The gonad (hermaphrodite gland) in the present species has a single conical lobe as in *Vaginulus borellianus*[8]. Examination of serial sections of the gonad revealed the presence of a short tract of cells staining intensely with the 2 stains, which are most specific for the endocrine cells. The position of these cells in the gonad has been shown diagrammatically in figure 1. It may be noted that the endocrine centre is a narrow streak located in the right half of the gonad near the periphery. The cells in the centre are mostly club-shaped having an oval body and a narrow stalk (figure 2). The nuclei are fairly large and centrally placed in the broader part of the cells. They fail to take the stain and appear as white discs. The cytoplasm stains very intensely. The narrow stalks of many of these cells prolong further and give rise to fine branches which are often confluent among themselves (figure 2).

To confirm that the cells in question contain material of the nature of hormone, appropriate histochemical tests specific for hormones are performed on the sections. The Kober's test[9] was postive on these cells. This is corraborated by the positive reaction yielded by these cells to Hooker-Forbest and thiocol-copper sulphate tests for hormones[10,11].

In the light of these observations, it may be inferred that the cells comprizing the streak found on the right side of the gonad in *L. alte* are endocrine in function.

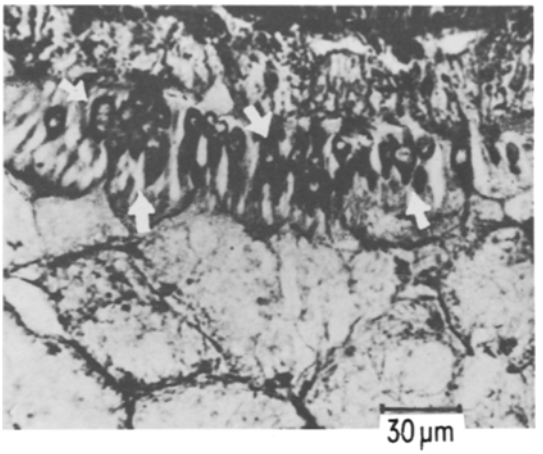

Fig. 2. Long section through the neurosecretory centre in the gonad of *Laevicaulis alte* stained in chrome-haematoxylin-phloxin. The arrows point to the groups of endocrine cells.

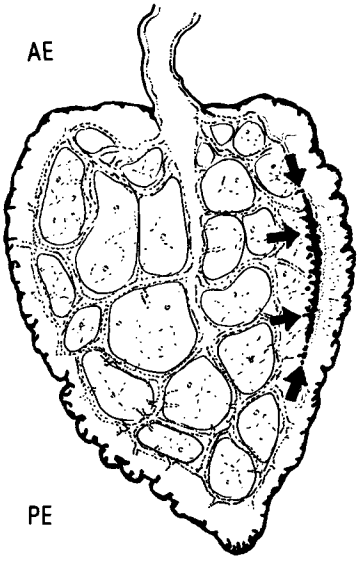

Fig. 1. Semidiagrammatic figure of the frontal section through the gonad of *Laevicaulis alte*. The arrows denote the position of the endocrine centre. AE, anterior end; PE, posterior end.

1 Acknowledgment. Thanks are due to Prof. Dr G. Sundara Rajulu for guidance and helpful suggestions. I am grateful to the Director of Collegiate Education, Tamil Nadu, for permission to undertake this investigation.
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Cocci and diphtheroids in blood cultures from patients in various pathological situations

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Summary. The evolution of cocci and diphtheroids taking origin from cell-wall-deficient forms seems not to be related to a particular state of illness, but to be the consequence of a generalized crypto-infection.

Previous research by other authors and ourselves[1-4] have demonstrated the presence, within the circulating blood of clinically normal subjects, of bacterial minimal reproductive units (MRU) and cell-wall-deficient (CWD) forms associated with the erythrocytes and platelets, which evolve in the haemocultures towards conventional forms of Corynebacteria-like microorganisms (diphtheroids) and *Staph. epidermidis*. It has been claimed[5,6] that the incidence of Corynebacteria-like microorganisms, recognizable as *Bacillus licheniformis* var. *endoparasiticus*, is higher in arthritic than in healthy subjects, and that such microorganisms may also be associated with malignancy[1]. The authors already cited, and others who have carried out analogous research[7], have observed that in the majority of cases the evolution of CWD and the reversion to conventional bacterial forms take place following very prolonged periods of incubation:

Growth of conventional bacterial forms within 3 months' incubation of broth cultures from whole blood drawn in connection with a febrile state from 148 patients in various pathological situations. In about 1/3 of the cases the proofs have been repeated 1-3 times at 2-4 days interval and previous results (positive and negative) have been confirmed

Pathological situations	Patients	Positive haemo-cultures	Growths of cocci	diphtheroids	cocci and diphtheroids	other bacteria	
1) Infectious diseases							
Respiratory system	19	7	1	2	4	-	
Alimentary tract	18	6	-	2	3	1	*S. tiphi*
Other infectious diseases*	15	8	-	5	2	2	*K. aerogenes, B. melitensis*
2) Disorders of the heart and of the vascular system	9	4	-	-	3	1	*P. pyocyanea*
3) Disorders of the respiratory system	10	5	-	4	1	-	
4) Disorders of the kidneys and urinary tract	8	3	-	-	3	-	
5) Disorders of the alimentary tract	18	9	-	4	2	3	*E. coli, P. mirabilis, E. liquefaciens*
6) Disorders of the hepatobiliary system	15	4	-	2	-	2	*E. coli, K. aerogenes*
7) Disorders of the hematopoietic system	7	2	-	-	1	1	*Pseudomonas* sp.
8) Hormonal disorders	4	2	-	-	1	1	*P. mirabilis*
9) Rheumatoid arthritis	9	1	-	-	1	-	
10) Tonsillitis	5	2	-	1	1	-	
11) Other diseases**	11	4	2	-	1	1	*E. liquefaciens*
Total	148	57	3	20	23	12	

* Rheumatic fever, 1; hepatitis, 2; infectious mononucleosis, 2; virosis (?), 1; tetanus, 3; melitensis, 2; encephalitis, 1; meningitis, 2; postop. sepsis, 1. ** Uterine fibroma, 2; traumas, 3; disorders of the nervous system, 3; echinococcosis, 1; abscesses, 2.

On the whole: positive cultures: 38.8%. Growth of cocci, alone or together with diphtheroids: 17.5%. Growth of diphtheroids, alone or together with cocci: 29.0%. Growth of other bacterial forms: 8.1%.

this may be the reason why recently Gleckman and coll.[8] failed to observe within 1 month's incubation bacterial growths from the blood of febrile patients.

The present research was undertaken in order to compare the percentage of growth of cocci and diphtheroids within the blood cultures from patients in various pathological situations, with the data concerning the growth of the same microorganisms in the blood cultures from clinically normal subjects. As will be seen, no significant differences have been detected.

Materials and methods. Specimens of blood from 148 patients with various pathological situations, in a febrile state, were utilized (table).

The cultures were made in BBL prepared culture bottles (Division of Becton, Dickinson and Co, Cockesville, MD 21030, USA) containing 25 ml trypticase soy broth with agar surface, carbon dioxide and sodium polyanethole sulfonate.

Samples of 2.5 ml of blood were introduced under sterile conditions into the bottles by means of the BBL blood taking units: anaerobic and aerobic specimens were collected. The bottles were incubated for 3 months at 37 °C and examined, not shaken and unopened, at successive intervals of 3 to 5 days in order to evaluate the bacterial growth on the agar surface and/or the modifications of the colour and turbidity of the broth.

When a bacterial growth was recognized, conventional methods were utilized for optical and in some cases electron microscopy[4], for the subcultures (aerobic and anaerobic, in liquid and on agar media) and for the characterization of the bacteria[9]. By means of the same methods, the negativity of all the residual cultures was confirmed at the end of the 3rd month.

Results and discussion. The data concerning the growth of conventional bacterial forms in the primary cultures are summarized in the table. Such growth in the majority of cases was accompanied by a more or less pronounced process of haemolysis. It needs to be taken into particular account that when the primary cultures from the same patient were repeated at suitable intervals, they often gave rise to a growth of cocci accompanied by a very small number of apparently non-multiplying diphtheroids, followed by the growth of cocci and diphtheroids together in various proportions and/or by the growth of a large number of diphtheroids in the presence of a few degenerating cocci. Probably the same observations would have been made in a larger number of cases if it had been possible to draw more than a single specimen of blood from a larger number of patients.

Such situations may be interpreted as the consequence of the ratio cocci/diphtheroids carried within the blood specimens, and/or of the modification of the oxygen pressure in the haemocultures in the course of the incubation. The correctness of such an interpretation has been evaluated experimentally: blood specimens cultured partly aerobically and partly within anaerobic jars, gave rise respectively to an intense growth of cocci and very few, if any, diphtheroids, or to an intense growth of diphtheroids and no cocci; the introduction of air into the anaerobic jars carrying growing diphtheroids was immediately followed by an intense growth of cocci.

In the primary cultures and subcultures, some bacterial forms grew rapidly, and these were recognized as: *S. tiphi, E. coli, Pseudomonas pyocyanea, Pseudomonas* sp., *Brucella melitensis, Klebsiella aerogenes, Enterobacter liquefaciens, Proteus mirabilis.*

In the primary cultures, a growth of cocci within the first 3 weeks of incubation was seldom observed (3 cases); in the other cases the cocci became recognizable following incubations lasting 3 to 6 weeks. In the haemocultures from 24 patients, the cocci were recognized as belonging to strains of *Staph. epidermidis;* in 2 cases the growth of *Micrococcus* sp. was observed.

The growth of diphtheroids in the majority of cases became

evident only after a very prolonged period of incubation (4–10 weeks). The strains were variable in their morphology and physiological reactions: these characters, being unstable, were not considered safe to use for the purpose of distinguishing types from different human subjects or clinical conditions.

The fact that the cocci and the diphtheroids took a very long time to grow in the primary haemocultures and grew rapidly in the subcultures, is compatible with our and other authors' previous data indicative of an evolution starting from MRU and CWD forms carried within the erythrocytes and platelets.

Our present data concerning the percentage of growths of cocci and diphtheroids within the haemocultures from subjects in various pathological situations, may be compared with the following data concerning blood cultures made in duplicate from 70 normal subjects in Birmingham (England) and 56 in Ancona (Italy) and incubated contemporaneously in the Department of Bacteriology of the University of Birmingham and in our own laboratory[1]: growth of cocci: 15%; growth of diphtheroids: 25%.

Such comparison indicates that, as regards the presence and evolution of the bacterial forms under consideration, no significant difference has been detected between normal subjects and subjects suffering from various types of pathological situations. It has to be noted that the cultures from patients suffering from rheumatoid arthritis behaved in a way opposite to that which one would have expected on the basis of other authors' points of view. The same may be said for 14 cases of neoplastic diseases, which in the table are distributed within groups 3, 5, 6, 7: 1 growth of *P. mirabilis*, 1 of *E. coli* and 1 only of a diphtheroid have been observed.

From the results here described, it may be concluded that the presence within the circulating blood of CWD bacterial forms evoluting in the haemocultures towards conventional forms of cocci and diphtheroids, seems not to be particularly related to any of the states of illness which have been examined, but to be rather the consequence of an already recognized generalized crypto-infection.

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Intracellular behaviour of *Leishmania enriettii* within murine macrophages

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Summary. Both promastigotes and amastigotes of *Leishmania enriettii* were readily ingested by mouse peritoneal macrophages (MPM). Promastigotes after their entry within MPM were rapidly immobilized and their multiplication was never observed. Microscopic examination revealed that ingested promastigotes were degraded within MPM. Nonmotile amastigotes of *L. enriettii* taken up by MPM, on the other hand, multiplied intracellularly and eventually destroyed the infected cells.

The role of host macrophages in which *L. enriettii*, an obligatory intracellular protozoan parasite, normally resides as amastigotes, in resistance and/or immunity against in vitro infection, has received some attention. Thus, it has been reported that macrophages from normal, allergized or immune guinea-pigs, when infected in vitro with amastigotes, are unable to restrict their intracellular multiplication[1-3]. In addition, it has also been shown that macrophages from normal mice when infected with amastigotes of *L. enriettii* could support their growth, whereas macrophages 'activated' to kill *Listeria monocytogenes* could destroy the amastigotes[4]. It was the purpose of the present experiments, to investigate the in vitro fate of *L. enriettii* promastigotes and amastigotes in macrophages obtained from the peritoneal cavities of normal (non-immune) mice.

Materials and methods. Cultures of mouse peritoneal macrophages (MPM) obtained from unelicited peritoneal cavities of normal (nonimmune) DBA/2 or C3H mice were prepared by conventional procedure[5]. The culture of *L. enriettii* was maintained as promastigotes at room temperature on conventional NNN-medium slopes[6]. The parasites were maintained as amastigotes in adult guinea-pigs by s.c. inoculation of promastigotes into the shaven dorsal surface of the right ear. Amastigotes were obtained by aseptic homogenization of excised leishmanial nodules in cold phosphate buffered saline (PBS). 24-h-old MPM cultures were infected with 10^6 viable promastigotes or 10^7 amastigotes to give a parasite to cell ratio of around 10 to 1. The infected cultures were incubated at 37 °C in a CO_2 air atmosphere for 1 h and then washed thoroughly to remove extracellular parasites. Thereafter, fresh growth medium without parasites was added to each culture and reincubated at 37 °C. Infected coverslip cultures were taken out periodically and examined under the phase contrast microscope, or stained with May-Grünwald-Giemsa, and then examined. At appropriate time intervals, the total number of intracellular parasites and the total number of macrophages were counted in at least 15 microscopic fields (×40) per coverslip. From this data the number of parasites per macrophage was calculated for each time interval sample.

Results and discussion. Under the experimental conditions used, a high proportion (70–90%) of MPM of both C3H and DBA/2 origin became infected with promastigotes or amastigotes of *L. enriettii*. Entry of majority of the promastigotes within MPM proceeded via the body of the parasites (figure 1). Phase contrast microscopy on living cultures revealed that the promastigotes subsequent to their entry within MPM maintained their motility for a period of 20–