. Injected with formic acid. Dissected on the 16th day. 90 c.c. of red-brown turbid fluid with much sediment. *Remarks.* The carcase drops to pieces. No organ was recognizable, and the muscles have been macerated from the bones.

M. Injected with formic acid. Dissected on the 12th day. 114 c.c. of fluid had exuded. *Liver* dark, shrunken, soft but no gas bubbles seen. *Kidney* very soft. *Stomach wall* tough. *Intestines* tough and full of gas. *Muscles* soft but look normal. Collections of gas in the retroperitoneal and other connective tissues. *Remarks.* The carcase was acid to litmus and well preserved.

Note. Reckoning about 65 % of the carcase as water sufficient formic acid had been injected to make the percentage of acid in the water of the body 0·35 % in the case of *L* and 0·5 % in the case of *M*. The former was much macerated and the latter well preserved. (See *P* and *Q*.)

N. Injected with lactic acid. Dissected on the 5th day. 96 c.c. of red fluid had exuded. *Remarks.* The carcase was similar in all respects to *K*.

O. Injected with soda. Dissected on the 5th day. 83 c.c. of red-brown, opaque, dirty-looking fluid exuded. *Liver* brown, full of bubbles, and collapsed. *Kidney* soft and dark. *Spleen* dark and very soft. *Stomach walls* disintegrated where in contact with liver. *Muscles* pink and soft. Collections of gas present. *Remarks.* Condition similar to *A*.

P. Injected with ammonia. Dissected on the 10th day. 100 c.c. of red-brown, turbid fluid with much sediment had exuded. None of the organs was recognizable, and the muscles had been macerated from the bones. *Remarks.* This carcase was the most macerated of the series.

Q. Injected with ammonia. Dissected on the 11th day. 82 c.c. of fluid had exuded. *Skin* over abdomen very thin. *Liver* very soft, very numerous gas bubbles. *Kidney* very soft. *Stomach* anterior wall disintegrated. *Intestines* moderately well preserved. *Muscles* well defined but soft, and easily detached from the bones. Little smell, apparently from stomach contents. All tissues alkaline to litmus. Collections of gas. *Remarks.* The body was moderately well preserved.

Note. In *P* the ammonia in the water of the tissues was 0·08 %, in *Q* 0·5 %. The former was macerated, the latter moderately well preserved.

R. Injected with sodium fluoride. Dissected on the 5th day. 92 c.c. of red-brown fluid had exuded. *Thoracic organs* well preserved. *Liver* soft, collapsed, full of gas bubbles. *Kidney* not very soft. *Spleen* soft. *Stomach wall* much disintegrated. *Muscles* very well preserved, but slightly soft. Psoas well preserved. *Remarks.* The carcase was moderately well preserved.

S. Injected with sodium nitrite. Dissected on the 10th day. 44 c.c. of clear, light yellow fluid had exuded, with some whitish sediment. Faint fresh blood odour. *Lungs* and *heart* very well preserved. *Liver* normal in size and appearance, no gas bubbles seen; smallest fragments sink in water. *Kidney* soft, but structure visible. *Intestines* slightly soft. No collections of gas or subcutaneous emphysema. *Muscles* very firm, and difficult to tear, remarkably well preserved, and not oedematous; psoas soft. *Hair* comes off easily over usual patch and abdomen, but not elsewhere. *Remarks.* This is probably the best preserved carcase of the series, the organs and muscles being in remarkably good condition. The fluid and organs were free from discoloration from blood pigment. The fluid exuded very slowly; no gas had been produced.

T. Liver treated with strong mercuric chloride. Dissected on the 16th day. 90 c.c. of brown fluid, with some smell, exuded. *Liver* very soft but shows no gas bubbles, and sinks in water. *Thoracic organs* very soft, and fluid in cavity. *Intestines* soft. *Muscles* diffluent and macerated from the bones. *Remarks.* The carcase is decomposed, but not so badly as *P*. The treatment of the liver seems to have preserved it to some extent but has not influenced general putrefactive changes.

The conditions found on post-mortem examination of the bodies *F*, *G* and *S* were so remarkable that we analysed filtered samples taken from the whole of the fluid which had exuded. 5 c.c. portions were taken, 22 c.c. of water added and made up to 250 c.c. with 97 % alcohol. The results are given in Table XXV.

As we believe that no bacterial putrefaction occurred in these carcases, we propose to adopt a ratio of 0·45 : 1 as a standard for comparing the extent of putrefaction in carcases after a few days' exposure. It is of interest to compare these ratios with that obtained in the left gluteus of the white horse after eight and a half months' exposure. In

TABLE XXV.

Showing the results of the analyses of fluids which had drained from the bodies of guinea-pigs injected with arsenic acid (F), arsenious acid (G) and sodium nitrite (S).

	Fluid collected	Quantity taken	In two-fifths of filtrate: Volatile bases c.c. N/10 acid neutralised	In two-fifths of filtrate: Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
F	24 c.c.	5 c.c.	2·00	4·65	0·43 : 1
G	28 ,,	5 ,,	1·80	4·40	0·41 : 1
S	44 ,,	5 ,,	2·50*	5·50	0·45 : 1

* A very careful inquiry, involving several different tests, was made to determine the presence of nitrite in the fluid, but all including the extremely sensitive metaphenylene diamine reaction gave negative results, showing that no traces of the nitrite remained. We suggest that the nitrite had been reduced to ammonia. If this is the case we calculate that the volatile base figure should be reduced by 0·4 c.c. to allow for the ammonia from this source. It is of interest to note that if this correction is made *G* and *S* give almost identical ratios. As no nitrite was present in the fluid there was no necessity to adopt van Slyke's method for the amino-nitrogen instead of the formyl titration method.

discussing this subject (p. 207) we attempt to explain that the ratio of about 1·5 : 1 found in fresh muscle should fall, if uncomplicated by the action of putrefactive bacteria, until the proteolytic ferments cease to act.

In four cases we collected the exuded fluid on the 4th or 5th days, and on the 11th or 12th days, and analysed samples from the portions taken at these times.

TABLE XXVI.

Showing the results of analyses at different times of the fluids draining from the bodies of guinea-pigs injected with formic acid (M), chromic acid (C), sulphuric acid (B) and ammonia (Q).

	Date of collection	Total collected	Quantity taken	In two-fifths of filtrate: Volatile bases c.c. N/10 acid neutralised	In two-fifths of filtrate: Formyl titration c.c. N/10 soda	Ratio of volatile bases to amino acids
M	1—5 days	79 c.c.	5 c.c.	2·20	5·45	0·40 : 1
	5—12 ,,	35 ,,	5 ,,	2·95	6·40	0·46 : 1
C	1—4 ,,	46 ,,	5 ,,	1·40	2·60	0·54 : 1
	4—11 ,,	28 ,,	5 ,,	5·00	5·00	1·0 : 1
B	1—4 ,,	28 ,,	5 ,,	3·25	5·75	0·57 : 1
	4—11 ,,	28 ,,	5 ,,	13·35	3·50	3·82 : 1
Q	1—5 ,,	50 ,,	5 ,,	5·70	2·65	—
	5—12 ,,	36 ,,	5 ,,	14·80	3·55	—

All the tissues of the carcase injected with formic acid (*M*) were acid to litmus. The two samples of fluid gave similar results on analysis, indicating no bacterial decomposition.

The tissues of the body injected with chromic acid (*C*) were alkaline to litmus at the time of dissection. The ratios in the first and second samples appear to indicate that some slight bacterial action was in progress. The figures in column 5 seem to show that enzyme action was partly inhibited at any rate during the first four days.

At the time of dissection the tissues of the body injected with sulphuric acid (*B*) were strongly alkaline to litmus. Bacterial action was apparently slight during the first period, but considerable during the second period. The injection of the dilute acid corresponding to 0·08 % in the water of the whole body (see *M*) has perhaps contributed largely to the maceration, owing to its cytolytic effects.

Sufficient ammonia was injected in *Q* to make a concentration of 0·5 % in the whole water of the body, assuming it became evenly distributed. The injected ammonia contained in the 2 c.c. of fluid used for analysis should neutralise 5·9 c.c. N/10 acid. After making some such allowance for the injected ammonia it is evident that bacterial change has occurred, at any rate in the second sample of fluid.

Remarks. (*a*) The substances injected do not prevent the action of enzymes, when in their natural environment in the tissues. (*b*) The reagents seem to have been fixed in the tissues, since tests for arsenic, nitrites, and sulphuric and chromic acids in the fluids were negative. Possibly traces of formic acid were present. (*c*) The presence of small quantities of creosote oil in the fluids collected in the bottles appeared to prevent further bacterial change after exudation. (*d*) It would be of interest to repeat these experiments omitting the skin treatment with creosote oil.

In regard to these experiments the following points are noteworthy:

(1) Treatment of the skin alone with creosote oil inhibits putrefaction to a great extent (see p. 132).

(2) The injection of weak solutions of certain reagents hastens putrefaction in spite of the treatment of the skin. These reagents exhibit well-marked haemolytic properties in test tube experiments, and probably assist putrefaction by causing disintegration of the cells with which they come into contact.

(3) Bodies injected with weak formic acid (*K*) and weak lactic acid (*N*) became completely disintegrated within 5 days. The increase of formic acid up to 0·07 (N) in the blood is not advantageous, but if the amount is increased to 0·1 (N) in the whole water of the carcase the body is well preserved, though gas is produced and fluid exuded.

(4) Both arsenic preparations showed remarkable preserving properties, though in very weak strength.

(5) The attempt to differentiate between the effects of the two potent replaceable hydrogen atoms of the orthophosphoric acid gave negative results (see *H* and *J*).

(6) The injection of ammonia so as to produce a strength of 0·3 (N) in the blood or 0·046 (N) in the whole water of the carcase resulted in advanced disintegration in a few days, though when the strength reached 1·6 (N) in the blood or 0·25 (N) in the water of the carcase the body was moderately preserved. The macerating action of the weak ammonia is perhaps a factor in disintegration. We have ascertained from other experiments that some organisms can live in the latter strength of ammonia. The ammonia and other 'volatile bases' produced by putrefactive organisms would exercise a disintegrating effect, especially when sufficiently diluted with water. This is probably one of the contributing causes of the more rapid disintegration of carcases when exposed to rain.

(7) The most remarkable result was obtained by the injection of sodium nitrite. The absence of pigmentation of the fluid and tissues was noteworthy.

The following reasons induced us to experiment with sodium nitrite. Many enzymes are regarded as protein in nature and may possess free amino groups in their molecular structure. If this hypothesis is correct the nitrous acid formed by the interaction of the nitrite with the acids developed in the tissues after death might prevent the action of these enzymes by attacking these amino groups.

(8) Gas was produced in all cases except *G* and *S*.

(9) Fluid exuded from every carcase. The amount of fluid which exudes from a body should be considered in relation to the condition of the tissues on dissection. Autolytic ferments tend to macerate the tissues, and the extent to which maceration goes on determines the amount of fluid which exudes, and the appearance of the tissues on dissection. Agents with considerable cytolytic powers contribute to the maceration. In the carcases injected with weak ammonia and weak formic and sulphuric acids so much maceration had occurred that the muscles were detached from the bones. Weak acids and bases seem to facilitate cytolysis to a great extent. The action of arsenic must be considered in regard to its specific effect. Sodium nitrite is a salt. The fluid exuded from the bodies injected with these reagents probably consists of serum, the water of the injected fluid and water from the gut. The

arsenic injected bodies yielded less fluid than the one injected with nitrite, and in this case fluid was present within the muscles. The liquid found in the carcases injected with weak ammonia and weak formic and sulphuric acids had nearly the same appearance as the fluid that exuded, but contained more sediment. The subject requires further investigation.

The Results of injecting Various Reagents into the Blood Vessels of Carcases exposed out-of-doors.

In this series of experiments the blood vessels of freshly killed rabbits were injected through the femoral artery. Consequently the reagent did not pass into the vessels of the leg on the side used for injection and the limb acted to some extent as a control. In most cases the skin and wound were treated with sufficient creosote oil to cover the whole surface, and a small quantity was poured into each of the natural openings. The carcases were exposed, lying on their right sides, on the grass without protection of any kind. They were inspected daily and the conditions found carefully recorded. These experiments were carried out at the end of August when the weather was warm and showery, and numerous flies were ready to lay their eggs on all suitable material.

These experiments, which preceded those recorded in the last section, were designed to test the value of injections of various types of disinfectants into the blood vessels of carcases exposed to varying weather conditions. The experiments also gave much information on the protective action of creosote oil, when applied to the skin, its power to repel flies and prevent the laying of eggs. The effects of the injection of creosote oil with and without treatment of the skin were also compared.

The carcases remained on the ground for nine weeks and were then dissected.

A. No fly eggs or maggots were noticed at any time on the carcase, and the external appearance was very satisfactory throughout. On dissection the face muscles were soft, thoracic organs and liver soft, but normal in colour and shape. Intestines normal in appearance, but a little soft. Subcutaneous tissues slightly disintegrated under the skin of the back. The injected leg was much decomposed. *Remarks.* The carcase was moderately well preserved.

B. No fly eggs or maggots were noticed at any time on the carcase, and the external appearance was very satisfactory throughout. On dissection the tissues resembled those of a freshly killed animal in colour, contour, size and consistency. The serous surfaces and fasciae showed the normal glistening appearance. There

TABLE XXVII.

Showing the methods used in treating the bodies of rabbits exposed out-of-doors.

	Weight of body in lbs.	c.c. of creosote oil per lb. used in skin treatment	c.c. of reagent injected per lb.	Reagent*
A	4·5	21	10	10 % hydrochloric acid
B	3·5	15	14	1 % arsenic acid
C	5·0	20	18	1 % mercuric chloride in 5 % sodium chloride solution
D	4·5	18	21	5 % potassium bichromate
E	6·25	0†	0	100 c.c. of 5 % pot. bichromate + 2 % bile applied to peritoneal surfaces and natural orifices
F	6·25	19	15	10 % liver of sulphur ‡
G	4·75	14	20	5 % phenol
H	4·5	0†	19	creosote oil
I	5·5	15	9	„ „
J	4·0	16	0	16·25 c.c. creosote oil applied to peritoneal surfaces

* All the reagents, except creosote oil, were in aqueous solution.

† No skin treatment.

‡ Free sulphur filtered off.

was no evidence of oedema or gas formation. The leg on the injected side was however much decomposed. *Remarks.* Preservation perfect.

C. A few fly eggs were deposited on the 15th day, but no maggots were ever seen on the carcase. The external appearances were satisfactory throughout. On dissection the under surface of the head was decomposed. The thoracic organs were well preserved; liver soft; intestines well preserved, but soft. The hair came off moderately easily over the abdomen. Muscles of back rather soft. The injected leg was much decomposed. *Remarks.* The carcase was not quite so well preserved as *A*.

D. A few fly eggs were found on the 21st day, but no maggots were ever seen on the carcase. The external appearances were satisfactory throughout. On dissection the facial muscles were very soft; the thoracic organs well preserved, but fluid was found in the pleural cavity. Liver was well preserved. Intestines were well preserved with dark patches on their surfaces. No smell. Muscles soft and greenish in colour, probably due to the reduction of the bichromate. The injected leg was much decomposed. *Remarks.* The carcase was moderately well preserved.

E. On the second day numerous flies were seen on the carcase, and large numbers of eggs had been deposited. By the 10th day there were numerous maggots, the hair was coming off and the muscles were disintegrating. By the 14th day the flesh was much decomposed, and the smell was bad. By the 21st day the maggots had eaten the greater part of the carcase.

F. In 14 days a few eggs were found in the mouth and coat, and large maggots in small numbers were found in the thigh muscles on the 28th day. Maggots

had also made a large hole into the abdomen. On dissection the muscles round the mouth were slightly disintegrated. All the organs were very soft, but their contours were preserved; muscles pale and soft, but not disintegrated. The hair came off easily in most situations. The injected leg was much decomposed. *Remarks.* The carcase was comparatively poorly preserved.

G. A few eggs were deposited on the 12th day, but except on the injected leg maggots were never seen. On dissection the face muscles were found well preserved; thoracic organs soft and some fluid in the cavity; liver soft; intestines soft, but moderately preserved. Muscles well preserved, but soft. Injected leg muscles soft, but not very much disintegrated. The hair came off moderately easily; very little smell. *Remarks.* This carcase was moderately well preserved.

H. Many eggs were deposited by the 2nd day. These had not hatched on the 5th day. On the 9th day a few small maggots in the coat. 13th day many small maggots found dead. On the 19th day the muscles of the injected leg had been eaten by maggots. On 31st day no maggots were found on the carcase, indicating that they could not eat the portions of the body reached by the injection. Dissection; hair came off very easily everywhere, and the skin was disintegrated over the abdomen, and slightly over the thorax; face muscles very well preserved and firm; thoracic organs firm and normal in shape; intestines wonderfully well preserved with asphalt-like smell; muscles soft but well preserved. *Remarks.* With the exception of the skin and underlying tissues the carcase was well preserved.

I. On the 19th day a few eggs were found on the coat, but no maggots were ever seen on this carcase. Dissection. The skin of one side was removed and the carcase photographed (Pl. V, Fig. 14). The figure shows the wonderful state of preservation of the carcase. In this state it was allowed to remain in a room for a month, and caused no nuisance. During this month the muscles became shrunken and very hard, and the body looked like a dark mummy. On dissecting the surface covered with skin it was found little changed. Though flies were present in the room no eggs or maggots were found on the carcase. *Remarks.* It will be noticed that twice the quantity was injected into *H* and yet *H* was not nearly so well preserved as *I*, which received skin treatment together with injection. The beneficial effects of skin treatment are very evident, when carcases such as these are compared side by side. The access of water and air has permitted the organisms present in the skin to produce some disintegration in the peripheral tissues of the injected carcase *H*.

J. This body lay on its back. On the 2nd day rain water had collected in the exposed abdominal cavity. On the 19th day a few eggs were deposited. No maggots were ever found on the carcase, and its appearance was satisfactory throughout. Dissection. The hair came off moderately easily; the facial muscles were well preserved; the exposed coils of intestine hard, but those underneath were normal in shape and consistency; muscles normal in consistency, colour and shape. *Remarks.* This carcase was very well preserved.

In all cases, except *E* and *H*, the skin was very tough, in spite of the fact that the carcases were frequently wet with rain. In no case, except *E*, was there any appreciable smell.

In regard to these experiments the following points are noteworthy:

(1) Even under weather conditions very favourable to putrefaction small carcases exposed in the open can be preserved for months.

(2) Treatment of the skin with creosote oil prevents the external conditions from nullifying any antiseptic properties which the injected fluid may possess.

(3) Treatment of the skin with creosote oil repels flies, and preserves the carcase from the attacks of maggots.

(4) An extraordinarily good result was obtained when a very dilute solution of arsenic acid was used for injection, amounting to a concentration of 0·046 % in the water of the body (see p. 173).

(5) Excellent preservation of the body can be obtained even when the abdominal cavity is opened and the organs exposed, if the peritoneal surfaces and skin are treated with creosote oil.

(6) An open wound was left at the site of injection and none of the injection fluid passed into the vessels of this limb. In every case the muscles of this limb were in an advanced stage of decomposition.

The Distribution of Fluids injected into the Blood Vessels.

In considering the effects of injections of reagents into the blood vessels it is desirable to know to what extent distribution occurs when fluids are introduced soon after death into vessels containing blood. We injected the bodies of guinea-pigs through the carotid artery and the bodies of rabbits through the femoral artery, and never experienced any difficulty in making the fluids pass into the vessels. Very early in the process of injecting the fluid may be seen passing up the arteries and capillaries of the ear, and finally passing down the veins. If a loop of intestine be exposed the same phenomenon may be noticed, and by cutting the skin of the feet it may be shown that the fluid passes into the vessels of the extremities. In the case of fluids insoluble in water, such as creosote oil, we believe some of the blood is pressed to the sides of the vessels, but the greater part accumulates in the large veins. This is best shown by injecting colourless fluids, such as medicinal paraffin. In our endeavour to ascertain with more certainty the distribution of the fluid the vessels of a guinea-pig were injected with milk, and sections prepared of the liver, lung, kidney and muscle. Fat was found in large quantities in the larger and smaller vessels of the lung and kidney, but its distribution in the vessels of the liver and muscle was very irregular.

Substances soluble in the water of the tissues doubtless diffuse out of the vessels, and act upon the cells of the body.

Injections with creosote oil, or the phenolic bodies separated from it at full strength, even in small quantities cause immediate rigor in all the muscles, which remain in this condition for days. The other groups of constituents of creosote oil do not produce this effect.

Summary.

Temperature has a profound influence on the rate at which changes appear in a carcase. A very low temperature indefinitely postpones the apparent changes.

The optimum temperature seems to coincide with that which is most suitable to the activities of ferments and bacteria present in the living body. Alterations in temperature, such as occur in exposed carcases, during the day and night, probably influence putrefaction by their effects on bacterial activity, antagonism, and symbiosis. Variations in temperature conditions may produce changes more profound than would appear likely on superficial consideration. The optimum temperature for one group of organisms is different from the optimum conditions for another. Changes in temperature, therefore, especially if they are of some duration, are apt to favour some groups at the expense of others, and influence in this way the whole sequence of subsequent events.

Water is necessary to the growth of bacteria. It permits of their migration in the tissues, allows their ferments to act, distributes by diffusion the substances on which they live, and dilutes the toxic products of their activity.

The effects of temperature and moisture cannot be dissociated for it is in combination that these factors exert their greatest influence.

In a hot dry atmosphere the removal of water by evaporation may be so rapid that a small carcase may become desiccated before putrefaction has advanced far. In hot arid countries a variety of influences inimical to putrefaction are at work, especially when carcases are resting on dry sand. The strong sunlight tends to destroy the skin organisms, which we believe to be the most important agents in producing putrefaction. The skin soon becomes horny in consistency, imprisoning the organisms within it, and preventing their invasion of the tissues. The horny skin protects the carcases from moisture from any source, and the exudation of fluid finally results in mummification.

In a hot moist atmosphere sunlight is robbed of much of its bactericidal power, and the conditions most favourable to putrefaction prevail. Moisture from the air, rain water and water from the ground are absorbed and keep the skin moist. Suitable conditions for the growth and penetration of skin and soil organisms are thus created. Very soon the superficial layers of the epidermis are loosened and the sodden skin permits the autolytic enzymes, passing from the deeper parts, to exert their action on it and assist the putrefactive organisms. The aerobic conditions now prevailing in the skin aid disintegration. The protective influence of the skin is thus lost, and water has free access to the underlying tissues, where it exerts its elutriating and solvent effects. The products of putrefaction are diluted and removed and the dissemination of organisms favoured. Oxidation now plays some part in the process and disintegration proceeds rapidly. The presence of maggots seems to hasten putrefaction.

Such considerations ought to be sufficient to suggest the inadvisability of treating decomposing animal matter with watery solutions of antiseptics as is so often done. Here we may point out that the flesh can be completely macerated from the bones in six days, if a carcase is kept in water at 37° F. The progress is especially rapid, if the water is changed after three days, and the products which seem to inhibit bacterial growth removed.

Within a few days of death, the time varying with the conditions, certain phenomena are noticed if a carcase is kept under observation. It becomes distended with gas, fluid exudes from it, and the skin over certain areas becomes green. These phenomena are usually regarded as evidences of putrefaction. Since in properly treated carcases they are not followed by disintegration of the proteins, which form the main constituents of the body, and since they appear to be due partly to enzyme action and partly to the action of intestinal bacteria of the colon-type on such constituents as carbohydrates, and not to the action of putrefactive organisms, we consider that we are justified in regarding these phenomena as precursors of true putrefactive changes. By the latter term we understand the disintegration of the tissues by putrefactive organisms, a process accompanied by the elimination of foul-smelling products.

The evidence on which this conclusion is based has been given in detail in this section, and here we propose to discuss only the more important considerations.

The evolution of gas.

Under suitable conditions a carcase soon becomes distended with gas. This is due in the first place to the production of gas in the intestines from the carbohydrates of the food, and secondly to the production of gas in the liver and other tissues mainly from the dextrose, which is present before death or is produced after death by ferment action from glycogen, glucoproteins, etc., and hydrogen sulphide from free cystine. At 26·5° C. the production of gas from the tissues reaches its maximum about the third or fourth day. We believe that this early gas formation is due principally to the action of intestinal organisms, which are known to pass into the organs after death.

This view is supported by the results of experiments on carcases treated in various ways, which remained excellently preserved in spite of a large initial formation of gas. In one experiment (p. 138) on an opened carcase as much as 4·5 c.c. of gas per grm. more than would be sufficient to distend the carcase was produced, and subsequently every part of the body was found to be well preserved. We therefore contend that though the carcase becomes unsightly early distension is no criterion of true putrefaction.

Subsequent gas evolution, especially large daily productions in the second and third weeks, is some guide to the extent of putrefactive changes. The later gas arises from the disintegration of proteins, and the decarboxylation of the resulting amino acids, etc. A comparatively large total gas production per unit weight is associated with considerable bacterial disintegration.

Gas production in the tissues may be prevented by the injection of suitable reagents into the blood vessels.

The exudation of fluid from carcases.

Fluid, which is at first clear and yellow, begins to drain from the body after a few days. In small carcases it first appears, separating the superficial epidermis from the deeper layers of the skin, on the left side in the stomach region. We have shown that fluid was always exuded even from excellently preserved carcases, which produced no gas, the organs of which appeared to be sterile. The exudation of fluid is therefore not an indication of bacterial action or of putrefaction. We think that this fluid consists of serum, water from the cells and liquid from the intestines, and escapes owing to the action on the cells of intestinal and autolytic enzymes. Fluid also exudes from sterile

organs excised from the body and kept in sterile bottles. In untreated carcases fluid drains from them continually and becomes opaque, red-brown and offensive.

Chemical analyses of putrefying substances.

The study of the actions of pure and mixed cultures on sterilised materials, while affording evidence of great value on the biology of certain species of bacteria, and the effects of symbiosis, omits to take into consideration factors which operate in the very complicated processes of putrefaction as it occurs in nature. Natural putrefaction is a process which results from the combined actions of enzymes and various groups of bacteria. By applying suitable methods to the products figures should be obtained, which indicate the collective results.

We have studied these processes as they occur in (1) finely divided animal material in suspension and (2) small carcases under various conditions. We submit a method of determining the ratios of volatile bases to amino acids in the products, which we claim shows the collective results at any stage. The application of this method to the tissues of treated and untreated carcases and the fluids exuding from them reveals the comparative power of disinfectants. Descriptive methods only enable us to discriminate between antiseptics of very different power, but this method enables us to place disinfectants of moderate power in order of merit.

By applying this method to cultures in tryptic digests and amino acid mixtures it should be possible to compare the effects of various species of organisms in pure cultures and in combination.

Stench and deodorants.

The stench from a decaying carcase is a combination of odours and varies in character at different times. The component odours of the stench mainly arise from organic bases and organic acids and sulphur compounds.

Deodorants of a purely acid nature can only fix the bases, while setting free the organic acids responsible for rancid odours. In like manner basic deodorants fix organic acids and set free bases. On the other hand some deodorants, such as oxidising agents, may destroy substances giving rise to odours and not merely fix them. Chemical action resulting in such destruction may be facilitated by the deodorant containing solvents for the constituents of the odour. Some deodorants

only dissolve noxious substances and hold them in solution for longer or shorter periods, according to their rate of evaporation. The period during which a deodorant remains operative depends to a large extent on its rate of evaporation, degree of solubility in water and its power of stopping putrefactive changes in the substances with which it comes into contact.

In estimating the actions of a deodorant it must be remembered that some reagents used for this purpose affect the nasal mucous membrane.

We consider that a perfect deodorant should contain chemical substances capable of eliminating all the constituents, which go to make up the stench. We are only acquainted with one fluid, with a not unpleasant smell, creosote oil, which possesses the necessary constituents and characters of a cheap and satisfactory deodorant.

The skin in relation to putrefaction.

Experimentally the conditions assisting putrefactive changes are exceptionally favourable when the bodies of small animals are kept in bottles at a temperature of 26·5° C. Even under these conditions the carcases can be excellently preserved if the skin is treated thoroughly with such fluids as creosote oil. The application of such fluids acts in three ways, (1) by killing many of the skin organisms, (2) by hardening the skin and thus increasing its protective properties and imprisoning organisms in it, and (3) by preventing the entrance of water and air into the carcase. Treatment of the skin does not interfere with the invasion of the tissues by intestinal gas-forming organisms, and yet in skin treated bodies disintegration of the tissues does not occur. We therefore believe the skin to be the chief source of putrefactive organisms. Chemical analyses of the tissues of skin treated bodies and the fluids draining from them confirm the opinion formed on dissections of the bodies.

These carcases though excellently preserved become distended with gas and exude fluid. In the hope of finding some means of inhibiting the activity of the intestinal bacteria, which cause the production of gas, and of checking the production of fluid through cytolysis and enzyme action, we injected the vessels of skin treated animals with various reagents.

Injections into the blood vessels.

In regard to the effects of injections into the blood vessels, uncombined with skin treatment, we suggest the following points for consideration.

The injection of a disinfectant produces an effect depending upon its nature, and the amount employed. The amount required to prevent putrefaction is influenced by the degree of fixation of the reagent by the tissue constituents. Aqueous solutions are diluted by the fluids of the body, and fixation commences at once. Oily fluids when injected are distributed throughout the smallest capillaries and diffusion of the bactericidal constituents contained in them is very slow. The injection of sufficient quantities of antiseptics in this manner is advantageous because (1) being protected in the oily particles they are not immediately fixed; (2) they continue to act for long periods, and (3) they are carried to and remain in those peripheral parts of the body it is most desirable to reach. The effects on organisms depend on (1) the concentration of free disinfectant in their vicinity, and (2) the length of time during which it acts. We have found the organs and muscles sterile after injection with phenolic bodies (p. 164). A large injection of creosote oil results in a fair degree of preservation of a carcase.

While preservation by means of injection alone requires the use of large quantities of disinfectants, extraordinary good results can be obtained by combining injection with skin treatment. For example bodies skin treated with creosote oil and injected with arsenious oxide or sodium nitrite never produced gas and exuded little fluid and were exceedingly well preserved though kept for long periods under conditions very favourable for putrefaction.

Small quantities of weak acids and ammonia seem to hasten dissolution even when injection has been combined with skin treatment.

The value of a reagent for stopping putrefaction should be gauged according to its capacity to preserve a body kept under conditions favouring rapid putrefaction.

Part III. Experiments on Maggots and Exposed Carcases.

Experiments on Maggots with Coal-Tar Oils and their Constituents.

In summer time fly maggots play such an important part in the destruction of exposed carcases, both by devouring the tissues and by altering the conditions under which putrefaction is proceeding that it may be best at this point to consider the action of coal-tar products on them.

In the series of experiments described in the following pages very large maggots, taken from carcases, were employed, since in preliminary tests we had found them to be much more resistant to the action of chemical agents than young and half grown specimens. In most cases six maggots were treated for a definite time with the reagent, then placed on blotting paper to remove the greater part of the reagent and finally in clean glass vessels in which the results could be watched.

TABLE XXVIII.

The effects of coal-tar oils on maggots. In each case six large maggots were dipped into the oil, immediately placed on blotting paper and then in glass vessels.

Reagent		2 hours	24 hours
Crude carbolic oil	motionless, 5 mins.	still; contracted	dead with brown patches
"Middle oil"	motionless immediately	,, ,,	dead, 2 brown, 4 yellow
Creosote oil	motionless almost at once	,, ,,	dead, slightly brown
"Heavy oil"	motionless in 2 mins.	,, ,,	dead, yellowish
Anthracene oil	moving freely in 10 mins.	still, but able to move head	All still, but heads capable of slight movement

By "still" we mean incapable of voluntary movement.

The first four oils killed these large maggots in a very short time, although they were placed in them only momentarily. Anthracene oil, by far the least effective, is the last fraction distilled from tar above 270° C., and is very deficient in phenolic bodies.

Six maggots were sprayed very lightly with creosote oil, immediately placed on blotting paper and then in a glass. Four of the six were motionless in two minutes. After two hours four were capable of moving. In six hours three were moving, and three were motionless, but capable of retracting their heads when touched. After 24 hours three were dead, two capable of retracting their heads, and one capable

TABLE XXIX.

Effects of fractions of crude carbolic, "middle" and "heavy" oils on large maggots. The same method was employed as in Table XXVIII. The controls were very lively when last observed after 48 hours.

Crude carbolic

		24 hours
(1)	Fraction up to 140° C.	3 pupated, 3 moving
(2)	,, 140—165° C.	3 retractile*, 1 dead, black, 2 dead, white.
(3)	,, 165—180° C.	4 dead, black, 2 dead, white
(4)	,, 180—187° C.	3 dead, black, 3 dead, brown
(5)	,, 187—197° C.	All dead with brown patches
(6)	,, 197—201° C.	All dead, soft, with brown patches

"Middle oil"

		Immediate	2 hours	24 hours
(1)	Fraction up to 174° C.	Motionless	Head brown, red patches on body	All brown-black and shrivelled
(2)	,, 174—184° C.	,,	Red patches on body	,, ,, ,,
(3)	,, 184—188° C.	,,	,, ,,	,, ,, ,,
(4)	,, 188—200° C.	Motionless	Red-brown	,, ,, ,,
(5)	,, 200—210° C.	,,	Red-brown patches	Blacker and more shrivelled
(6)	,, 210—220° C.	Almost motionless	,, ,,	Brown and slightly shrivelled
(7)	,, 220—230° C.	,, ,,	,, ,,	,, ,, ,,
(8)	,, 230—240° C.	,, ,,	,, ,,	,, and more shrivelled
(9)	Residue	Moving slightly in 5 mins.	,, ,,	3 dead and 3 retractile, flaccid, extended, not discoloured

"Heavy oil"†

		Immediate	15 mins.	4 hours
(1)	Fraction up to 200° C.	Motionless	All dead, contracted‡	Becoming red
(2)	,, 200—220° C.	,,	,, ,,	,, ,,
(3)	,, 220—240° C.	,,	,, ,,	,, ,,
(4)	,, 240—260° C.	Almost motionless	5 dead, 1 retractile	All dead
(5)	,, 260—280° C.	Not apparently affected	All retractile	Retractile
(6)	,, 280—300° C.	,, ,,	5 retractile, 1 moving	,,
(7)	Residue	,, ,,	Very lively	Retractile or moving

Creosote oil

		Immediate	45 mins.	3 hours
(1)	Fraction up to 170° C.	Motionless	Motionless, contracted	Contracted, not coloured
(2)	,, 170—200° C.	,,	,, ,,	,, ,,
(3)	,, 200—220° C.	,,	,, ,,	,, ,,
(4)	,, 220—240° C.	Almost motionless	,, ,,	,, ,,
(5)	,, 240—260° C.	Moving in 2 mins.	,, ,,	,, ,,
(6)	,, 260—280° C.	Moving rapidly in 2 mins.	Moving slightly	Retractile
(7)	,, 280—300° C.	Moving rapidly	Head retractile	,,
(8)	Residue	Lively	Very lively	Not moving, but irritable

* =capable of retracing the anterior end of the body when touched.

† =two minutes' exposure.

‡ =a condition like rigor and hard to the touch.

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of moving slowly. Six others, which were not placed on the blotting paper, soon became motionless. All were dead and brownish in colour in 24 hours. We next proceeded to test the actions of various fractions obtained by distillation of crude carbolic, "middle" and "heavy" oils.

These experiments show that the potent constituents for killing maggots appear to be mainly contained in the fractions which distill over below 240° C.

Since other experiments had shown us that creosote oil was the best as a deodorant and preservative, we decided to investigate the effects of some of its constituents on maggots.

These experiments (Table XXX) show that in the absence of water the phenolic bodies are extremely toxic to maggots. Immediately on being taken out of the fluid the maggots become contracted, hard and tense. Very soon a red tinge appears in patches, and within 15 minutes they assume a deepened colour. After 24 hours they become black (cf. rigor in bodies, p. 178). The bases produce the opposite effect. The maggots remain white, but become extended and flaccid. The higher boiling fractions of the hydrocarbons are decidedly more toxic than those of lower boiling points. Each group of constituents of creosote oil possesses some degree of toxicity to maggots.

In another experiment, using maggots from another source, the bases caused a small percentage of deaths, but the fractions 190–220° C. and onwards of the hydrocarbons killed all the maggots treated. In considering these results the very short exposure should be borne in mind.

It may be seen from Table XXXI that in most cases momentary immersion in dilutions of the highly toxic constituents in water or inert fluids produces very little effect on the maggots. The presence of water appears to rob phenolic bodies of their toxic action to a large extent, for example 12·5 % phenolic bodies in water had no apparent effect. With more prolonged treatment maggots may be killed, if dilution is not carried too far.

In order to test this point further experiments were carried out on large maggots with emulsions (*A*, *B* and *C*) containing the cresols separated from creosote oil. Emulsion *A* contained 5 % of the phenolic fraction distilling over between 191–200° C. emulsified with the aid of 2% of soft soap. Maggots kept in this emulsion for 45 minutes and then placed on blotting paper never appeared to be affected in any way, and pupated. Other maggots kept in the emulsion for 5 minutes were unaffected, and subsequently fed well for eight days on meat which was provided for them, and finally pupated. In another experiment

TABLE XXX.

Showing the effects of fractions of creosote oil on large maggots. Six large active maggots were used. They were dipped into the fluid and immediately placed on blotting paper, and finally in glass vessels.

Separated from creosote oil fraction 170—220° C.

	Immediate	1 hour	24 hours
Fraction of creosote oil from 170—220° C.	motionless	all dead, flaccid	brown
220—240° C.	„	„ „	„
Residue	—	just capable of moving	moving or retractile

Phenolic bodies	Immediate	2 hours	24 hours
Complete mixture	motionless almost immediately, red in 8 minutes	dead, hard, retracted, brown-red	dead, black
Fraction 77—191° C.	motionless, red in 15 minutes	dead, hard, retracted, red	dead, black or black patches
„ 191—200° C.	„ „	„ „	„ „
„ 200—210° C.	motionless, tinged with red 15 mins.	„ „	dead, 5 black, 1 white
Residue	motionless quickly	retracted, less red	dead, black in patches

Bases	Immediate	2 hours	24 hours	3 days
"Water sol. fraction" (p. 159)	2 moving, 4 relaxed, motionless	extended, soft, retractile	2 dead, 2 retractile, 2 moving	3 dead, 3 pupating
"Water insol." „	2 motionless, relaxed	„ „	non-retractile, extended, flaccid	All dead

Hydrocarbon			
Complete mixture	—	2 motionless, 4 retractile	6 moving	4 dead, flaccid 2 pupae
Fraction 80—170° C.	—	1 motionless, 3 retractile, 2 moving	6 moving	2 dead, flaccid 4 pupae
„ 170—180° C.	—	1 retractile, 5 moving	6 moving	2 dead, flaccid 4 pupae
„ 180—190° C.	—	2 retractile, 4 moving	5 moving, 1 dead	1 dead, 5 pupae
„ 190—200° C.	—	6 slightly retractile, retracted	5 moving, 1 retractile	3 retractile 3 pupae
„ 200—210° C.	—	6 contracted	5 moving, 1 retractile	4 dead 2 pupae
„ 210—225° C.	—	6 contracted	3 moving, 3 retractile	3 dead 3 pupae
„ 225—240° C.	—	5 motionless contracted, 1 retractile	6 dead	6 dead, decomposed
Residue	—	4 motionless, 2 retractile	5 dead, 1 retractile	6 „ „

	Immediate	2 hours	24 hours
Fraction creosote oil 170—220° C. 2 pts. „ phenolic bodies 77—191° C. 1 pt.	motionless	dead, retracted, red	hard, black
Fraction creosote oil 170—220° C. 2 pts. Hydrocarbons, compl. mixt. 1 pt.	almost motionless	—	dead, soft, brown in patches
Fraction creosote oil 220—240° C. 2 pts. „ phenolic bodies 77—191° C. 1 pt.	motionless	dead, retracted, red in patches	black, brown, hard
Fraction creosote oil 220—240° C. 2 pts. Hydrocarbons compl. mixt. 1 pt.	„	—	dead, soft, brown

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TABLE XXXI.

Further experiments with various extracts and emulsions of constituents of creosote oil. In each case six maggots treated.

	30 mins.	2 hours	24 hours
5 % aqueous emul. of fraction 170—220° C. + 1 % bile	no apparent effect	all moving freely	all moving freely
5 % aqueous emul. of fraction 170—220° C. + 2 % soft soap	,, ,,	,, ,,	,, ,, ,,
Aqueous extract containing phenolic bodies (0·63 % calculated as cresols)	,, ,,	,, ,,	,, ,, ,,
Aqueous solution 'calcium cresolate'* (0·91 % cresols)	,, ,,	,, ,,	,, ,, ,,
Mixture of equal parts hydrocarbon frac. 190—200° C. phenolic frac. 191—200° C.	still immediately; red in 6 mins.	dead, brown-red	black
Above mixture 1 part, medicinal paraffin 2 pts.	still almost immediately; contracted, red 15 mins.		dead, brown
Above mixture 1 pt., medicinal paraffin 9 pts.[1]	still 4 mins.		all alive third day
Above mixture 1 pt., medicinal paraffin 99 pts.[2]	no effect		,, ,,
Above mixture 1 pt., water 3 pts.†	35 mins. all moving		,, ,,
,, ,, 1 pt., ,, 9 pts.[3]	,, ,,		,, ,,
,, ,, 1 pt., ,, 99 pts.[4]	,, ,,		,, ,,
Medicinal paraffin (10 mins. exposure)	,, ,,		,, ,,
Olive oil (10 mins. exposure)	,, ,,		,, ,,

* Creosote shaken up with milk of lime and filtered. The filtrate contains the calcium salt of phenolic bodies equivalent to 0·91 %, calculated as cresols.

† Emulsified with the aid of bile salts.

[1] Exposure of one minute causes the maggots to become motionless and retracted; red tinge appears in 20 minutes, and all die.

[2] Exposure of seven minutes has no effect.

[3] Exposure of 30 seconds causes the maggots to become motionless and retracted; a red tinge appears in 20 minutes and all die.

[4] Exposure of seven minutes makes the maggots at first motionless and retracted, but they recover in 30 minutes. Even small maggots are not killed.

five small maggots were kept in the emulsion for 15 minutes. When placed on blotting paper they were motionless and flaccid. 20 minutes later they showed signs of recovery. A piece of meat soaked in the same emulsion was now placed in the vessel. Within 24 hours four of the maggots had completely recovered and were eating the meat. The other recovered later, and all were well and feeding on the eighth day. Finally all pupated, and flies emerged from the pupae. Maggots

kept for 15 minutes in water were little affected, and after feeding for a few days pupated. Maggots placed on fresh meat soaked in this emulsion attacked it immediately, never showed any signs of ill health and pupated on the 8th day. Flies emerged from all these pupae.

An emulsion, *B*, containing 2·5 % of the phenolic fraction distilling over between 191–200° C. and 2·5 % of the fraction distilling over between 200–209° C. emulsified with the aid of 2 % soft soap, and another emulsion, *C*, containing 5 % of the complete mixture of all the phenolic bodies (tar acids) contained in creosote oil were tested. During five minutes' exposure the maggots kept turning over, and on being removed were somewhat rigid. Within 24 hours all had recovered, and eventually pupated.

Not one maggot was killed by exposure to these emulsions[1].

Experiments were also carried out to test the action of creosote oil on maggots present in carcases.

The bodies of six guinea-pigs which had been dead ten days, and were much decomposed and contained innumerable maggots of all sizes, were placed in a large glass vessel and sprayed with 20 c.c. of creosote oil. A careful examination made 24 hours later showed that all the maggots were dead and black. In another case the carcase of a guinea-pig full of large maggots was sprayed with 5 c.c. of creosote oil. Within 15 minutes all the maggots were dead. Next day their bodies were dark red or black (Plate IV, fig. 9).

The body of a guinea-pig lying in the open and full of maggots was treated with 25 c.c. of creosote oil. On examination next day thousands of dead maggots were found in various parts of the carcase, but not a single living specimen.

Also the body of a goat was treated. Most of the thoracic contents and the greater part of the back had been eaten by maggots and very large numbers of maggots were present in these situations. About 400 c.c. of creosote oil were poured into those situations and over other parts of the carcase. Within an hour all the maggots seemed to be dead. Careful examination next day proved that all the maggots were dead and brown.

These and other experiments prove that by suitable treatment with creosote oil all the maggots present in a carcase can be killed.

The conditions under which creosote oil preserves carcases from the attacks of maggots are dealt with in the following sections.

[1] We have not had the opportunity of comparing the toxicity to maggots of pure ortho-, meta- and paracresol. (See experiments with the toluidines p. 114.)

EXPERIMENTS DESIGNED TO TEST THE EFFECTS OF TREATING SMALL CARCASES EXPOSED IN THE OPEN WITH VARIOUS TAR OILS AND OTHER REAGENTS.

In the following experiments the carcases of freshly killed guinea-pigs were treated and left on the ground. All these experiments were started on 25 August. The bodies were examined almost daily.

1. Weight 315 grms. Intact. Skin treated with 15 c.c. of creosote oil, except head, anus and inner sides of thighs. 2nd day, some eggs on under side of head. 5th day, head almost completely eaten by maggots, and some eggs on anus. 8th day, head completely eaten and maggots seen on body under fore leg. No maggots found elsewhere. 15th day, skin intact wherever treated. Maggots had eaten the rest of the carcase, only bones and faecal material being found within the skin. *Remarks.* This experiment shows that the treated parts of a carcase are preserved from the attacks of maggots, but that eggs may be laid on untreated portions, and the maggots make their way into the body from such places. The necessity for thorough treatment of the skin and natural orifices is very clearly indicated.

2. Weight 365 grms. Intact. Whole surface of skin and orifices treated with 15 c.c. of creosote oil containing 2 % aniline. 3 c.c. injected into abdominal cavity and 1 c.c. into each pleural cavity. Every day for a fortnight, except when rain fell, water equivalent to one-eighth inch of rain was evenly distributed over the body and a circular area 8 inches in diameter. No eggs found up to the 15th day, when a few eggs found on nose, anus and thigh. On 22nd day a few scattered eggs on the coat. The body had a slight smell. 26th day, eggs had not hatched, and none freshly laid. 34th day, examination showed a little gray, pasty material on the under side probably arising from the exuding fluid. Smell slight. Few eggs, no maggots. On 49th day, condition similar. Skin of under side moist and tough. *Remarks.* In spite of frequent applications of water the body was well preserved for several weeks, and was never attacked by maggots. The few eggs deposited never hatched.

3. Weight 390 grms. Intact. Skin and orifices treated with 15 c.c. of creosote oil. The intestines of another guinea-pig were placed one inch from the body as a source of maggots. On 2nd day numerous eggs were present on the intestines, and on 3rd day hundreds of half-grown maggots. 5th day intestines almost eaten and numerous maggots wandering on them and on the surrounding ground. The carcase was quite free from eggs or maggots. 8th day, three large maggots brown and dead were found on the body, and a living maggot on the ear. These had migrated from the intestines. 14th day, no eggs or maggots. Body dissected and found to be excellently preserved. Muscle looks normal, peritoneal surfaces glistening and intestines well preserved. Abdominal walls not green. Slight rancid smell which disappeared quickly on exposure to air. *Remarks.* Surface treatment alone kept the body in an excellent state of preservation for a fortnight. No eggs were deposited on it, and no maggots migrated on to the body, in spite of large numbers being present in the intestines close by.

4. Weight 360 grms. Abdomen and thorax opened. External surfaces of the body and serous cavities treated with 25 c.c. of creosote oil, containing 5 % aniline. 5th day, an egg found on the lip. 8th day, same egg unhatched. 15th day, a few eggs on the nose. 22nd day, a few scattered eggs. 26th day, condition excellent. 34th day, same. 49th day, many eggs on surface, but no maggots seen. A moist patch on the skin underneath. Smell very slight. *Remarks.* Even though the body was opened it was well preserved by superficial treatment for seven weeks. Towards the end of this period eggs were deposited but no maggots developed from them, and a very slight amount of putrefaction had occurred as shown by the smell.

5. Weight 380 grms. Abdomen and thorax opened. Skin treated with 20 c.c. of creosote oil, and orifices and serous surfaces with 12 c.c. No eggs deposited up to the 8th day. Two eggs found under nose on the 15th day. On 22nd day a few scattered eggs. Condition excellent. 26th and 34th days, same. 49th day, skin and intestines very well preserved. Very slight smell. Numerous eggs but no maggots on the body. *Remarks.* This is another example of an opened body very well preserved by superficial treatment for seven weeks. Again though eggs were deposited towards the end of this period no maggots developed from them.

6. Weight 394 grms. 19 c.c. of creosote oil containing 2 % aniline used in treatment of skin and orifices. Then abdominal and thoracic cavities opened and treated with 30 c.c. of 10 % hydrochloric acid. On 5th day, five small patches of eggs were found on the intestines. 8th day, the eggs had not hatched. 15th day, most of the eggs had not hatched, but a few large maggots were found in the body, but none on the oil treated parts. 22nd day, numerous half-grown maggots in the abdomen. None on surface. 26th day, the maggots have eaten a large part of the thorax, but have not touched the skin. 34th day, skin and intestines well preserved, but contents of the thorax completely eaten. *Remarks.* The parts treated with creosote oil were well preserved, but maggots made their way into the body from parts not so treated. (See No. 1.)

7. Weight 390 grms. Skin treated with 16 c.c. of creosote oil and 2 % aniline, orifices with 2 c.c. Abdominal cavity opened and 2 c.c. injected into stomach, and 3·5 c.c. into coecum. Exposed serous surfaces not treated. 5th day, no eggs; intestines appeared normal. 8th day, a few eggs on the lips and near the abdominal wound. 15th day, eggs on anus and in mouth. None on the intestines. 22nd day few scattered eggs on surface. 26th day, no freshly laid eggs. Hair loose underneath. 34th day, few eggs, no maggots, very well preserved. 49th day, many eggs; no maggots; very slight smell; very well preserved. *Remarks.* The result of this experiment is most interesting, but in the absence of further experiments of this nature we are not in a position to make important deductions from it.

8. Weight 370 grms. Stomach and intestines removed and the cavity filled with hay and abdominal walls approximated with sutures. Skin treated with 20 c.c. of creosote oil and orifices with 8 c.c. Up to the 15th day no eggs deposited. 22nd day, a few scattered eggs. 26th day, very well preserved. No eggs. 34th day, a few eggs, but no maggots. 49th day, eggs had been deposited, but the body was well preserved. *Remarks.* There seems to be no advantage gained by removing the stomach and intestines.

9. Weight 375 grms. Abdominal and thoracic cavities opened and serous surfaces and natural orifices treated with 9 c.c. of creosote oil and 2 % aniline. Skin not treated. The carcase was laid on its side on a patch of ground painted with the same solution. 2nd day, seven eggs on chest. 5th day, few eggs on the chest and back. 8th day, numerous small maggots in skin over lumbar region. They had made a large hole through the skin. No maggots between the skin and the ground, nor on intestines or head. 15th day, many maggots under head and in eye and mouth. Several holes through the skin of the back. No living maggots on the intestines, though some dead maggots were found amongst the intestines and on the ground. 22nd day, the muscular tissues had been eaten by maggots. *Remarks.* This experiment again shows the necessity for complete surface treatment.

10. Weight 371 grms. The carcase of an animal dead 48 hours but apparently without eggs or maggots, was sprayed so as to moisten the whole surface with 12 c.c. of creosote oil. On 2nd day no fresh eggs laid on it, but medium sized maggots, dead and brown, were protruding from the mouth. These were apparently in the body before treatment. During the next five days no fresh eggs were laid. 13th day, a few fresh eggs but no maggots. 17th day, a large hole was seen in the abdomen and large maggots inside the carcase. These no doubt were present in the carcase before treatment. Skin well preserved. 26th day, internal organs partially eaten by maggots; skin well preserved. 40th day, no maggots now on the carcase. The internal parts much decomposed. Skin well preserved. *Remarks.* Spraying into the mouth failed to kill some of the maggots present in the body. These maggots devoured as much of the body as they could, but no fresh maggots from eggs laid on the body after spraying attacked it.

11. Weight 390 grms. Intact. Skin and orifices treated with 20 c.c. of "heavy oil." 5th day, no eggs. 8th day, carcase distended with gas, no eggs. On 12th day, no eggs. The body was opened. The muscles looked fairly well preserved, but gas was found between the muscles, and in the abdominal cavity. Peritoneal surfaces not glistening, though intestines well preserved. Abdominal muscles greenish. Slight putrid odour. Hair comes off over abdomen. *Remarks.* This carcase, although moderately well preserved, was not so well preserved as No. 3. It should be noted that both bodies were of the same weight, and 20 c.c. were used in the treatment of No. 11, and 15 c.c. in the treatment of No. 3.

12. Weight 380 grms. Intact. Skin and orifices treated with 20 c.c. of "heavy oil" containing 2 % aniline. The carcase was daily treated with water in the same way as No. 2. No eggs were found up to the 8th day. 15th day, some scattered eggs were found on the head, ears and thigh. On 22nd day, hair loose underneath. Slight smell. 26th day, no maggots or fresh eggs. 34th day, gray, pasty material on under side. Few fresh eggs. 49th day, condition similar to No. 2. *Remarks.* The "heavy oil" exerted a similar influence to creosote oil under similar conditions as may be seen by comparing Nos. 2 and 12. The former was however the better preserved of the two.

These experiments show that in warm and showery weather, particularly favourable for rapid putrefactive changes, at the height of

the fly season the bodies of small animals can be well preserved for several weeks by efficient surface treatment with coal-tar oils, especially creosote oil. Flies are deterred from laying eggs for a considerable time, and though finally many eggs may be deposited maggots seldom develop from them. In order to obtain such results surface treatment must be thorough for eggs may be deposited on untreated areas and maggots developing from them make their way into the carcase from these areas. Even opened carcases can be similarly preserved from putrefaction and the attacks of maggots, if the exposed surfaces are treated. No advantage is obtained by removing the stomach and intestines. Maggots do not migrate on to treated carcases, even though their supply of food is exhausted.

The influence of creosote oil extended over several weeks even though the bodies were frequently wetted by rain, and some of them wetted with water daily during the first fortnight. Dissections showed how efficiently treatment with creosote oil protected the bodies from the influence of rain and soil water.

It was particularly noticeable that while untreated carcases were covered with flies, very few alighted on the treated carcases, even after three weeks, and these never remained long on them.

Our experiments with small bodies had given such satisfactory results that we decided to test the actions of various reagents on the carcases of larger animals, such as goats. In all cases the carcases were laid on the ground on their right sides in the middle of a large lucerne field, and lightly covered by sacking supported by hurdles.

Goat I. Weight 25 lbs. Killed 20 August. 48 hours later, when a few eggs had been deposited, 270 c.c. of double strength "Solution B" (p. 120) were injected into the femoral artery, and the skin and orifices treated with 900 c.c. of the same solution. On the 1st day after treatment many eggs were found on the lips, flank and anus. On the 3rd day many more eggs had been deposited, and many flies, mainly *Lucilia*, were visiting the carcase. On 4th day eggs were very numerous, but no maggots were seen. The abdomen was slightly distended, subcutaneous emphysema was present in places, and the skin was green. On the 6th day, wound, mouth, and eyes were free from eggs. Many small maggots were present on the under side and between the thighs. The eggs laid on the upper side had not hatched. 8th day, condition similar, but superficial epidermis of legs becoming loose. 11th day, hair loose except in the driest parts and maggots on the skin; small maggots very numerous underneath and round the anus. 19th day, almost all the maggots are dead. The flesh does not seem to have been eaten. Smell slight. On cutting into it the thigh muscle was found to be well preserved. 21st day, very few maggots on the surface, though some seemed to be present under the skin. 24th day, beetles have made shallow holes through the skin. A few sluggish maggots

found underneath the body. 36th day, numerous small maggots in certain areas of the body. 43rd day, some half-grown maggots present in the thorax, and mouth. The exposed thigh muscle was very much decomposed. 59th day, the body disintegrating and numerous maggots in the thorax and round the anus. 107th day, the remains of the body appeared to be in the same condition as on the 59th day.

Remarks. Although innumerable eggs were laid on the carcase and maggots developed from them they grew very slowly and hardly any survived. Even though some of the maggots eventually attained a large size they were sluggish and matured slowly. Putrefaction was delayed to some extent. The effect of this treatment is best seen by comparison with the control Goat VIII.

Goat II. Weight 25 lbs. Killed 20 August. 46 hours later 90 c.c. of crude carbolic acid were injected into the femoral artery, and the skin and orifices treated with 800 c.c. of "Solution B." On the first day after treatment eggs were deposited on the flank and round the anus. On 3rd day many more eggs on legs and abdomen, but not elsewhere. Wound free. 4th day, very small maggots found near the ground, but not elsewhere. Skin green in places and some subcutaneous emphysema. 6th day, numerous very small maggots in wound and on under surface of the body. 8th day, hair loose near ground, and many small maggots on the skin in these regions. 19th day, a large hole had been made into the thorax, and innumerable maggots were seen in the lung, in femoral wound and under the thigh, where they had eaten through the skin. 22nd day, the maggots had made further progress. 24th day, most of the thorax and a large part of the hind quarters had been eaten by maggots. 400 c.c. of creosote oil were poured on to the body and into the holes caused by the maggots. About one hour later the maggots were found to be dead. 27th day, all the maggots were dead and brown, except a few on a spot on the leg which had not been treated. 36th day, no living maggots seen. 44th day, no living maggots seen. There was no smell, and the treatment with creosote oil seemed to have completely preserved the remains of the body. On the 59th and 107th days, the body seemed to be in the same condition.

Remarks. The early skin treatment delayed the growth of the maggots to a considerable extent, and the injection seems to have delayed putrefaction, but no apparent difference was made to the maggots when they penetrated into the tissues. The further treatment with creosote oil killed the innumerable maggots present in the carcase, prevented eggs from being deposited subsequently and completely checked further putrefactive changes.

Goat III. Weight 122 lbs. Killed 20 August. Treated 28 hours after death. 560 c.c. of a fluid consisting of commercial formalin one part and 10 % aqueous solution of sodium chloride four parts injected into the femoral artery. Skin and orifices treated with 2000 c.c. of "Solution B 1." On the 1st day after treatment no eggs or maggots were seen. 2nd day, innumerable eggs in coat over abdomen and patches in mouth and near the wound. 4th day, numerous flies on the carcase, which smells considerably. Skin becoming green. Many more eggs have been deposited on the back of the neck and near the ground, and there were thousands of small maggots near the wound. 5th day, many maggots of the same size in other situations, and a small hole had been made through the skin near the anus. Skin very green, and the body greatly distended with gas, though the hair was not loose.

Owing to the condition of the body the skin was treated with 350 c.c. of creosote oil, and the gas let out by small openings into the peritoneal and pleural cavities, and through each hole thus made 50 c.c. of creosote oil were poured. 7th day, all maggots dead, and smell slight. On 9th and 12th days, no eggs or living maggots seen. Smell hardly perceptible. On 19th day, the body seems very well preserved; slight rancid smell. 25th day, some very small maggots near the ground. The body now smells more than before. 200 c.c. of creosote oil were put into the cavities and 200 c.c. on to the hair touching the ground, without moving the body. 28th and 37th days, no living maggots seen. 45th day, no living maggots seen; some smell. The body can be dragged along the ground by the horns. 60th day, no living maggots or fresh eggs seen. Slight smell. Skin moist underneath. 107th day, no visible change. The body lay in the field without further treatment and unprotected till 27 October, 1916, a period of 14 months. At this time the body was opened, found to be well preserved, and burnt.

Remarks. The injection of formalin did not prevent gas formation or discoloration of the skin, changes which are preliminary to disintegration (p. 179). Subsequent treatment with creosote oil killed the maggots and, though there was some smell while the gases from the emphysematous tissues were escaping, the body was so well preserved that it remained on the ground without apparent change for a year.

Goat IV. Weight 22 lbs. Killed 20 August. Treated 28 hours after death. 110 c.c. of a fluid consisting of one part commercial formalin and four parts 10 % aqueous solution of sodium chloride injected into the femoral artery, and the skin and orifices treated with 190 c.c. creosote oil containing 2 % aniline and 1 % ox bile.

On the 1st, 2nd and 4th days, the body was observed for some time and no flies were seen to approach it and not a single egg was found on it. The surface was oily. After 4th day, the covering of sacking was removed. On 5th day, no eggs or maggots were seen. Skin not green; surface moist with oil. 7th day, no eggs or maggots seen, a few flies alighting on the upper drier surface. 9th day, a few eggs on neck, none seen elsewhere; a few flies. 12th day, not distended. A few dead brown eggs on neck. No living maggots. Hair not loose. 20th day, very numerous eggs on back, neck, thigh and near ground. A few tiny maggots on upper shoulder. Skin is now not moist with oil on the upper surface. 23rd day, maggots have grown slightly on back and neck. The body could be lifted by one leg. 400 c.c. of creosote oil put on the skin. 28th and 37th days, no living maggots seen. 45th day, no living maggots seen; slight smell. 60th day, no maggots and no signs of decomposition, but a few eggs on the back. 107th day, condition same. The carcase remained in much the same condition during the next year.

Remarks. In the early period the condition of this carcase was better than that of No. III. No eggs were deposited on it for nine days. In both cases the original treatment was the same, but while in No. IV the skin treatment with creosote oil was carried out the day after death, in No. III this was not done till the 6th day. It should be noticed, however, that in relation to its weight the surface of No. IV was much greater than that of No. III and more creosote oil was applied per unit weight.

Goat V. Weight 76 lbs. Treated 28 hours after death. 150 c.c. of creosote oil were injected into the femoral artery, and 45 c.c. of creosote oil containing 2 %

aniline and 1 % bile into the subcutaneous tissues in several places, the sides of the neck, flanks and proximal parts of the limbs, and into the abdominal and right pleural cavities. The skin and orifices were treated with 350 c.c. of the same solution.

On the 1st and 2nd days no eggs were found and it was noticed that flies did not approach the body. 4th day, abdomen distended, but no eggs have been deposited. 5th day, skin turning green; no eggs found. 7th day, much distended; skin green near thigh wound. Some eggs in left nostril. 12th day, a few eggs on skin, lip and tongue. The eggs seen previously in the nostril have become brown and shrivelled. No maggots seen. 20th day, a few very small maggots in wound. Many eggs near the ground. The eggs previously noted appear to be dead. 25th day, a very few small maggots near ground, but none elsewhere. Some smell. Small openings were made into the thorax and abdomen to let out the gas. The organs appear to be in an excellent state of preservation. 450 c.c. of creosote oil put into these openings and 350 c.c. on to the skin. 28th, 37th, 45th and 60th days: no living maggots found. Very slight smell. 107th day, same. The body was left on the ground without further treatment until 27 October, 1916, 14 months in all, and on dissection was found to be very well preserved.

Remarks. The early phenomena of distension and green discoloration of the skin were again evident, but the body remained for 14 months in a remarkably good state of preservation.

Goat VI. Weight 65 lbs. Killed and treated on 7 September. The skin and orifices were treated with 1000 c.c. of creosote oil and 100 c.c. were injected into the thoracic and 150 c.c. into the abdominal cavity. 3rd day, distended with gas; no eggs found. 5th day, no eggs or maggots found, but flies were seen resting on the carcase. 8th day, a few eggs on the lip, and some very small maggots on the anus. On enlarging the injection hole some gas escaped from the abdominal cavity. Some smell. Upper surface treated with 200 c.c. of creosote oil, and 50 c.c. poured into the peritoneum. 11th day, very numerous eggs on the under surface of the head, which is moist. Few small living maggots on the lower eye, and some, which are discoloured and appear to be dying, on the chest. Many eggs on back near the ground. 28th day, most of the eggs on the skin are dead, but a few large living maggots were found on a portion of the gut which was protruding. 43rd day, flies visiting the carcase. A few small maggots in the mouth. Numerous dead eggs on the chest. Some maggots still alive on the protruding gut. 91st day, little change. A year later (26 October, 1916) the carcase was entire, but dropped to pieces when raised with a fork.

Remarks. On comparing this carcase with No. V after 13 months' exposure the beneficial effect of the original injection of the blood vessels with creosote oil, combined with the skin treatment, is thrown into relief.

Goat VII. Weight 60 lbs. Killed and treated on 9 September. The abdominal organs were removed and placed on the ground about four feet from the carcase. The cavity packed with hay, and the abdominal walls roughly approximated with string sutures. Skin, orifices and abdominal wound and hay treated with 1000 c.c. of creosote oil. On 3rd day, no eggs found, and no flies on the body. 5th day, no eggs and no maggots. 8th day, no eggs found. 11th day, a few eggs in mouth and nostrils, but none elsewhere. 20th day, many eggs under head and udder. No

maggots found. On 28th day, innumerable eggs on the head, and some tiny dead maggots near them. Some dead eggs on anus. The thin abdominal walls now mummified. 43rd day, eggs under head dead, and no living maggots found except under the chin and on the lower eye. On the 91st day, condition similar. A year later (26 October, 1916) the carcase was entire but dropped to pieces when raised with a fork.

Remarks. This carcase was only treated once with creosote oil. A few maggots developed in some situations, but very few survived and these made very little progress. Evidently the carcase provided little, or no, food for maggots. No advantage seems to have been derived from the removal of the abdominal organs.

Abdominal organs of Goat VII. 100 c.c. of creosote oil were distributed over the exposed surfaces. 3rd day, exposed surfaces dry and flies walking over them, but no eggs found. No smell. 6th day, some small maggots at one side, but the greater part of the mass was free from maggots. 8th day, some small maggots at the place mentioned, but not elsewhere. The rest of the surface dry and hard. 200 c.c. sprayed on the area where the maggots were present. On 11th day all maggots dead. 20th day, a few eggs found. On 28th day, the surfaces like parchment. The whole mass was carefully examined and no maggots found. No smell. 43rd day, well preserved, numerous dead eggs in two situations. 91st day, condition similar.

Remarks. This experiment shows that, contrary to the popular idea, the intestines are more easily preserved by surface treatment than other portions of the body (p. 206).

Goat VIII. Control. Weight 33 lbs. Killed on 20 August, and exposed 28 hours after death. On 2nd day, hundreds of flies were sitting on the carcase, and innumerable eggs had been deposited on the body. On 3rd day, flies numerous, and countless eggs everywhere, and innumerable small maggots in the coat, on the anus, eye, mouth, nose, groin and scrotum. 5th day, stench intolerable; flies numerous. Maggots have eaten through the abdominal walls, and the intestines have ruptured. Numerous half-grown maggots found under the carcase and elsewhere, except where the skin was dry. Hair very loose. 6th day, flies numerous; abdominal cavity full of half-grown maggots; head half eaten. 8th day, a hole a foot in diameter over the thorax was seen, full of maggots. Innumerable maggots of all sizes on the skin. 10th day, the whole carcase was eaten except the fore legs, and part of the neck, where very large numbers of very large maggots were at work. 12th day, nothing left except hair, bones, horns, hoofs, some fibrous tissue and stomach contents.

Remarks. The great power of creosote oil in repelling flies was very evident when comparing the numbers found on this and on the treated carcases. The carcase was completely eaten by the maggots within 11 days, while in creosote treated carcases hardly an egg had been deposited in that time.

Conclusions from experiments on the carcases of goats.

The control attracted large numbers of flies of many kinds, and was reduced to a skeleton in eleven days, smelling intolerably during the process.

Treatment with "Solution B" produces very great mortality amongst the eggs, and also prevents the development of such maggots as do hatch, but possesses small value as a repellent for flies, and only delays putrefaction to a small extent. A carcase efficiently treated acts as a trap for the destruction of eggs and small maggots on a large scale for a variable period of time. The time this treatment remains operative depends mainly on the rainfall, since the potent constituents are letched away, and the carcase left without protection. The remains then become available as food for maggots. By surface treatment with creosote oil flies are repelled almost completely for a week, and to a smaller extent for a long period. The deposition of eggs on carcases treated with creosote oil after two or three weeks has certain advantages. A very large proportion of the eggs shrivel and never produce maggots. Great numbers of the maggots which do emerge die within a few days, and in properly treated bodies it is doubtful whether any reach maturity, and give rise to flies. Eggs continue to be deposited for a long time. Carcases in this stage therefore act most efficiently as agents for destroying the coming generations of flies and diminishing the total fly population. Apart from these effects surface treatment with creosote oil cuts off the access of water from any source, the potent constituents are not extracted, the skin is made leathery and the internal organs preserved for a long time. The combined effect of surface treatment with injection preserves the body for many months. As in the case of small carcases distension with gas, and green discoloration of the skin though suggestive of putrefaction, are not followed by disintegration in properly treated carcases. At any stage of decomposition maggots may be destroyed, smells eliminated and the process arrested by suitable treatment with creosote oil.

The stomach and intestines, whether in or out of the body, can be more easily preserved than other tissues by treatment with creosote oil. We are inclined to the opinion that the removal of the abdominal organs is disadvantageous, for intact carcases are well preserved by surface treatment, and the procedure of removal permits of the introduction of organisms into the exposed tissues.

Fly Repellents.

Throughout all our experiments, some of which extended over long periods, we compared as well as we could the numbers of flies approaching, alighting on and walking over treated carcases and untreated

controls. It is impossible to record these observations in precise terms, and we can only state that some of the coal-tar oils exert a very marked and prolonged repellent action, the period apparently depending on the rate of evaporation. We attempted to increase this action by varying the proportion of the constituents and by adding to creosote oil small quantities of reagents, which possess well marked repellent powers and are soluble in creosote oil, such as aniline, bone oil, pyridine and various other bases. Of these the most satisfactory appear to us to be the bases derived from "light oil" which add very distinctly to the repelling and other properties of creosote oil without appreciably affecting its flash point or pleasant smell. In the treatment of faecal material and in deterring flies from entering habitations and approaching food it is desirable that the greatest possible deterrent effect should be exercised.

For general use as an inhibitor of putrefaction, deodorant, repellent of flies, and destroyer of maggots we recommend the addition to creosote oil, of the type we have previously described (p. 123), of sufficient bases derived from "light oil" to make the proportion of phenolic bodies to bases two to one. This mixture has been called "Solution C."

If a means could be devised for preventing biting and other flies from settling on the exposed parts of the body, such as the hands, face and neck it would add immensely to health and comfort in tropical and subtropical countries. For this purpose an accurate knowledge of the relative repellent powers of various reagents, alone and in solution, is necessary. In the study of the relative powers of disinfectants descriptive methods failed to give reliable means of comparison. The failure of descriptive methods was even more evident when attempting to determine the relative powers of repellents. We have devised an apparatus with which we hope to obtain results of sufficient accuracy to make a reliable comparison of repellents possible.

The repellent powers of a substance appear to depend upon its nature and rate of evaporation, the latter affecting the length of time the repellent remains operative. Some of the best repellents are poisonous, inflammable or very volatile. These properties may be submerged by dissolving suitable quantities in such fluids as creosote oil. By such a procedure the high flash point of creosote oil may not be appreciably affected. The evaporation of the added substance is so retarded that it remains operative for a considerable time. To prevent flies from alighting on the exposed skin repellents might be employed in two ways; by application to (1) the clothing and (2) the skin. Clearly for these purposes irritating, poisonous and inflammable reagents must be avoided.

Conclusions.

1. In summer time maggots play a very important part in the destruction of exposed carcases.

2. Coal-tar oils, such as crude carbolic, "middle," creosote and "heavy" oils, when used at full strength kill large maggots almost immediately. Of these the most suitable for general use is creosote oil. Anthracene oil is much less effective.

3. The most potent constituents are contained mainly in the fractions which distil over below 240° C.

4. Each group of constituents of creosote oil possesses some degree of toxicity to maggots even when the exposure is momentary. The phenolic bodies in the absence of water are extremely toxic to maggots, which immediately become contracted, hard and tense, and within 15 minutes assume a deep red colour. The bases are also toxic, but the maggots remain white, and become extended and flaccid. The higher boiling fractions of the hydrocarbons are decidedly more toxic than those of lower boiling point.

5. Momentary immersions in dilutions of highly toxic constituents in water or inert fluids produce little effect on maggots. The presence of water seems to enable maggots to resist the toxic action of phenolic bodies to a large extent. By more prolonged treatment, however, the maggots may be killed, if dilution is not carried too far.

6. Maggots can survive 15 minutes' immersion in emulsions containing 5 % phenolic bodies and reach maturity. They can feed on meat soaked in such emulsions without ill effect.

7. Maggots present in carcases are killed by suitable treatment with creosote oil.

8. Even in warm and showery weather, particularly favourable to putrefactive changes, at the height of the fly season, the bodies of small animals can be well preserved for several weeks by efficient surface treatment with coal-tar oils, especially creosote oil.

9. To obtain such results the surface treatment must be thorough, for eggs may be deposited on untreated areas and make their way into the carcase from these areas.

10. Open carcases can be similarly preserved, if the exposed surfaces are treated.

11. This treatment protects the bodies from the influence of rain and soil water.

12. Experiments on the bodies of goats show that large carcases can be preserved in the same way as small ones.

13. The combined effect of surface treatment with injection preserves the body for many months.

14. The removal of the abdominal organs is disadvantageous for intact carcases are well preserved by surface treatment, and the process of removal permits of the introduction of putrefactive organisms into the tissues.

15. Surface treatment with watery emulsions, such as "Solution B," delays putrefaction to a slight extent, but causes the destruction of innumerable eggs and small maggots. Sooner or later rain letches away the potent constituents and the carcase, left without protection, becomes available as food for maggots.

16. On the other hand treatment with creosote oil repels flies almost completely for a week or two, and to a less extent for a long period. After two or three weeks eggs are deposited. A large proportion of these die, and great numbers of the maggots, which emerge from them, also die. In fact in well treated carcases it is doubtful if any maggots reach maturity. Hence in their different ways carcases treated with such fluids as "Solution B" or creosote oil act as traps for destroying fly eggs and maggots.

17. At any stage of decomposition maggots may be destroyed, smell eliminated and the process arrested by suitable treatment with creosote oil.

18. Several reagents, possessing fly repellent properties, can be dissolved in creosote oil, in order to increase its efficiency in this respect. Of these the most satisfactory are the bases derived from "light oils." These increase the repelling and other properties of creosote oil without appreciably affecting its flash point or smell.

19. For general use as an inhibitor of putrefaction, deodorant, repellent of flies and destroyer of maggots we recommend the addition to creosote oil, of the type described (p. 123), of sufficient bases from "light oil" to make the proportion of phenolic bodies to bases two to one.

20. The study of fly repellents by methods sufficiently accurate to make reliable comparisons possible might suggest means for preventing flies from alighting on exposed surfaces of living persons.

Part IV. The Control of Nuisances due to Flies and Putrefaction.

Our work was undertaken for the purpose of devising methods for overcoming the dangers and nuisances arising from flies and putrefying substances, and throughout we have kept this aim steadily in view. In this part of our paper we mention only those experiments which illustrate practical methods for dealing with large carcases, maggots, manure, fly infested habitations, etc. As far as possible we have quoted the impressions of competent observers, who watched our experiments or independently tested our methods.

Experiments on the Carcases of Horses.

Two horses, a white (*A*) and a black (*B*), were killed at 8 a.m., on 7 September, 1915, and allowed to lie where they fell in an open field. They were treated at 4 p.m. in the presence of Col. C. H. Melville, A.M.S., who visited them frequently during the next two months, often invited officers who might be interested to see them, and kept his own notes.

One horse, (*A*), was injected through the carotid artery with 2 gallons of creosote oil[1], the process occupying a few minutes. Then the fluid was applied in small quantities at a time on to the body from a watering can fitted with a rose and distributed with a coarse brush in the direction of the hair. The *whole* surface of the body was treated in this way. A small quantity was poured into the eyes, ears, mouth and anus. About half a gallon was used in the external treatment.

The other horse, (*B*), was not injected, but about half a gallon was poured through openings into the pleural and peritoneal cavities, after cutting the intestines to allow the gas to escape. A large wound was made in the right thigh, and the whole surface treated in the same way as horse (*A*). About one gallon of the fluid was used in the complete treatment, which occupied less than 15 minutes. "There was an enormous number of fly eggs, probably enough to fill a pint measure, near the mouth and in sheltered positions, laid already in the eight hours that elapsed since death." To hide the bodies from the inquisitive, hurdles were leant against them and a few old sacks thrown over them. In both carcases gas had developed in large quantities in the intestines between the time of death and treatment.

[1] Most of the experiments mentioned in this part were carried out with the creosote oil mixtures, especially that recommended on p. 199.

The results of these experiments may be stated in the words of Col. Melville. "The two horses were allowed to lie out in a field, covered with sacks, for 29 days. They were visited from time to time, but no important changes occurred."

On 15 September 600 c.c. were poured into the peritoneum, 400 c.c. into the thorax and 400 c.c. sprinkled on to the skin of the black horse (*B*). The white horse (*A*) was not treated. On 18 September creosote oil was sprayed on the sacks covering the black horse, and some also on the blood patches on the ground near the head, which had not previously been treated. About 1400 c.c. were used in this manner. On the same day the sacks covering the white horse were sprayed with 1000 c.c.

During this period very few flies visited the carcase of the white horse (*A*) and only a few scattered eggs were deposited on it. There was no smell at any time and no subcutaneous emphysema.

In the carcase of the black horse (*B*) gas developed, subcutaneous emphysema was present and the gas gradually escaped through the incisions we had made. The escaping gases probably contributed to the slight smell, and accounted for the considerable number of flies attracted to this carcase after the first week. Many eggs were deposited on a piece of intestine which protruded through the opening made in the abdomen, and some maggots developed from these. Thousands of eggs were deposited on untreated blood patches near the carcase, on the hairs of the tail and on the untreated sacks.

"On 6 October, 1915 (the horses having been killed and treated on 7 September), various dissections were made as follows:

White horse (*A*). The skin was removed from the left gluteal region and as far down as the hock. The fat and fascia were also removed and the various muscles defined. The flesh was firm and normal in appearance; no signs of putrefaction anywhere. The colour of the muscle was slightly different from that of fresh muscle, and there was a very superficial change of colour on exposure to air.

The abdomen was freely opened and the intestines allowed to escape. There was no sign of putrefaction and the peritoneal surfaces retained the gloss present in fresh tissues. The spleen, stomach and lungs showed no change. The skin was then reflected from the sides of the face; there was no sign of change here, nor in the muscles of the neck."

We may add that the hair was loose on the under side of the abdomen, and here and in the neck the tissues were oedematous. The skin was very tough and the abdomen not distended with gas. No unpleasant

odour was noticed during the dissection, though the intestines had their characteristic smell.

"A similar series of dissections was made in the case of the black horse (*B*) with identical results. The flesh in this case showed absolutely no change in colour or consistency, and could have passed anywhere for fresh butcher's meat except for a slight rancid smell."

We may add that the neck muscles were oedematous, and there was some rancid smell when the dissection was being made.

"It will be remembered that this horse was not injected with creosote oil as in the case of the white horse; the fluid was merely brushed over the surface of the body and some poured into the thoracic and abdominal cavities, about a gallon in all being expended. The secondary applications to the sacks and blood patches on 18 September undoubtedly kept flies off the carcase, but did not in any way, in my opinion, influence the question of putrefaction. This is a matter of considerable importance, as the technique of application demands no apparatus more recondite than a watering can and brush. No skill is required, and the time necessary to treat a horse completely is between ten minutes and a quarter of an hour, with two men working. Injection, however, is also an easy and rapid process and very satisfactory, though requiring rather more of the fluid."

Fleet-Surgeon D. W. Hewitt, R.N., representing the Admiralty, visited Cambridge on 23 September, and saw a number of experiments then in progress. The Medical Director General of the Navy, Sir Arthur W. May, K.C.B., most kindly permitted us to read the reports he had received, and to make quotations from them. The following extracts are from Fleet-Surgeon Hewitt's report:

"These experiments have undoubtedly been governed by a great deal of care and forethought, in several instances 'controls' have been carried out at the same time. The bodies examined were those of guinea-pigs, rabbits, goats, pigs and horses, grouped in three different areas, separated by from two to three miles....They had all been exposed to the open air, rain, and sunshine for periods varying from a fortnight to six or seven weeks, thus as far as possible simulating the actual conditions met on a battle area.

The methods of treatment may be described under three headings:

(*a*) The fluid brushed over the whole surface of a dead animal.

(*b*) A combination of (*a*) with injection into the carotid artery.

(*c*) A combination of (*a*) with injection of the fluid into openings in the peritoneal and pleural cavities.

I was shown carcases stated to have been treated by each of the above methods, and came to the following conclusions:

The bodies in either case were all deodorised, putrefaction was greatly retarded or prevented, and the fly maggots were all killed.

The most effective of all the above methods was (*b*); and the body of a horse treated thus a fortnight ago was fresh and without odour. Process (*c*) was also efficient, and there were numerous flies round the body of a horse so treated, but no smell or maggots. Process (*a*) was effective as regards guinea-pigs and small animals, and would probably suffice for a human body."

Professor J. Stanley Gardiner, who inspected the bodies on 17 December, more than three months after the death of the animals, was particularly struck by the absence of smell and the excellent appearance of the meat, when an incision was made into the body.

The carcases of these two horses were allowed to lie without further treatment in the field for 12 months after the dissections described, and were examined from time to time.

White horse (*A*). In the exposed meat a slight very superficial change in colour occurred, and the surface became soft. Moulds grew on the exposed intestines. During the winter the carcases were often very wet, but there was very little smell at any time.

On 20 April, 1916, seven and a half months after death, the carcase was shrunken, but there was no smell, and on section the larger muscles were very firm and compact, but otherwise normal in appearance, and the smaller exposed muscles very tough and the colour of mahogany. The appearance of the left gluteal muscle may be seen in the accompanying photograph (Pl. V, fig. 10). Microscopically the muscle fibres were very well preserved, the striae being exceedingly evident. Very few of the fibres were degenerated (Pl. V, fig. 12). Sections stained by Weigert's method showed in places minute slits in the connective tissues with numerous undegenerated non-sporebearing bacilli at the margins. It is probable that these organisms, which may have been introduced into the blood stream when the animal was shot, were present at the time of injection and were killed and fixed *in situ*.

On 23 May, 1916, eight and a half months after death portions of meat were taken from (*a*) the left shoulder, (*b*) the left gluteus, and (*c*) the upper part of the right thigh for chemical analysis. The meat from the left shoulder was easy to grind with sand and had an unpleasant smell, suggestive of acetamide; that from the left gluteus was dry and not easy to grind and smelt of creosote oil, while that from the right

thigh looked like fresh meat. The results of the chemical analyses are given on p. 207.

On 27 October, 1916, thirteen and a half months after death the body was very carefully examined, cut up, the parts removed on a barrow and burnt. The skin was very tough, the muscles excellently preserved, except in a part of the area dissected a year previously, where maggots had worked to some extent. The muscles of the shoulder and neck, and lowest part of the body, were softer and more oedematous than those of other regions. The lungs were well preserved, the liver hard, almost normal in appearance, and contained much creosote oil. The stomach and intestines, except the superficial coils on which moulds had grown, were so well preserved that they could be lifted without rupturing. The coecal contents appeared normal in colour, smell and consistency. There was no unpleasant smell, and no fluid oozed out of the body. The whole carcase was so well preserved that it could be dragged by one leg.

Black horse (*B*). By the end of October, 1915, the surface of the exposed meat had become gray-brown and soft, but the change was confined to the superficial layers. Moulds had grown on the intestines and on some of the exposed muscles. Though numerous eggs had been laid near the carcase and on the intestine there were very few living maggots on it. Small dead maggots were very numerous. There was some rancid smell, but insufficient to make very close examination of the body unpleasant. On 7 December little change was noticed, and the carcase could be dragged along by one leg.

On 20 April, 1916, the carcase seemed less shrunken than that of horse (*A*) and had a slight rancid odour. The gluteal muscles where covered by skin were normal in colour, but soft in section and showed small cavities due to the presence of gas. The appearance of the gluteal muscle may be seen in the accompanying photograph (Pl. V, fig. 11). Microscopically some of the fibres were well preserved and showed distinct striae, others were degenerated and stained badly (Pl. V, fig. 13). Degenerated, badly staining bacteria, mostly spore-bearers, were found in considerable numbers, especially in the connective tissues.

On 23 May, 1916, portions of meat from (*a*) left shoulder, (*b*) left gluteus and (*c*) the upper part of the right thigh were taken for chemical analysis. The meat from the left shoulder was very easy to grind and had a rancid smell, only appreciable on close examination, differing from the smell in the same region of the white horse. The gluteus was

easy to grind and had only a slight smell like that of fresh blood, and the meat from the right thigh was very easy to grind, very moist and had the same smell as (*a*).

On 27 October, 1916, thirteen and a half months after death, the carcase was carefully examined, cut up, and the parts removed in a barrow and burnt. The skin was very tough, and intact in all situations not interfered with by our previous dissections. From the wounds maggots had penetrated during the summer for some distance into the muscular tissues. In situations not affected by the invasion of maggots the muscles and organs had become disintegrated, to a greater or less extent, some parts being moderately well preserved but others being represented by pasty material.

The carcase was dissected and cut into small portions without discomfort from smell or semi-liquid putrid material.

Remarks. In both carcases much intestinal gas developed in the few hours intervening between death and treatment. In the case of the white horse this intestinal gas did not appear to be supplemented by appreciable tissue gas subsequently. Gas, however, was produced in the tissues of the black horse in the first week or two, as it was in smaller skin treated carcases.

The great benefit derived from local supplementary treatment of areas where isolated colonies of maggots developed after two or three weeks was again shown. Both carcases were satisfactory from a practical point of view, but the white horse was distinctly better preserved than the black, especially in the later stages of the long exposure. This agrees with the results obtained with smaller carcases.

Surface treatment is the more easily carried out and the more economical in fluid.

Special attention may be directed to two noteworthy facts; that the skin which had been in contact with the wet ground for many months was tough and well preserved and that the intestinal contents showed little, or no, change.

Chemical analyses.

Specimens from the samples of meat taken on 23 May, 1916, were examined in the following way. Portions weighing about 5 grms. selected for their freedom from connective tissue, were thoroughly ground with 2 grms. of washed sand, triturated with 23·5 c.c. of water and made up to 250 c.c. with 97 % alcohol. After standing 24 hours, the insoluble matter was filtered off on tared papers, dried to constant

weight at 100° C. and weighed. After allowing for the sand the percentage of this dry matter was calculated. The volatile bases and amino acids were then estimated in 100 c.c. of the filtrate by the method previously described (p. 144). The results are given in Table XXXII. We have examined also in the same way pieces of meat taken from the upper shoulder and centre of the upper thigh of an untreated horse on the first, second and third days after death. The incisions made in taking the different samples from the same region did not encroach on one another. The carcase lay during this time in a stable and the weather was very warm and fine. On the third day the carcase was greatly distended, and gas was present in the tissues.

TABLE XXXII.

Analyses of muscles of horses.

Material	Weight of muscle taken	Percentage dry matter insoluble in 86% alcohol	Volatile bases c.c. N/10 acid neutralised	Formyl titration c.c. N/10 soda	Volatile bases per grm. muscle, c.c. N/10 acid neutralised	Formyl titration per grm. muscle, c.c. N/10 soda	Ratio of volatile bases to amino acids
White horse							
(*a*) left shoulder	5·122	15·52	5·8	2·3	2·83	1·12	2·52 : 1
(*b*) left gluteus	4·748	23·94	3·3	5·1	1·74	2·69	0·65 : 1
(*c*) right thigh	5·208	19·57	—	—	—	—	—
Black horse							
(*a*) left shoulder	4·789	18·83	13·05	2·45	6·81	1·28	5·33 : 1
(*b*) left gluteus	4·949	19·30	10·4	7·1	5·26	3·6	1·46 : 1
(*c*) right thigh	4·997	13·69	17·45	2·7	8·73	1·35	6·46 : 1
Untreated horse							
(*a*) shoulder 28 hrs.	4·914	24·14	0·6	0·35	0·30	0·18	1·71 : 1
(*b*) thigh 28 ,,	4·886	25·73	0·7	0·45	0·35	0·23	1·55 : 1
(*c*) shoulder 40 ,,	4·794	24·89	0·5	0·45	0·26	0·23	1·11 : 1
(*d*) thigh 40 ,,	5·032	23·36	0·65	0·45	0·32	0·22	1·44 : 1
(*e*) shoulder 62 ,,	5·041	23·84	0·6	0·45	0·29	0·22	1·33 : 1
(*f*) thigh 62 ,,	5·040	24·14	0·7	0·5	0·34	0·24	1·4 : 1

The percentage of dry matter present in tissues taken from various situations in a carcase depends upon the distribution of the fluids as well as upon the amount of disintegration. A low percentage may indicate the presence of fluid or much disintegration. On the other hand a high percentage of dry matter may indicate disintegration after the removal of fluid containing the products, leaving compacted connective tissue, or that little change has occurred. It is obvious therefore that no reliance can be placed upon the percentage of dry matter as an index of the extent of putrefactive changes. For example material from the left shoulder of the white horse contains a low percentage of

dry matter because fluid collected in this situation, and this is correlated with a low ratio of volatile bases to amino acids. The still lower percentage of dry matter in the material taken from the right thigh of the black horse is correlated with the highest ratio. In the case of the black horse the percentage of dry matter in the material taken from the left shoulder and the left gluteus is almost the same, but the ratios show a considerable difference in the amount of putrefactive change.

It will be noticed that considerable differences were found in the results of the analyses of muscles taken from different situations in the two treated horses. In each case the smallest ratio was obtained in the left gluteus where the tissues were driest. In the white horse it is very low, the whole change being probably due to proteolytic activity without the influence of putrefactive organisms. Taking this figure as a standard for this region a little putrefactive change in the black horse is shown (see p. 170). The material taken from the other situations contained much more fluid, and the ratios are higher. Sterile fluid draining from any organ will contain the products of autolysis and hence we would expect to find slightly higher ratios in regions where fluid collects, more particularly if it has come from the abdominal organs where more deamination occurs. The ratio in the shoulder of the white horse is higher than in the gluteus partly for this reason and partly because a little putrefaction had occurred. In the shoulder and thigh of the black horse the ratios are decidedly higher showing that putrefaction had occurred during the long period of exposure.

The differences in the figures obtained by the analyses of materials taken from various parts of a carcase show the importance of a standard disposition of the bodies and of the selection of materials from the same situation, when comparing the rates of putrefaction in muscle or determining the relative effects of disinfectants. The necessity for standard conditions applies also when the fluid exuding from a body is used for analysis.

The results of the analyses of the muscles of the recently killed horse indicate that in three days, in spite of gas formation, no true putrefactive changes had occurred. They show further that little, if any, changes due to the activity of proteolytic ferments had taken place in the muscles. The ratios given in the last columns resemble those found in fresh tissues. The ratios would become lower as proteins were broken down by proteolytic ferments, and subsequently higher as the result of the action of putrefactive bacteria.

Atkinson (1916) reports as follows on an experiment he carried out in Gallipoli on the body of a mule.

"On October 9th the body of a small gray Indian mule, killed by high explosive in the morning, was treated. Holes were made into the thorax and some 'Liquid C' was squirted in. There was a large wound over the abdomen with a portion of colon lying outside it; a counter opening was made and some liquid squirted into the interior, the intestines also being opened by means of a long butcher's knife. The surface of the body, with especial care to all the openings, was sprayed and brushed over with the fluid. In all two gallons were used and 15 minutes taken....

On October 15th the mule was visited again, was slightly distended, but no flies were near or around the body.

October 17th. No flies, and the distension partly subsided.

October 20th. Gas formation was going on rapidly and gas was bubbling from the wound in the intestine. The smell was not great, but was distinct. The mouth was almost half full of the eggs of the blow-fly, and on other patches eggs had been deposited. There was a sloughing patch from an old wound.

October 21st. A further addition of one and a half gallons of 'Liquid C' were used, and some gas from intestine floating in the liquid let out. There was bunch of fly-blow in the mouth which was killed by the fluid.

October 24th. Complete absence of smell and only one small patch outside the nostril where a fly had laid its eggs."

Observations under War Conditions on Human Bodies.

Atkinson (1916) also reports some experiments on human bodies.

"October 9th. The body of a Turk, killed about eight days previously and buried three days, was partially exhumed on the parapet of an old trench. Decomposition was taking place rapidly and the body was covered with a thick swarm of flies. The stench in the area around was very great. The conditions were ideal for trying the usefulness of the liquid under conditions of trench warfare. After about one gallon was sprayed from a short distance over the body, the smell was practically gone. The flies were repelled and the corpse left exposed to the air. The previous night there had been a heavy thunderstorm, so that conditions were favourable for rapid decomposition. It was impossible in day light to spray the whole body as the sniping was too keen.

October 11th. The body was visited again and there was a complete absence of smell and flies. The body was markedly shrunken, and was again sprayed with about a gallon of the liquid. On this day there was again a fall of rain, and three or four other bodies, lying behind the parados between saps 4 and 5, were sprayed also. The smell in their vicinity immediately subsided, and the atmosphere in the trenches adjacent much improved. The bodies had been lying there for over two months.

October 13th. Visited the body of the Turk again after a sharp shower of rain. There was no smell and only an occasional fly settled on the body and immediately went off again.

October 14th. Body gradually shrinking. A few blow-flies were about but did not settle on the body, and there was no smell.

October 18th. After considerable rainfall, no smell or flies.

October 24th. Body shrunken and almost mummified, no smell or flies."

REPELLING FLIES FROM HABITATIONS.

In order to ascertain whether the repellent action could be made use of to free dugouts and shelters from flies, several experiments of the following type were tried. A small shelter, about 5 by 5 feet, constructed in a bank, and having a corrugated iron roof, without a window and with a single open doorway, was chosen. This place became very hot on a warm day. Decomposing animal matter (dead rabbits, guinea-pigs, and in one experiment a pig, exhumed after being buried for some days) and fresh excrement were placed inside, a sack hung over the entrance, and the flies, which were soon attracted, were hunted out. The sack was then very roughly sprinkled with creosote oil mixture.

Col. Melville reported on one of these experiments as follows:

"On 12th October, 1915, an imitation dugout was improvised from a shelter on the golf links. This was a small hut, partially turf built and partially excavated, about 6 ft. in height and 5 ft. square. It has a galvanised iron roof and no door. Various animals and other substances in an advanced state of putrefaction were placed inside on 12th October, 1915, and on the 16th when I visited it, the shelter was full of flies and the smell intolerable. The flies were driven out as far as possible, and a piece of sacking hung over the doorway; about half a pint of fluid 'C' was then sprinkled roughly out of a bottle on to the sacking. There were several openings left between the edges of the sacking and the door-posts and lintels of the doorway. We watched the shelter

for 15 minutes, during which period we could see numerous flies trying to effect an entrance, but without success. At the end of the 15 minutes no flies were found in the shelter and there was a complete absence of smell. The shelter was left as it was, the putrid matter being left *in situ*: no more fluid was put on the sacking.

On the 20th October, I revisited the 'dugout.' A few flies had penetrated into the shelter, but these left immediately the curtain was raised; they must have entered through apertures, of which there were several, between the roof and the turf walls of the shelter. There was absolutely no smell in the shelter."

Atkinson (1916) reports as follows: "As a repellent to flies. In this respect the liquid was exceedingly useful; it is best that the beams or wooden structure of messes or dugouts be actually rubbed by the fluid. Spraying leaves patches and the flies seek them out....Such a mess was fly-free for ten days. The other mess at Divisional Headquarters was also sprayed, and was kept free for over six days.

Flies congregate in masses, sometimes two or three deep, in dugouts at night, more especially in stores covered with tarpaulin. The liquid is a most efficient means of dealing with these, as sprayed over them it kills them effectually. Flies that escape with even a small quantity of fluid on their bodies subsequently die.

The tins and seats and immediate surroundings of the latrines belonging to the Divisional Headquarters were sprayed with the liquid, and they were fly-free for four days. About two gallons were used.

Conclusions. The benefit to be derived from liquid 'C' in trench warfare if used in sufficient quantities would be difficult to over-estimate. Bodies are mummified by its action and rendered inoffensive, even after and during a fall of rain. It is a very definite fly repellent and will kill the adults in great numbers with quite small quantities from a spray. ...It is the best fly antidote that has been used so far."

It may be pointed out that in Gallipoli the most prevalent flies were *M. domestica, F. canicularis, F. scalaris, C. vomitoria, C. erythrocephala, L. caesar, S. carnaria, M. stabulans,* all species which are very common in Europe.

Ross (1916) reported as follows: "Staff-Surgeon E. L. Atkinson, R.N., made a very energetic and successful attempt to deal with the putrefying corpses which lay in the open between lines of trenches. These in many cases lay unburied for months owing to the extreme danger a burying party would run, even if working only at night. He supplied me with a fluid, named liquid 'C' for spraying these corpses.

From thirty bodies, which I was able to keep under daily observation, I came to the conclusion, that though the fluid appeared to be non-toxic to flies, they did not congregate on substances sprinkled with it."

In connection with the experiments just quoted Lt.-Col. L. S. Dudgeon has very kindly allowed us to publish the following extracts from a letter he sent to one of us on 30 December, 1915.

"I have only just returned from the Mediterranean area to which I have been attached as a member of the War Office Commission on Epidemic Diseases and Sanitation. I arrived in July and therefore experienced the intense heat and the curse of flies. I was all over ... and in the winter at .... I mention the facts because it will show you that I experienced and saw every possible discomfort and disease produced by flies and dead bodies, both human and animal. All remedies were tried but par excellence in my opinion is solution 'C.' It kills flies at once while distant ones revolve in circles and then join the home only fit for flies. At ... in the advanced line during construction of fresh trenches, the highly offensive bodies of the Turks were removed and if covered with sufficient 'C' work could continue. If no solution 'C' the odour cannot be expressed in words."

The Effects of Creosote Oil on Adult Flies.

As we pointed out previously (p. 113) many reagents have a temporary action on adult flies, and it is necessary to watch the insects for a considerable time after treatment in order to ascertain their effects. On many occasions we observed that even very small quantities of creosote oil sprayed on to flies killed them immediately, and that flies only touched with minute droplets soon died. When a putrescent mass of animal matter is sprayed most of the flies caught in the spray die instantly.

Even the vapour of creosote oil is fatal to flies, if they are exposed to it for some hours. In one experiment blow-flies were placed in a balloon trap in a bell jar, one edge of which was lifted so as to allow air to enter. A watch glass containing creosote oil was also placed in the bell jar, some distance from the trap. After two hours all the flies were very feeble and some incapable of movement. All were dead within 18 hours.

Deodorisation.

It has been shown that stinking animal matter may be deodorised by the application of creosote oil. Circumstances may arise when it is impossible to reach such material or when it would be most desirable to treat it from a distance. We have carried out a number of experiments to ascertain how far spraying is effective for such a purpose. The fluid is capable of application through the finest sprayers and small quantities applied by this means will very rapidly deodorise putrefying materials, so that they can be approached without discomfort and more thoroughly treated. Bodies which have been sprayed become offensive, if disturbed, but if undisturbed attract few flies and only become offensive gradually.

Professor I. Walker Hall wrote to one of us as follows: "This solution has been of great use in removing the smell from putrefying tissues in both animal and human bodies. When sprayed on rats, which had been found dead and sent for examination in an advanced stage of decomposition, the nauseous smell disappears almost immediately.

In two post-mortem examinations made during August, 1916, upon persons who had died six days previously, the smell was so offensive that it could be detected 50 yards away, although the bodies were shut up in a room. In each instance the cadaver was almost covered with flies. Before we had time to spray completely the surface of the body and the internal organs, the offensiveness had disappeared and the autopsy was carried out without any discomfort or repulsion.

When making post-mortem examinations upon rats suspected of plague this solution was used to protect the workers from the bites of rat fleas. It was sprayed over the whole skin of the animal. If fleas or lice were present they came out from the deeper parts of the hairs and appeared on the surface. They did not make any attempt to jump and seemed stupefied. A 50 % solution in alcohol served equally well."

Maggots in Manure.

The large heap of manure already infested with larvae presents a difficult problem. Many points such as the kind, age, method of storage, the extent and kind of fermentation in relation to the food supply of the larvae and their distribution and migratory habits have to be considered. Some of our preliminary observations show that

the distribution of the larvae depends upon the temperature, which depends upon the nature and extent of the fermentation and this in its turn upon the air supply, and ultimately the air supply depends upon the wetness and compactness of the heap. Horse faeces contain much less water than cattle faeces, and horse manure is termed "hot" because the large amount of air in it permits of the growth of aerobic organisms, which are responsible for the oxidation of carbohydrate material and the consequent increase in temperature. If manure is sufficiently wetted and compacted diffusion of air into the heap is prevented, the air remaining in it is soon used up, the carbon dioxide produced by the aerobes saturates the heap, and anaerobic conditions, which are not accompanied by any considerable rise in temperature, prevail.

We have found the superficial and deep distribution of maggots very irregular in heaps composed of the manure of different animals, and especially so in manure mixed with offal.

The attempt to destroy maggots in large heaps of farmyard manure is likely to be attended with considerable difficulty as water soluble larvicides must be employed to insure sufficient penetration to reach all the maggots, if the temperature is not high. We have already pointed out the resistance of maggots to extremely toxic larvicides, when these are applied in the form of solutions (p. 186). It is therefore evident that the quantity of soluble substances it is necessary to add to reach the necessary concentration throughout the bulk of the manure prevents their effective use from the economical standpoint. The possibility of the fixation of the potent constituents by the organic matter must also be borne in mind.

Fluid larvicides, insoluble or only slightly soluble in water, including those of an oily character, when applied to the surface would not diffuse sufficiently into the mass.

The successful treatment of manure should result in the prevention or destruction of larvae without injury to the manure for agricultural purposes. The difficulties attending the successful treatment of heaps already infested with larvae are evidently so considerable that we sought for other means of solving the problem, and concluded that the easiest method would be to treat the manure at the earliest possible moment, before the heaps were constructed. Our experience with creosote oil indicated that this would be a suitable reagent to try, and we are indebted to Mr J. E. M. Mellor for carrying out some experiments on the lines we suggested to him.

It has been shown that flies are only attracted to human faeces for three or four days (Graham-Smith, 1916, p. 492) and Mr Mellor's observations indicate that for the purpose of depositing eggs house flies are attracted mainly to fresh horse manure. His experiments, which will be published shortly, show clearly that the superficial treatment of a manure heap, already infested with fly larvae, with creosote oil at the rate of 4 gallons to the ton, is of little value, since large numbers of flies eventually emerge. On the other hand he has shown that if fresh horse manure exposed for 24 hours is sprayed "incrementally" at the rate of one gallon to the ton the results are satisfactory. The smell which attracts the flies is diminished to a large extent, most of the eggs already deposited are killed, a large proportion of the maggots which do hatch die, the creosote oil is distributed throughout the heap subsequently made, flies are repelled from it, and few emerge. Such "incremental" treatment can be easily and cheaply carried out, is more effective than any attempt to treat maggot infested heaps, and so far as we have been able to ascertain does not apparently have any injurious effect on the manure[1]. Further experiments are necessary before we can determine the minimal quantities that should be used, but we believe the method is worthy of trial on a large scale.

The Prevention of Flies in Towns.

In towns flies deposit their eggs in stable manure, dust bins, middens, refuse heaps, and similar situations, and the larvae develop in collections of horse manure and refuse tips. If the method we have advocated for dealing with manure by "incremental" spraying is found to be effective, the same means could be made use of in dealing with the breeding places of flies in towns. The fresh manure in stables should be sprayed daily thus eliminating the odour attractive to flies, killing the eggs already deposited and rendering the heaps unsuitable for the larvae. We believe that in the absence of large quantities of straw, spraying at the rate of 100 c.c. per horse per day, when the manure is collected, would suffice. The cost per ton of manure would be very small. Since maggots pupate at the margins of the yards these might be treated with advantage. Dust bins used for house refuse are very attractive to flies of many species which deposit their eggs in them. The film of semi-liquid putrescent material, which invariably covers

[1] Applied "incrementally" at the rate of one gallon to the ton the treatment does not interfere with processes responsible for the increase in temperature.

the bottom and sides of the bins, is never removed, causes the bins to be attractive at all times and affords food material to the maggots. On each occasion when the bin is emptied a small quantity of creosote oil should be poured into it. This procedure would tend to disinfect the film, kill any eggs or maggots present in it, eliminate the odour attractive to flies, and prevent them from visiting the bins. If the owners could be induced to spray the contents daily with a sprayer delivering about 10–20 c.c. these very prolific breeding grounds would no longer be a source of nuisances from smell or flies. The expense would be negligible, since a gallon would suffice for the fly season from the beginning of April to the end of October.

If dust carts were provided with suitable sprayers of one gallon capacity so arranged that the contents of the cart could be sprayed at frequent intervals through the cover by turning a handle and thus distributing the gallon throughout the load, an additional safeguard would be provided against the development of eggs and larvae present in the material from neglected bins. The refuse tips would require no further treatment.

There should be no difficulty in devising suitable means for treating privies, earth closets, middens, etc., and preventing nuisances, when they were being cleaned out.

Some Objections to the Use of Creosote Oil Mixtures.

Atkinson (1916) has summarised the main objections to the use of creosote oil in the following words. "The liquid is an irritant and it is well to wash the hands after use. It burns the face slightly, if left on. Protective glasses should be worn by men using the spray....The liquid is extremely inflammable and very great care must be taken not to have a naked flame near when spraying dugouts and messes."

It is true that the liquid has a transitory irritant effect on the mucous membranes, especially of the eye, but we have never experienced irritant effects on the skin, though working with it for hours at a time with it on our hands. The fluid can be removed very easily from the skin with a little spirit. Nevertheless it would be best for those who might have to work much with such fluids to protect the eyes with glasses, when using a spray in windy situations.

The flash point in an "open cup" is 193–194° F. In an apparatus which gives results corresponding closely to those obtained by the Abel tester the flash point is higher, namely 199–200° F. This flash point

is more than two and a half times that allowed in the transport of dangerous fluids, and we are of opinion that the danger is negligible with ordinary methods of transit. In the form of a very fine spray the liquid is inflammable, and the precautions suggested by Atkinson should be taken.

The colour, taste and smell of the liquid are such that no precautions need be taken to prevent its being swallowed.

It has been suggested to us that, since dilution is undesirable, difficulties in transport render the extensive use of such liquids costly. We have shown that the most important factor in the preservation of a carcase is the treatment of the skin in such a manner as to prevent the ingress of maggots, putrefactive organisms, water, and perhaps air into the tissues. This can be most easily done by applying an oily material containing disinfectants, slightly soluble in water, by means of a brush so as to produce a continuous film. No 5 % emulsion, prepared by means of soft soap or otherwise, or solution can accomplish this purpose, because the water of the emulsion softens the skin and a continuous film is not produced. The putrefactive bacteria protected by greasy constituents in the follicles, etc., are neither destroyed nor imprisoned as the skin is not hardened. The disinfectants are more liable to fixation and removal by dilution and letching. The deficient distribution and concentration would not prevent smells and maggots from developing in a short time, necessitating very frequent applications with comparatively poor results. In our opinion any economy in transport by the use of 5 % emulsions is far outweighed by the great economy in labour and incomparably better results obtained by the use of undiluted oily disinfectants.

For many purposes a spray of a suitable fluid may be used, for example for destroying eggs and maggots working superficially, for deodorising putrescent material, for repelling flies from habitations, putrefying substances, faecal material, latrines, dust bins, etc., treating infected soil, or fresh manure containing fly eggs. A fluid suitable for such a purpose should be capable of use with the finest sprayer, adhere to greasy surfaces, spread and tend to form a film over comparatively large areas, retain its properties for a long time, not be washed away by rain, or have the concentration of its potent ingredients rapidly affected by extraction with water. Watery emulsions are incapable of exercising most of these functions.

INSTRUCTIONS FOR USING CREOSOTE OIL MIXTURES.

Our experience has shown that creosote oil mixtures may be used with very great advantage for a variety of purposes, and we therefore indicate briefly the most satisfactory methods of applying them for different purposes. These methods are so simple that no difficulties should arise in their practical application.

(*a*) A carcase that cannot be disposed of immediately should be sprinkled over with creosote oil mixture by means of a watering can, and the fluid distributed in the direction of the hair with a hard brush. When one side has been treated, including the extremities, the carcase should be turned over and the other side treated in the same way. The abdominal and thoracic cavities may be opened and some of the fluid poured into them, but this does not appear to be necessary. If the abdominal cavity is opened it may be desirable to puncture the gut in order to allow the gases to escape, and the fluid to find its way amongst the coils. By means of a funnel some of the fluid should be poured into the gut through the punctures. Then small quantities should be poured into the mouth, eyes, ears, anus and any wounds there may be. In this climate about a gallon suffices for treating a horse, half a gallon for the external treatment and the rest into the serous cavities, if they are opened. Two men can easily treat a horse in 15 minutes. While the gases from the intestines are escaping there may be some smell but this phase soon passes off, and the very disagreeable odours due to putrefaction do not arise. This treatment will preserve the carcase satisfactorily for some weeks, keep off flies for some days and prevent the deposition of their eggs, or, if eggs are present, the hatching of the larvae. If fly larvae are present before the carcase is treated they will be killed. If the carcase cannot be disposed of within two or three weeks, it may be necessary to make use of small local supplementary applications.

(*b*) As the method described above has proved satisfactory, we do not now advocate under ordinary conditions the process of injection. Injection through the carotid artery is, however, easily accomplished, and a body thus treated in addition to the skin treatment is preserved for months.

(*c*) Putrefying bodies should be sprayed from a distance. Almost immediately the stench will be much diminished, if not entirely obliterated. If the body can be reached, it should be treated thoroughly. Other foul-smelling materials can be treated in the same way.

(*d*) Fly larvae can be destroyed by spraying or preferably sprinkling the fluid over and into the spaces in infested materials. In a short time all the maggots will be dead. For the treatment of manure, dust bins, latrines, etc., see p. 216. All flies touched by the spray will be killed.

(*e*) All flies should be as far as possible driven out of shelters or rooms and pieces of cloth sprinkled with creosote oil mixture hung over the places where they can enter. By this means the majority of flies can be prevented from entering for some days.

(*f*) Such fluids should not be emulsified with water.

(*g*) For disinfecting liquids an alcoholic solution of the fluid can be used, which on addition to water makes a milky opalescence throughout the fluid owing to the separation of the oil in the form of extremely minute globules.

Conclusions.

1. Experiments with the bodies of horses show that they behave in the same manner as those of smaller animals, and can be efficiently treated in the same ways. A body was preserved by surface treatment alone for a long period, and produced no appreciable nuisance from smell.

For large carcases relatively less fluid is required for surface treatment owing to the relatively small ratio of surface to weight.

2. The treatment of human bodies under war conditions has given satisfactory results.

3. Flies have been kept from entering such places as dugouts by hanging sacks treated with creosote oil mixtures over the entrance.

4. Adult flies in dugouts and other situations and on putrescent material are killed by spraying.

5. Latrines have been kept free from flies by spraying.

6. Manure should be treated by spraying with creosote oil at the earliest opportunity. If made into heaps each incremental addition should be spread uniformly on the heap and sprayed at the rate of at least 100 c.c. per horse per day. The manure does not seem to be injured by this treatment.

7. In towns the breeding places of flies could probably be treated with little expense, and the numbers of flies very greatly diminished.

8. The chief objections to the use of creosote oil for such purposes are (*a*) its irritant action on the skin and mucous membranes, (*b*) its inflammability and (*c*) difficulties in transport. In view of the excellent results obtained the objections are of little importance. In our

experience its irritant action on the skin is very slight, and the eyes can be protected by the use of glasses when spraying; its inflammability is low except when used as a spray, and suitable precautions could be easily employed. The difficulty and cost of transport have to be weighed against the economy in labour, since a single treatment with creosote oil is more efficient than many with 5 % emulsions of disinfectants.

General Summary.

1. Flies may be killed either by poisons (*a*) absorbed from the alimentary tract, or (*b*) acting through the respiratory system. They are very resistant to many alimentary poisons which possess considerable toxicity to animals, but are more susceptible to respiratory poisons.

2. As very little difference could be made to the general fly population by killing adults alone we have not persisted with experiments designed for this purpose. Aniline is the most suitable of the reagents, not dangerous to man, used in the way suggested, which we have tested.

3. Flies are most easily and effectually destroyed by attacking them in their immature stages as eggs or larvae.

4. The eggs of species likely to be dangerous to man by conveying infected material to his food are laid on (*a*) exposed animal matter, (*b*) manure, and (*c*) refuse.

The eggs and maggots in these situations may be considered to represent large numbers of flies in traps.

5. For killing eggs or larvae in their breeding grounds we have found coal-tar oils, especially creosote oil, to be the most satisfactory reagents. Aniline emulsions are useful, but have little effect on putrefactive processes and the nuisances due to them.

6. Flies may be repelled from substances which attract them, such as decaying bodies, faecal material, etc., and kept out of habitations by means of the repellent constituents of coal-tar oils.

7. Flies sprayed with these oils are killed.

8. In carcases true putrefaction or disintegration is preceded by (*a*) early gas formation, mainly due to the action of intestinal organisms on the carbohydrates of the intestinal contents and tissues, (*b*) exudation of fluid, probably due to the effects of cytolysis and enzyme action, and (*c*) green discoloration of the skin which appears to be connected with the effect of hydrogen sulphide or organic acids on the blood pigments. By suitable treatment the tissues may be rendered sterile, when neither gas nor green discoloration is produced though fluid exudes.

9. By true putrefaction in carcases we mean the breakdown of the tissue constituents, accompanied by the elimination of foul-smelling products. The process is due to the activity of putrefactive bacteria assisted by the action of tissue enzymes. Gas production and exudation of fluid continue as true putrefaction proceeds, but in much smaller daily increments than in the preliminary stages.

10. Descriptive methods are lacking in precision and do not give definite information regarding the progress of putrefaction. The need arose therefore for a method by which the actual products of putrefaction could be estimated. The importance of the combined activity of autolytic enzymes and putrefactive organisms in the disintegration of a carcase was impressed upon us by noting the great rate of production of volatile bases in tryptic digests containing such bacteria.

The putrefactive powers of various species of bacteria can be measured definitely by incubating an amino acid mixture containing the organisms under standard conditions for a suitable time and determining the ratio of bases to amino acids.

We claim that by similar means the relative powers of different disinfectants to inhibit the action of putrefactive organisms on carcases (kept under standard conditions, p. 209), can be compared precisely, using for analysis the fluids which exude or tissues from comparable situations.

The proteolytic as well as the deaminating enzymes of autolysis produce small amounts of ammonia. The results of their combined activity, in the absence of organisms, yield a low ratio of volatile bases to the substances which respond to the formyl titration. If putrefactive organisms do not develop in a treated carcase the same low ratio is obtained. The ratio is correspondingly greater the more active the organisms.

Our method enables us to measure the progress of putrefaction under all conditions, provided the reagents used to inhibit putrefaction do not interfere in the estimations.

11. The stenches arising during putrefaction are mostly derived from acid and basic products and from sulphur bodies. An ideal deodorant should be capable of fixing or absorbing all foul-smelling bodies.

12. We believe that putrefactive bacteria mainly gain entrance into the tissues through the skin.

13. The presence of water and a high temperature provide optimum conditions for the progress of putrefactive changes.

14. In the superficial treatment of intact or opened carcases and other putrescible materials reagents should be used which adhere to

the greasy surfaces, form films, render the skin waterproof and kill the bacteria in it, thus checking putrefaction by preventing the access of water and putrefactive bacteria to the tissues. Further the reagent should be capable of eliminating any stenches which may arise, repelling flies, killing the eggs or larvae, resisting the action of water and remaining operative in all respects for a long period.

15. Watery emulsions of disinfectants are necessarily deficient in most of these properties. Undiluted oily reagents only possess them.

16. By superficial treatment combined with injection of certain reagents into the blood vessels exposed carcases may be preserved for months.

17. The burial of carcases does not prevent the development of larvae present on them, or the subsequent emergence of the flies.

18. In our experience the reagent, which possesses the required properties to the greatest extent, and gives the most satisfactory results in practice and is sufficiently cheap and easily obtained for use on a large scale, is coal-tar creosote oil of "country make."

19. For general purposes, especially when the repelling of flies is of importance, we recommend the use of coal-tar creosote oil of country make, containing a high percentage of phenolic bodies, to which sufficient bases, extracted from "light oil," are added to make the proportion of bases to phenolic bodies approximately one to two.

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DESCRIPTION OF PLATES I—V.

PLATE I.

Fig. 1. The bodies of the two horses, *A* above and *B* below, on 6 October, 1915, 29 days after death. The wounds made in the thorax of the black horse, *B*, in order to introduce the fluid into the thorax are visible. Some of the gut, which was pulled out of the abdominal wound, is seen lying on the abdomen.

PLATE II.

Fig. 2. The body of the white horse, *A*, on 14 Sept. 1915, seven days after death. The abdomen is somewhat distended with gas, and the injection wound in the neck is visible.

Fig. 3. The body of the white horse, *A*, on 4 October, 1915, 27 days after death. The abdomen is less distended.

Fig. 4. The body of the white horse, *A*, partly dissected, on 6 October, 1915. The gloss on the peritoneal surfaces of the intestines and the excellent condition of the abdominal wall is well seen.

PLATE III.

Fig. 5. Dissection of the superficial muscles of the gluteal region and leg of the white horse, *A*, on 6 October, 1915. The reflected skin is seen lying below.

Fig. 6. Dissection of the deeper muscles of the gluteal region of the white horse, *A*, on 6 October, 1915. The normal appearance of the hair should be noted.

PLATE IV.

Fig. 7. Further dissection of the white horse, *A*, showing the normal appearance of the abdominal organs.

Fig. 8. Dissection of the black horse, *B*, on 6 October, 1915, showing part of the intestines with glossy peritoneal surfaces, and a deep incision into the muscles of the hind quarters. The skin has been reflected downwards, and a large mass of muscle is hanging down behind the reflected skin. A portion of the gut, which had been exposed throughout on the surface of the abdomen, is seen at the upper margin of the abdominal wound. (Cf. Fig. 5.)

Fig. 9. The body of a control guinea-pig 24 hours after treatment with creosote oil showing great numbers of large maggots dead and black.

PLATE V.

Fig. 10. Vertical section ($\times \frac{2}{3}$) through the skin and left gluteal muscle of the white horse, *A*, made on 20 April, 1916, seven and a half months after death.

Fig. 11. Vertical section ($\times \frac{2}{3}$) through the skin and left gluteal muscle of the black horse, *B*, made seven and a half months after death. Small gas bubbles are visible throughout the section.

Fig. 12. Section of muscle taken on 20 April, 1916, seven and a half months after death, from the left gluteus of the white horse, *A*. The fibres are regular and the striae very evident.

Fig. 13. Section of muscle taken on 20 April, 1916, from the left gluteus of the black horse, *B*. The fibres are irregular, and some are degenerated. The striae are irregularly disposed but are well marked in some of the fibres.

Fig. 14. Photograph of rabbit (*I*) after removing the skin of the right side on the 19th day after death. The body was injected with creosote oil (9 c.c. per lb.) and the skin treated with 15 c.c. per lb., and it was allowed to lie on the ground without protection.

Fig. 1

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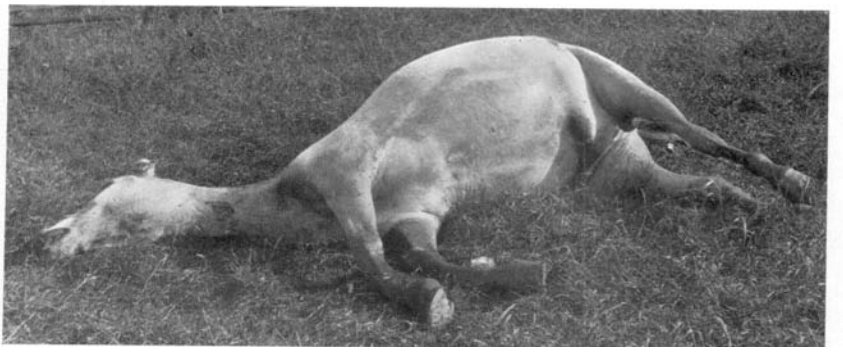

Fig. 2

Fig. 3

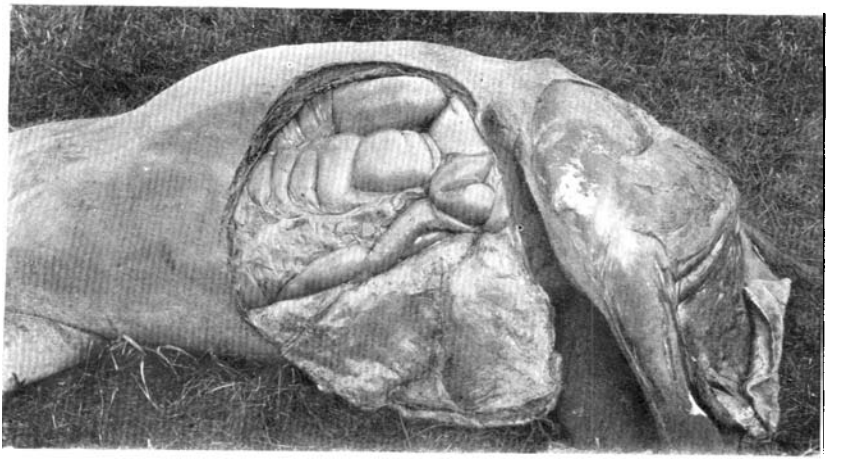

Fig. 4

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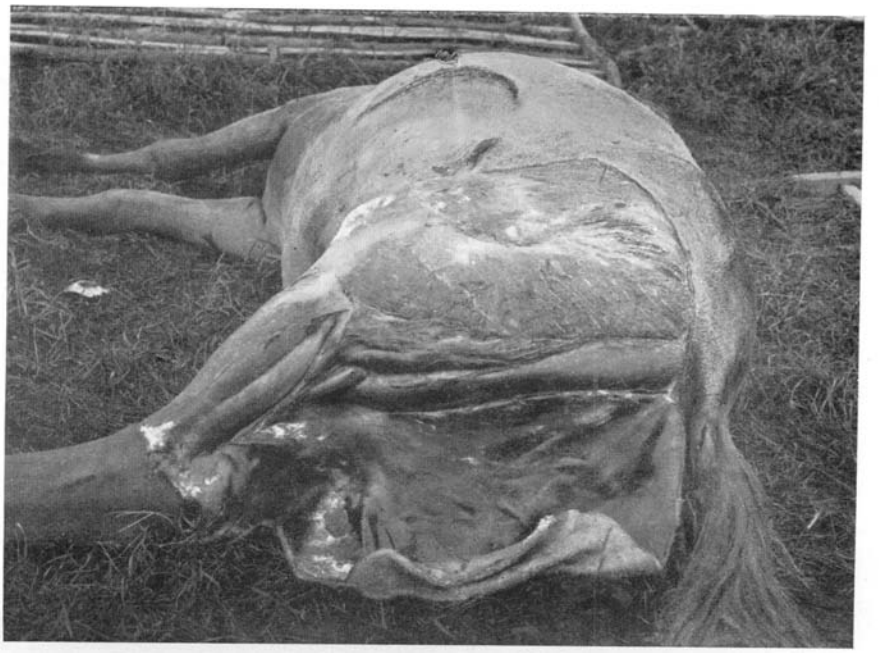

Fig. 5

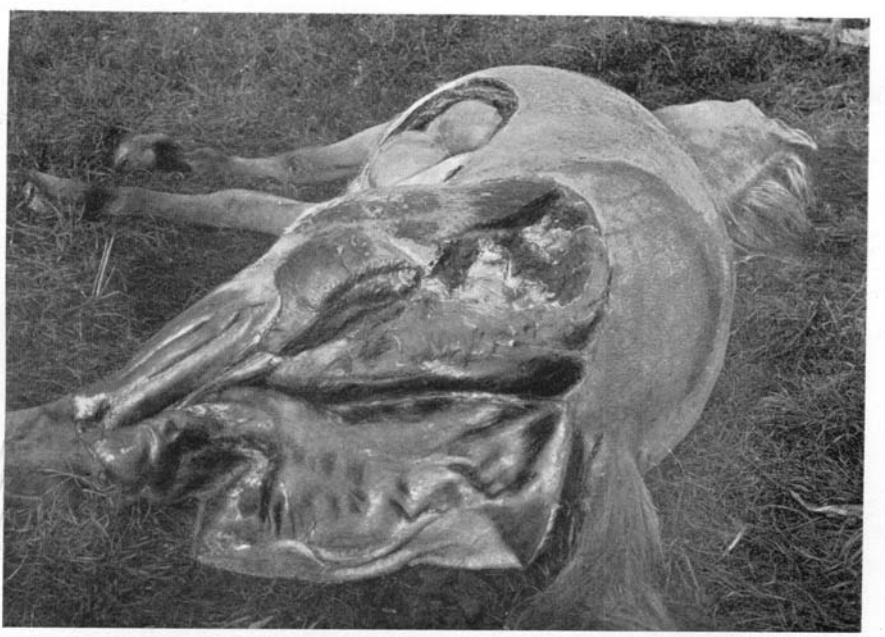

Fig. 6

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Fig. 7

Fig. 8

Fig. 9

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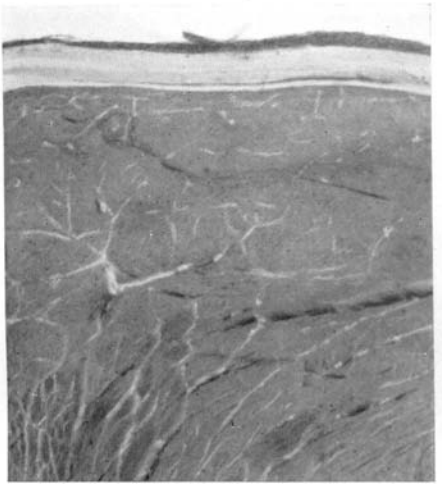

Fig. 10

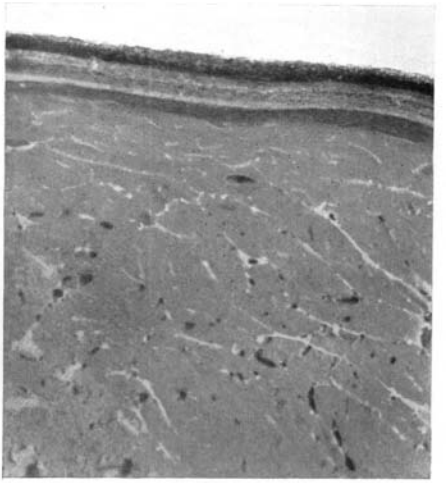

Fig. 11

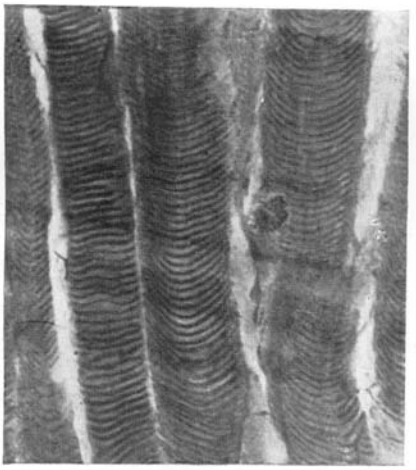

Fig. 12

Fig. 13

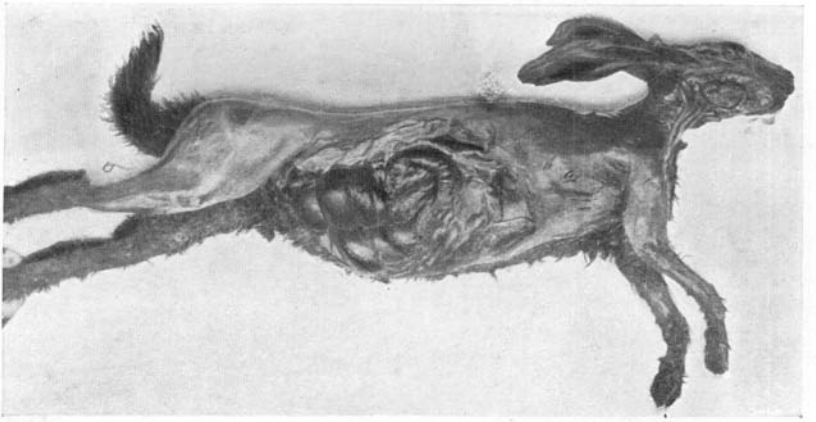

Fig. 14

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