A CRITICAL REVIEW OF KALA AZAR AND TROPICAL SORE.¹

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(Continued from p. 580, vol. xvii.)

II.—Infantile Kala Azar. L. infantum, Nicolle.

The first cases of this disease were recognized by Pianese and by Cathoire in Italy and by Nicolle in Tunis. In the former country a form of infantile splenic anaemia had long been recognized by Italian physicians, and by some of them its apparently infectious nature had been realized long before the discovery of the parasites. The giant’s share in the rapid extension of knowledge as to its association with Leishmania, and the mass of experimental and other evidence dealing with the aetiology of the disease, we owe to C. Nicolle and his colleagues at the Pasteur Institute of Tunis, who, during the last four or five years, have issued a long series of valuable reports in the Archives published from their Institute.

The earlier cases were mostly isolated cases detected by Nicolle and his associates in or near Tunis itself, but once attention had been drawn to the fact that this deadly disease existed on the Mediterranean littoral, it was not long before numerous additional districts and countries were signalled as infected. It is impossible at present to assign accurate limits to the Western or Mediterranean form of kala azar, since additional foci are

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continually being made known, but it is obvious, even now, that practically the whole of the countries bordering the Mediterranean are infected in greater or less degree, and, as was suggested in the case of the Indian form, it is probable that a mere fraction of the actual number of cases occurring in these countries is at present brought to light.

The known distribution may be roughly indicated as follows: On the southern shore, Algeria, Tunis, and Tripoli have all been proved to harbour the disease, while Abyssinia and the Sudan are in parts affected by a kala azar which appears to conform more closely to the Indian type. To the east, Syria is said to be heavily infected. Of the countries bordering or adjoining the northern shore of the Mediterranean we have reports of cases from Crete, the Grecian archipelago, Greece, Turkey, Italy, Malta, Sicily, the Lipari Islands, and finally Portugal. Of these, the island of Sicily and the coast of Calabria appear to be most heavily infected, though it may well be that the greater number of cases reported from these localities is more accurately explained as a measure of the interest and enterprise of the local physicians. However far this distribution may eventually prove to be short of the reality, it will be clear that a form of kala azar is at the present moment widespread in Southern Europe, as well as in Northern Africa, and, since it has been found as far north as Rome itself, there may be reason to dread its extension still farther north, if indeed it does not exist already in Central Europe.

It is not certain whether the infantile type exists elsewhere than round the Mediterranean basin, but Jerusalem speaks of its presence in China, in the province of Nganwei. Until we have more accurate means of distinguishing the two forms than is afforded by the age-incidence and small differences in symptomatology we cannot hope to be certain of this, and it is still far from certain that we have justification for accepting more than one form of the constitutional disease. In India, at all events, cases are met with very frequently in infants and young children, although the disease appears to display a predilection for young adult life; while cases, admittedly rare, have been found in adults in countries affected by the infantile type. For instance, Tashim mentions a case 17 years old at Tripoli, Gabbi one of 18 years at Stromboli, Fulci and Basile a case in a young man of 19 at Rome, and Gabbi another in a man of 38 in Calabria.

However this may eventually be settled, it is undoubted that the enormous majority of the cases occurring in Southern Europe
and Northern Africa affect young children of from 2 to 5 years, and in this respect form a striking contrast to the more advanced age-incidence of the Asiatic type. The sexes are apparently equally susceptible, and no race appears exempt, to judge from the details of the cases published.

Before dealing with the different branches of the subject seriatim, it may be well at this stage to indicate the principal points of difference between the Indian and the infantile forms. Briefly these are the following: (1) The infantile attacks almost exclusively young children, while the Indian is met with at all ages. (2) Certain differences of symptomatology have been described. (3) Cultures of the parasites are readily obtainable upon Novy-McNeal medium in the case of L. infantum and are easily sub-cultured, while, in the case of L. Donovani, cultures on this medium are as a rule unsuccessful, and sub-cultures cannot be made. On the other hand, cultures of L. Donovani succeed in citrated splenic blood and usually fail in the case of L. infantum. (4) Inoculation of the spleen parasites into dogs and monkeys reproduces the disease in the case of L. infantum and fails in L. Donovani. (5) A spontaneous infection of dogs has been found in the endemic areas of infantile kala azar, but no such infection of dogs has been encountered in India.

Morphology of the Parasites.—A number of careful studies of the morphology of the parasites, as encountered in the body tissues, have been published. Such descriptions correspond extremely closely one with another and confirm earlier impressions as to the great degree of uniformity of structure observable in specimens taken from cases in different districts and countries. All are in close accord with the earlier descriptions of both L. Donovani and L. tropica. This morphological identity is emphasized by some, for instance by Visentini, and can be confirmed by the writer, who has been unable to detect any constant differential feature between the three species. Forms indicating the mode of growth and multiplication have been closely studied, and division appears to be commenced by amitotic division of the nucleus, which subsequently extends to the cytoplasm. The great number of parasites frequently found in the cells—up to 200—has given rise to discussion as to how large a part is played by phagocytosis. It appears probable that the first parasites are taken into the cell in this manner, but that intracellular multiplication will account for the larger number. Degeneration forms are spoken of by several, and Tomaselli considers that death of the parasite takes place by a
vacuolization, first of the protoplasm and subsequently of the nucleus, the blepharoplast being the last structure to disappear. In view of the intense vacuolization of the protoplasm exhibited by parasites undergoing rapid development in cultures, the writer is doubtful whether this is to be taken as a sign of degeneration, and whether it may not rather point to an increase in the functional activity of the protoplasm. Forms have been noticed by many observers in which the blepharoplast was absent, and such have been taken to represent a hitherto unrecognized stage of the parasite, degeneration forms, or, as Jemma and di Cristina think, young parasites soon after fission of the mother-cell. The point remains unsettled.

The presence in certain parasites of a third structure in the form of a thread of chromatin substance running from the blepharoplast to the nucleus, originally noted by Christophers in L. Donovani, has since been described by Mesnil and by Novy in L. infantum; they consider it to represent a rhizoplast and to stand in an important relationship to the flagellum.

Not infrequently differences in size and shape of the parasites have been mentioned in relationship to the organ from which they were derived; Pianese, for instance, considered that they were smallest in the liver and largest in the marrow, whilst those in the spleen were of intermediate size; from this he suggested that the marrow was probably infected first and the liver last. Other observers, however, do not confirm this difference in size in different organs, at all events as a constant feature in a series of cases.

The blue-staining matrix in which a number of parasites are frequently seen to be embedded is generally accepted as a fragment of the endothelial or other cell in which they were originally contained, but Feletti thinks it may have some nutritive relationship to the parasites which are embedded in it.

Cultures and Cultural Forms.—Artificial cultures of the infantile form were obtained by Nicolle from some of his earliest cases, and he has elaborated cultural methods which have been very successful, both in his own hands and in those of others. Thanks to these methods it has been found possible to make careful investigation of the various stages of the development of the parasites into free-swimming flagellates, and numerous detailed accounts of these forms are now available.

Nicolle first succeeded in his culture of the parasites by employing the medium of Novy and McNeal, which is an agar mixed
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with defibrinated rabbit’s blood; but he was unable to secure any growth in Roger’s medium of citrated splenic blood. The material derived from a splenic puncture was inoculated directly into the condensation fluid at the bottom of a tube of Novy’s medium which was kept at a temperature of 20° C. to 22° C. Growth commenced about the seventh day and was abundant by the fifteenth day, numerous masses of parasites in all stages of growth and multiplication being present, the majority flagellated and motile.

Since then Nicolle has simplified Novy’s medium by employing only a solution of agar and salt, to which is added fresh rabbit’s blood without preliminary defibrination. In this way he dispenses with both peptone and meat extract, neither of which he finds essential. With this modified medium he gets even more rapid and abundant growth and almost invariable success, provided the conditions he lays down are carefully observed. With it he finds growth commencing on the fourth or fifth day and increasing up to the thirtieth day. The formula which is given is the following:

- Agar, 14 grm.;
- Sea salt, 6 grm.;
- Water, 900 cc.;

and he lays great stress on the preliminary purification of the agar from impurities and salts by maceration in cold water. This mixture is then sterilized in the autoclave, without previous standardization and distributed into tubes. The tubes are subsequently melted and then cooled down to between 48° C. and 52° C., at which temperature one-third of their volume of rabbit’s blood is added, taken directly by aseptic puncture of the heart. After the mixture has been allowed to set in an inclined position the tubes are covered so as to protect the contents from evaporation and incubated for two or three days, with the double purpose of proving their sterility and encouraging the formation of condensation fluid. After this the tubes should be kept in the dark, and it is best not to use them for some days. They will keep their nutritive qualities for more than a month. Further details have been given by Manceaux of the points which it is well to observe to get the best results from this modified Novy-McNeal medium, or, as Nicolle calls it, “N.N.N. medium.”

Another medium has been recommended by Laveran and Pettit as useful when massive cultures are desired for any purpose. The formula of this is:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
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<tbody>
<tr>
<td>Peptone, Chapoteaut</td>
<td>2 grm.</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>6 grm.</td>
</tr>
<tr>
<td>Water</td>
<td>900 cc.</td>
</tr>
<tr>
<td>Rabbit’s blood (defibrinated)</td>
<td>1 vol.</td>
</tr>
</tbody>
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The peptone solution is prepared first and poured into small Roux flasks, an equal volume of rabbit’s blood being then added. They have not found any advantage resulting from the substitution of sheep’s blood or of market preparations of haemoglobin. The flasks should contain approximately 30 cc. and should be filled to about one-tenth of their capacity. Incubation is carried out at 21° C. to 22° C.

Rogers’s method of employing simply the splenic or hepatic blood, kept liquid by the addition of sodium citrate, appears to have failed almost invariably when tried with material containing L. infantum; but successful cultivation into flagellate form has been obtained by di Cristina and Cannata, by Longo and by Gabbi. In connexion with this the writer may record that success has by no means invariably followed his employment of Rogers’s medium, even when dealing with cases of kala azar of Indian origin.

In most cases the presence of oxygen appears essential for free growth, but di Cristina and Cannata were only able to get development of a somewhat weak nature, in citrated rabbit’s blood on employing anaerobic conditions.

In almost all cases of successful cultivation the material has been derived either from the spleen, liver, or bone marrow of patients or of animals, naturally or experimentally infected, but Novy records a positive result from the inoculation of his medium with the circulating blood of an infected dog. The paucity of the parasites in the circulating blood is against the chances of success in this line, but it is quite possible that, if tried on an extended scale, it might prove a valuable diagnostic method in cases where microscopical examination of the blood failed and splenic or hepatic puncture appeared undesirable.

A great advantage of the methods of Novy and Nicolle lies in the fact that sub-cultures are readily obtainable and that it is possible in this way to maintain in active growth a given strain for an indefinite time. M. Nicolle was good enough to send the writer a culture from Tunis, which reached him, in London, in good condition, and which he was able to carry on through twelve generations, covering a period of over three months, the medium in this case being the original medium of Novy and McNeal.

As to the morphological details of the cultural forms of L. infantum no attempt will be made to summarize the mass of work recently recorded, since this would demand a separate article, and, after all, is not of great practical importance: only a few
points will be touched. On the whole it may be said that there is an extremely close relationship between the forms developing from the parasites of Indian and infantile kala azar respectively; indeed, some who have gone closely into this matter state that they are indistinguishable—for instance, Pulvirenti concludes that they are absolutely identical and believes in the unity of the species. The writer has also made a prolonged study of the two forms, contrasting cultures made by himself and by Statham from Indian cases with cultures of *L. infantum* derived from Nicolle at Tunis. He concluded that small morphological distinctions do exist between the two, but has not had the opportunity of determining how far these differences are real and how far they may have been attributable to the different media employed in the two cases. One fact of some importance he was able to elicit, namely, that the parasites of Indian origin will grow on Novy-McNeal medium, as in one case he obtained development up to the appearance of flagella; the culture, however, was not vigorous and attempts at sub-culture failed.

Di Cristina and Cannata, as a result of their study of the cultural forms of *L. infantum*, think they obtained evidence of a sexual method of reproduction with the formation of cytogametes and gametes, and further details of these are given by Jemma and di Cristina, but their work lacks confirmation and the subject is too complicated to admit of clear summary.

Parasites with two flagella have been seen by many; the writer has only observed them in the case of *L. infantum*, and their significance is doubtful. In the majority of instances it is almost certainly a case of longitudinal fission of a parasite and early appearance of the second flagellum, but other specimens are not so easily explicable. Jemma and di Cristina think that when seen in stained films they are artifacts, since they have failed to notice them in hanging-drop preparations.

The small fillet-shaped segments, apparently the result of unequal longitudinal fission, which were originally described by the writer from a case of the Indian disease, have been figured and mentioned by several of those who have made a study of the infantile form; they doubt, however, whether the granules which these fillets contain are of true chromatin nature and their significance is still uncertain.

The importance of the study of these flagellated cultural forms is not only considerable on phylogenetic grounds and for the determination of the number of species of *Leishmania*, but has, obviously,
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an intimate bearing on the etiology of the different diseases caused by this genus. Although test-tube experiments have their obvious limitations, somewhat unduly emphasized by some, in the opinion of the writer, there can be little doubt that the forms encountered in these artificial cultures are the forms which will be found in the transmitting ecto-parasites and which appear, as will be seen, to have already been found in the case of the infantile form of kala azar. In this connexion the almost universal failure in infantile kala azar to infect by means of cultures—Novy alone has succeeded in infecting dogs with massive doses—suggests that before successful infection can take place it may be necessary for the flagellate parasites to assume a post-flagellate form, to follow Patten’s nomenclature, and that only in this form can they effect a lodgment in the tissues of their new host. Some support of this view may be found in the fact that the fate of flagellates inoculated into the body is to be speedily phagocyted and destroyed; this has been noticed by Delanoe, and the writer is able to add that he has observed intense phagocytosis of the cultural forms of *L. infantum*, *in vitro*, when placed in contact with his own leucocytes at a temperature of 37°C. In this case there was no doubt of the destructive action of the cell juices, as the parasites, even when fully formed, motile and flagellated, were rapidly disintegrated, and in a very short time nothing was left but a little granular débris lying in the vacuoles formed in the polynuclears during the process of digestion.

Animal Experiments.—The parasite of infantile kala azar has proved to be capable of infecting animals, and in this respect presents a striking contrast to the Indian form, with which, as has been said, no positive results have so far been obtained. The animals which have shown themselves most susceptible are the dog and the monkey, though slight infections have also been recorded in mice, white rats, guinea-pigs and rabbits. In addition, the very important observation has been made of spontaneous infection in dogs. This spontaneous canine kala azar will be considered separately after the experimental forms of animal infection have been dealt with.

Experimental Infection of Dogs.—Nicolle, as has so often to be recorded, was the first to succeed in this by inoculating some emulsion of spleen tissue, rich in parasites and derived from one of the earlier child cases, directly into the liver substance of a dog and also into its peritoneal cavity. The dog showed no symptoms and, two and a half months later, was given a similar dose from another case. The animal was sacrificed on the 159th day after the first
inoculation, and parasites were found in the spleen and, more rarely, in the liver. This experiment has been repeated by Nicolle and his co-workers on numerous occasions, and has also been confirmed by numerous other workers, the virus being derived either from a human case or from a dog previously infected. A study of these cases, however, makes it clear that the almost invariable success which attended Nicolle's experiments—he mentions one series of eighteen results among nineteen dogs—is not attainable in all instances; for instance, Jemma and di Cristina found many dogs refractory even to massive doses of the virus. It appears extremely probable that differences as regards susceptibility exist in different breeds of dogs, if indeed some breeds are not altogether refractory. As has been pointed out, such differences in susceptibility may in part be accountable for the failure to infect dogs in India.

With the single exception of Novy's experiments, mentioned below, the material used for inoculation has invariably been an emulsion of spleen, liver, marrow, or other organ containing the intracellular forms of *L. infantum*; inoculation of cultures from which these forms are absent has failed. In most instances the original technique of Nicolle has been followed in the mode of inoculation, namely, the simultaneous inoculation of this emulsion into the liver substance and into the peritoneal cavity. Infection has, however, frequently followed inoculation into one or other site alone, and those who have experience of both methods think the peritoneal channel is preferable and more certain. Numerous attempts at infection by means of intravenous inoculation have failed. Subcutaneous inoculations have also given negative results, at all events as regards the induction of a generalized infection, but Nicolle and others have at times noted a local reaction at the site of inoculation, from which parasites were recovered, which is of interest in connexion with the etiology of tropical sore; the histological characters, however, of these rare local lesions in no way resemble those caused by *L. tropica*.

The symptoms of experimental infection in the dog correspond closely with those of the spontaneous infection in these animals. The disease may assume one of two forms, an acute form which is often fatal in three to five months and usually occurs in young animals, or a mild form which is very chronic and occurs more often in older dogs. The acute form is accompanied by irregular fever, progressive wasting, motor disturbances involving the hindlegs, occasional diarrhoea, and the animal dies in a comatose con-
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dition. In the chronic form, on the other hand, although some of the above symptoms may be present, the animal may show practically no signs of disease and may remain in apparent good health, except for some loss of weight. The existence of the latter type of infection naturally adds to the difficulty of determining whether infection has resulted from the inoculation, and emphasizes the importance of satisfactory methods for diagnosing the disease in dogs. Symptoms being obviously unreliable, recourse must be had to the demonstration of the presence of the parasites in the body. The parasites are almost always present in the spleen, liver, and bone-marrow of infected animals and, though rarely, may also be found in the peripheral blood, especially at such times as high fever exists. The infrequency of their presence in the blood necessitates search being made in one of the other infected organs or tissues. Spleen puncture in the case of a dog is impracticable in view of the impossibility of locating this organ with accuracy. Liver puncture is easy, and is the method usually employed, but it has the disadvantage that parasites may not be found, although a subsequent post-mortem examination may reveal their presence in abundance in the organ. Examination of a sample of bone-marrow is coming to be more frequently relied upon, a small sample being obtained by trepanning the femur or the tibia; in the hands of Italian workers this method has given good results, and it appears of especial value in experiments conducted in areas where the spontaneous disease exists or is suspected, since it is possible in this way to ascertain with a fair amount of confidence that dogs which are intended to act as controls or to serve for infection experiments are normal and free from all traces of infection.

It is possible that cultural methods applied to the blood or to material derived from some organ or tissue may give a positive result even when careful microscopical examination has been negative. Laveran and Pettit, for instance, mention a case in which splenectomy was performed and cultures made from the organ were positive in spite of the failure to detect the parasites in stained films.

The very chronic nature which the experimental form may assume is illustrated by two cases recorded at Tunis, in which the animals died after seventeen and eighteen months respectively, without having shown any marked symptoms; in one of these cases the infection of the organs was found to have been intense. There does not appear to be any relationship between the size of the infecting dose and the subsequent attack; a very small number of
parasites may give rise to an intense and fatal attack, and a massive
dose to a mild one, or it may even fail, and this when working with
the same virus.

Infection of Monkeys.—These animals were proved
to be susceptible by Nicolle shortly after he had succeeded with
dogs, and the method of infection and the character of the attack
follow much the same lines in each. On the whole, the symptoms
are more manifest, and of seven *Macacus sinensis* inoculated by
Nicolle two died within three months. The well-known delicacy of
these animals in captivity, even under the best conditions, makes it,
however, difficult to judge points such as this. A case was noted
in which the animal showed no signs at all during life, but was
found after death to be heavily infected. A point of some interest
is that in several cases parasites have been detected in the hepatic
cells, an extremely rare condition in man, if it ever occurs, and one
which Nicolle has only met with once in the dog. Later experi­
ments were carried out with another species of monkey, *Macacus
cynomolgus*, which proved to be equally susceptible to *M. sinensis*,
and possibly a little more so since in one animal a petechial
eruption was noticed in the course of the disease, and this had not
been encountered in *M. sinensis*.

Infection of the smaller experimental animals has in most
instances failed, but Laveran and Pettit, by inoculating material
derived from an infected dog, noticed a slight infection in the case
of the mouse, the white rat, and the guinea-pig, parasites being
found in mononuclear cells of the peritoneal exudate as late as fifty­
nine days after the inoculation. Volpino, too, has recently
succeeded in producing a keratitis in the cornea of the rabbit by the
inoculation of parasites into the scarified surface of this tissue;
three months later the portion of cornea examined was found to
contain *L. infantum* in large numbers.

A few other points may be noted in connexion with these very
numerous animal experiments. In a few instances opportunity was
afforded of examining fœtuses of infected dogs; in no case were
they found to be infected, we have therefore, no suggestion of the
possible hereditary transmission of the disease in dogs. Splenectomy
has been performed during the course of the infection in dogs and
monkeys without in any way modifying the progress of the disease.
Passage of the virus from dog to dog is readily procurable, and may
apparently be carried on indefinitely; no marked alteration in the
virulence of the strain is apparent. On the other hand, passage of
the virus through monkeys has been found by Nicolle and
Manceaux to result in the diminution of the virulence for the monkey, but not for the dog.

**Infection by means of Cultures.**—The whole of the experimental work dealt with above was the result of infection with the non-flagellated parasite, as it is met with in the tissues of man and infected animals. Numerous attempts to produce infection by means of the flagellated forms which develop in artificial cultures have been made in Tunis, Sicily, Italy, and elsewhere with universal failure except in the hands of Novy. He thought that the failures might possibly be due to the employment of too small doses, and in consequence gave repeated and large doses to a dog and succeeded in infecting it. The strain with which he worked was derived from Tunis, and had been sub-cultivated through so many generations that it was hardly possible that any of the pre-flagellate forms could have persisted in a living, but undeveloped, state. In all he gave fifteen inoculations to the animal, spread over a period of four and a half months, the doses varying from the contents of 8 to 40 culture tubes: the total amount corresponded to the growth from 270 culture tubes of his medium! The animal remained in good health in spite of this colossal dosage, but when killed was found to be infected, the parasites being numerous in the cells of the spleen and liver, but mostly free. Cultures made from the infected organs were also positive, proving the vitality of the organisms.

Repeating this experiment, he records in a later publication the infection of five other dogs, and that he was able to secure infection by a single inoculation of the material from 20 culture tubes. He recommends for diagnosis during life cultivation of the blood, 10 cc. being collected and distributed over 20 tubes of culture medium.

These observations of Novy's are naturally of great importance, and, as he points out, establish the susceptibility of the dog to cultural infection and complete the chain of evidence regarding the relationship of the parasite to the disease.

**Spontaneous Infection in Dogs.**—Here, too, we are indebted to Nicolle for the recognition of the important fact that parasites indistinguishable from *L. infantum* occur in dogs and cause in them a disease presenting a resemblance in many ways to the infantile form of kala azar. On finding that dogs were susceptible to the disease on inoculation of the infantile virus, he searched first for evidence of any association between actual cases in children and dogs, and in several instances such information was forthcoming, children who had contracted the disease being found to have lived...
in intimate association with dogs, some of which died during this time, or subsequently, of an indefinite disease. Next, he made a systematic examination of the dogs which were destroyed at the fourrière in Tunis, and soon found one which harboured the parasites in its organs.

Since then large numbers of dogs have been examined and other cases found. In his first series four infected animals were found in 220 examined, while in a more recent series carried out at Tunis by the Yakimoffs, five were found infected out of 299, a percentage of 1.67.

Search for the existence of this spontaneous infection of dogs was soon instituted in other districts in which infantile kala azar had been found, with the result that, almost without exception, infected animals were detected. In two instances parasites were found in dogs in localities in which infantile kala azar had not been recorded, but each of these announcements was speedily followed by the discovery of cases whose undoubtedly genuine character was demonstrated by the finding of the parasites on spleen puncture. These were, first, Algiers, where Edmond and Etienne Sergent first found nine dogs infected out of 125 examined, a percentage of 7.2, and where Lemaire subsequently found a human case. The second instance was at Rome, in which the presence of the infection in dogs was first recorded by Basile, and not till later was the first human case reported by Fulci and Basile. At the present moment it may be said that in every country in which search has been made and in which infantile kala azar has been proved to exist, spontaneous Leishmania infection of dogs has also been found. The only exception so far reported is at Palermo, where infantile cases occur, but in which Jemma and di Cristina examined 300 dogs with a negative result.

The percentage of infected dogs in a given area seems to vary considerably. In an endemic focus of the disease discovered at the village of Bordonaro in Sicily by Gabbi, an investigation of the dogs by Basile showed the heavy infection of 27 out of 33 dogs examined. At Rome, just referred to, Basile found also 16 infected dogs out of 60 examined, but by reason of the diagnostic methods employed was inclined to believe that this was an underestimate of the degree of infection. Alvares and De Silva found 1 infected dog out of 19 in Lisbon, where the infantile disease exists. Nineteen infected dogs were found among 284 examined by Cardamitis in Greece, of which 15 out of 184 were in the city of Athens itself. Critien, at Malta, found 3 infected out of 30
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examined, and in this instance also the human infection is known to co-exist with the canine.

Negative results were obtained by Fülleborn with 50 dogs in Hamburg, where there is no suspicion of the human disease, and Donovan's negative results at Madras may also be recalled, though connected with the Indian form, as he has examined 1,150 dogs there without finding any trace of Leishmania. Finally, Bousfield found parasites in a dog in the Egyptian Sudan which had been in association with one of the human cases of the kala azar which is met with there, although it remains doubtful to which group these Sudanese cases are to be joined.

Pathological Anatomy.—There is a very close correspondence, if not absolute identity, between the infantile and the Indian forms as regards the distribution of the parasites in the body and the histological changes which their presence causes. As regards distribution, whatever blanks may be noted in connexion with one series of cases could be filled in from the results of another investigator. The sites in which the parasites are most abundant, and in which they seem almost invariably to occur, are the spleen, liver, and bone marrow; but, besides these, they have also been found in the kidney, the lung, the pancreas, the mesenteric and other groups of lymphatic glands. Critien has also recorded finding them in mucous flakes passed in the stools of a three-year-old child at Malta, the first observation of this kind in either form of the disease.

The histological changes induced in these tissues have been the subject of much careful study by Pianese, Jemma and di Cristina, and others, and their results are in the closest agreement with similar studies made on the Indian disease. The irregular distribution of the parasites in a given organ has been remarked on many occasions, and the histological changes in such organs are, as one would expect, largely dependent upon the degree of cellular infection.

The changes in the spleen have been closely investigated, especially by Italian workers, and Pianese considers that the essential features are a well-developed fibro-adenitis, the elastic tissue remaining normal, the cells of the follicles become involuted and assume an epithelioid character; there is also a diminution of the spleen pulp in the areas where the "macrophages" are abundant and the venous spaces appear to be dilated.

Nothing differing from the Indian form has been noted as to the distribution of the parasites and the histological alterations in other
situations. From a study of the experimental disease it appears probable that the endothelial cells of the lymphatics and smaller capillaries of the organ concerned are first invaded.

The comparative rarity of bowel symptoms of a dysenteric character in the infantile form is notable, and this is reflected in the rarity with which intestinal lesions have been mentioned; at the same time, Jemma and di Cristina found the parasites in the follicles of the large intestine in one of their cases, and the observation of Critien has already been mentioned.

Treatment.—Up to the present, the treatment of infantile kala azar stands in no better position than that of the Indian disease, although the former now possesses the great advantage that dogs and monkeys can be infected and it is therefore possible to carry out on these animals tests of various particular therapeutic measures. Experimentally infected animals have been largely used for this purpose, but, although certain drugs give encouraging results with such animals, nothing but persistent failure seems to follow their application to the sick child. There is, however, no reason to despair of ultimate success, and encouragement may be drawn from the fact that cases of spontaneous cure in children do occur, while it is also possible that the apparent limitation of this form to young children may be partially due to adult immunity, acquired by unrecognized attacks in childhood.

A long list might be given of the drugs which have been tried in recent years, but only a few will be mentioned. No one has a good word to say for quinine, whose failure has indeed come to be looked on as one of the symptoms. Atoxyl has been tested exhaustively on many systems of dosage and interspacing of individual injections, but although some cases appeared to improve there is no record of recovery attributable to its action. Domela, however, appears to have a higher opinion of its value, though the case he recorded could not be claimed as a cure at the time he wrote. Arsenophenylglycin was employed in a fair number of cases but has not been of any benefit, and the experiments which Nicolle and Comte carried out with it in dogs experimentally infected were not encouraging, indeed the disease appeared more severe in the case of the treated dog than in the untreated control.

Electromercurol has been employed by Nicolle and by Cortesi and Lévy, by intramuscular injection, and has been pushed as regards dosage to its limits, but with the usual result of failure. Colloidal electrargol and colloidal thiarsol also failed to do any good when tried by Cortesi. Jemma, however, speaks of the value of
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Roentgen rays and believes that the disease is curable. Morpurgo employed small doses of arseniate of soda, but without effect.

The striking results which have attended the employment of Ehrlich's new remedy "606," or dioxydiamidoarsenobenzol, in syphilis and other spirilloses has led to its being tried in many other affections, and hopes were entertained that it might be beneficial in this disease. There are now on record a fair number of cases in which it has been tried, but so far with no better results than have followed other arsenical preparations. At the same time there remains some hope that further experience may prove more satisfactory, since experiments on dogs have apparently shown that this drug, alone of all that have been tried, is capable of killing the parasites, at all events in some instances. In the first of these experiments Nicolle and Conor inoculated 20 cg. into the thigh muscles of a dog of 11 kilos, which had been infected by intraperitoneal inoculation, and in which two successive punctures of the liver had shown the parasites to be increasing. Four days later, puncture of the liver in three places failed to disclose any parasites, and a subsequent trepanning of the tibia yielded a similar negative result. The animal, which remained in good health, was killed on the thirty-fifth day and no parasites could be found in any organ. This result naturally gave rise to great hopes that arsenobenzol might abort the human affection in similar manner, and at Tunis and elsewhere it has been tried on children, but there is no case of recovery up to the present moment. It appears to be in the main a question of dosage. In the case of the dog the sterilizing dose seems to approximate 2 cg. per kilo of body weight, and such a dose would be dangerously near the toxic dose for a human being. Nicolle also points out in this connexion that the experimental disease in the dog is comparatively mild and frequently terminates in spontaneous recovery, while in the child the tendency is towards a fatal issue, and that this fact, in addition to the greater tolerance of the drug by the dog, may explain the contradictory results so far attained. At the same time he thinks that further experience may be more successful and he intends to administer the arsenobenzol in his next cases by the intravenous method; hitherto it has always been given intramuscularly and in doses which could not be expected to be of much service. Further encouragement is to be derived from the good results reported of the action of "606" in tropical sore by Manceaux, and the results of its further trial in infantile kala azar will be awaited with great interest.

Fresh bone marrow, the use of which was advocated some years
ago in the Indian disease, has been tried by Sluka and Zarfl, but appeared useless, and hectine, which was used by both Mara and Conseil, does not appear to be any more reliable, although the case treated by the latter gave some signs of improvement.

**Etiology.**—Progress in the elucidation of the mode of infection in infantile kala azar has been remarkable during the last two years, fact has been added to fact and the whole now pieces together in a most convincing manner. It may still be too early to speak of the etiology as completely clear, but the observations summarized below will show how little doubt can remain that canine kala azar is identical with infantile kala azar and that the transmitting agent is a flea, either the dog-flea, *Pulex serraticeps*, or the human flea, *P. irritans*.

As soon as Nicolle had found that the disease could be transmitted to dogs and had further discovered the existence of the spontaneous infection in these animals, he naturally sought for evidence of any connexion between infected children and dogs. This was speedily forthcoming, as two of the first four cases were known to have lived in close contact with dogs, while in one instance the dog in question had been ill and had died. He suggested, then, the natural hypothesis that some ecto-parasite of the dog might prove to be the transmitting agent between dog and child. It soon became manifest that this association between dogs and infected children was of frequent occurrence and, further, that localities where infantile kala azar was found were also localities in which the spontaneous disease in dogs occurred in endemic form.

It is true that cases in children were found in which no close association with dogs could be proved, and others in which the dogs of the house were examined and found to be in good health; such observations, however, are not surprising in view of what has been said as to the difficulty of diagnosing the affection in dogs and the existence of the chronic and mild form in which the dog shows few if any signs of illness.

Cases were soon on record in which infected animals were found in houses in which the human disease was present or had been present, and this was shortly followed by the discovery of what appeared to be *Leishmania* parasites in fleas taken from infected dogs, as was done both by Basile and by Sangiorgi, the material in each case being derived from Sicily. The demonstration of the pre-flagellate forms of *Leishmania* in the gut of fleas collected from an infected dog, though interesting and suggestive, was more or
less to be expected, as we already know that at times a fair number of parasites may be found in the circulating blood of the dog, but it was further noted that the parasites appeared to undergo development in the flea, similar to that which takes place in the culture tube. This latter fact added to the probability that the flea was a true intermediate host. Further search was made in fleas collected in endemic areas of the human disease, not from infected dogs, but from the coverlets, pillows, and mattresses of houses in the affected areas, and in a certain small percentage of these protozoal organisms resembling the cultural forms of *Leishmania* were found. Sangiorgi, for instance, found such organisms in fourteen out of 378 dog-fleas collected in Catania, and Basile had a similar result with fleas collected from mattresses, &c., in Bordonaro, the village in which both the human and the canine affections are so frequent. Further confirmation came from Lisbon, where Alvares and Da Silva found all stages of *Leishmania* in a flea taken from an infected dog; they also examined sixty fleas collected on healthy dogs with negative results.

The notorious difficulties connected with the proof of insect transmission of flagellated protozoal organisms were to be anticipated here, for, however close the resemblance of such forms as are found in the digestive tubes of the insect to the cultural forms already known, it is hard to distinguish them from other flagellates which may be common parasites of the particular insect in question. Flagellate parasites have been described in fleas by Balfour, by Patton, by Mackinnon, and if the observations had stopped here there would have remained abundant material for scepticism as to the genuine character of the flea as a true host, but fortunately further proof of an experimental nature has recently been obtained, principally from the work of Basile.

Basile's first experiment, aiming at proof of the infectivity of a flea which harboured *Leishmania* parasites, was conducted in the following way: Fleas were collected from a dog which had been proved free from the disease; these were placed in vessels containing some spleen pulp taken from an infected dog and rich in parasites, and on this some of the fleas fed. After a time, the fleas were killed and dissected, one portion of the gut contents on being searched showed the presence of the parasites. The other portion was emulsified and injected into a young puppy, whose marrow had previously been examined with negative results. The puppy was infected and *Leishmania* parasites were subsequently found in its peripheral blood. It was further noted
that the parasites found in the intestine of the flea were far more numerous than in the spleen pulp on which it had fed, and numerous division forms were seen. The fleas employed in this experiment were *Ctenocephalides (Pulex) serraticeps*.

A later experiment, and one more closely imitating what might be assumed to occur in Nature, was next carried out. Two pups, which had been born in the laboratory, when 30 days old were placed in a special cage, which had been thoroughly disinfected, after having been proved to be free from kala azar by examination of the bone marrow. The cage was protected by netting fine enough to ensure that no fleas or other parasites could gain entrance from outside, and the temperature was maintained at about 20°C, as the experiment took place in the winter. After a few days a dog infected with the disease was placed in the same cage, so that there was no obstacle to its numerous fleas passing to the uninfected pups. Thirty days later, liver puncture of the two pups showed that each was infected, *Leishmania* parasites being found on microscopic investigation. Control pups, from the same litter, remained in good health and showed no infection.

At a later date this experiment was repeated by Basile under somewhat different conditions. Again he employed young pups which had been born in his laboratory, and he took the same precautions to ensure that they were uninfected. When they were about a month old, four of the litter were placed in a disinfected and gauze-protected cage, the two remaining pups being kept as controls. The cages were so situated that no contact with other dogs was possible. In this instance infection was introduced among the dogs, not in the person of an infected animal, but in the shape of fleas which had been collected at Bordonaro, an endemic focus of the disease, from coverlets or mattresses in houses where dogs were kept. Repeated examination of the blood of the dogs and hepatic puncture gave negative results until about two months after the fleas had been admitted to the cage, when two of the dogs were found to be infected. All of them had for some time shown irregular temperatures and were getting thin. Within six days of the demonstration of the parasites in the first two dogs the whole four died, and on examination all were found infected with *Leishmania*. The two control dogs, which had remained in good health, when sacrificed later were found to be completely free from the disease.

Basile concludes from the result of these experiments and the rigorous conditions under which they were carried out that they prove beyond dispute the fact of flea transmission.
Further communications, published in July of this year, gave further evidence in support of the above conclusion and again demonstrated that dogs could be infected at a distance by the bites of fleas collected in a house in which was a case of kala azar. In addition, the important observation was made that not only *Pulex serraticeps*, but also *P. irritans*, were found to contain parasites indistinguishable from the cultural forms of *Leishmania infantum*. Pianese's latest communication, in collaboration with La Calva and Visentino, gives further evidence that both *serraticeps* and *irritans* are concerned in the spread of the disease, and they conclude that the human and canine leishmaniasis of the Mediterranean are identical, and that these two fleas are the intermediate hosts and the transmitting agents from dog to man, from man to man, from man to dog, and from dog to dog.

Confirmation of part of Basile's work is already forthcoming, since Alvares and Da Silva have found in three fleas taken from an infected dog in Lisbon every stage of *Leishmania*, including fully flagellated forms and typical rosettes; the writer has had the opportunity of examining the excellent microphotographs which they have taken from the gut contents of these fleas and is quite in agreement with the authors that they are indistinguishable from the cultural forms usually seen. In addition to this they made the interesting observation that the parasites are passed in the feces of the flea; this they ascertained by confining the fleas in a vessel with a glass plate at the bottom on which the fleas voided their excrement; this being subsequently stained and examined proved to contain all the forms seen in the gut, including rosettes. They conclude from this that the mechanism of infection may be similar to that which occurs in the transmission of plague through the bites of *Pulex cheopis*, where, as shown by the Indian Plague Commission, it is probable the feces passed while the insect is feeding is rubbed into the bite together with the plague bacteria which it contains.

Whether fleas are the only intermediate hosts remains to be proved; there is nothing improbable in the conjecture that ticks, lice, bugs, or biting insects may at times act in a similar manner, but, at the present moment, there is nothing but the slightest evidence tending to incriminate any of them.

(To be continued.)