Mg^{++} ions act, but also by a modification of the catalytic receptor. Consequently, although trypsin acts on the dependence on Mg^{++} ions, we may not conclude exclusively that it changes the regulatory receptors. It may be that trypsin reacts directly with the catalytic receptors of adenylate cyclase, but we would rather suggest that limited proteolysis exposes receptors which were previously hidden. This activation lasts as long as cells restore their initial membranal organization. The membrane structure and functions of transformed cells are different from those of normal cells. Ultrastructural freeze fracture morphology[12] and activity, or certain membrane enzymes are changed[13]. Adenylate cyclase activity of transformed cells is lower than that of normal cells[14]. It has been suggested that the characteristics of the enzyme altered with different transformation agents[15,16]; consequently we cannot state, from our findings relating to the KB cells, that the mechanism of the action of trypsin on adenylate cyclase is the general rule. However, trypsination of cell cultures can affect cell metabolism by modifying the enzymatic system of adenylate cyclase; the use of another method of removing cells would appear preferable in order to eliminate some biochemical changes during studies of cell culture.

The generation and identification of the hemolysin of Trypanosoma congolense[1]

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Summary. The hemolytic activity of Trypanosoma congolense appears to be due to the presence of free fatty acids generated by the action of phospholipase A on endogenous phosphatidyl choline. Some lysolecithin also contributes to the lytic activity. Trypanosoma lewisi, being devoid of phospholipase A, does not generate free fatty acids and is therefore non-hemolytic.

Although the diseases caused by the African trypanosomes are of major economic and social significance, their pathogeneses remain very poorly understood[2]. Various hypotheses have been put foreword to account for the death of trypanosome infected animals including the suggestion that these organisms produce toxins[3-5]. Until recently, this was considered to be unlikely since the unequivocal occurrence of such toxins had not been demonstrated. It has now been shown however, that both Trypanosoma congolense[6] and T. brucei[7] generate, on autolysis, material which is potently cytotoxic and hemolytic.

Material and methods. Hemolytic activity is absent from freshly isolated T. congolense strain TREU 112, but is generated on incubation of a 7% v/v suspension of these organisms for 8–10 h at 20°C in phosphate buffered glucose (PBG) (0.04 M, pH 8). At the end of this time, such a suspension is capable of causing 100% lysis of an equal volume of 2.5% sheep erythrocytes in PBG within 2 min at 37°C. All hemolytic activity is contained within the particulate fraction of the suspension, being sedimented by centrifugation at $5{,}000 \times g$ for 5 min. This autolysate is also capable of lysing rabbit buffy coat cells and mouse peritoneal cells as well as being able to cause a local acute inflammatory response on intradermal inoculation into rabbits[6].

In order to determine the nature of the hemolytic material, 1 ml of an autolysed trypanosome suspension prepared as described above and possessing hemolytic activity was extracted by shaking with chloroform-methanol (2:1) for 20 min at room temperature. At the end of this time the chloroform layer was separated, evaporated to dryness under vacuum at room temperature and resuspended to 1 ml in PBG. The methanol was removed from the aqueous layer by evaporation to its original volume. Hemolytic activity, as shown by 100% lysis of an equal volume of 2.5% sheep erythrocytes within 30 min, was found to be confined to the material extracted by chloroform. No lysis was observed in the aqueous fraction even after incubation with erythrocytes for 18 h at 37°C.

A chloroform extract of autolysed trypanosomes was further analysed by thin layer chromatography. The material was streaked onto plates coated with silica gel H (Merck) of 0.5 mm thickness with a number of phospholipid and fatty acid standards. The chromatogram was developed in chloroform-methanol-water (65:25:4) and visualized in iodine vapour. The spots were identified against the standards, scraped off, and the scrapings extracted with two washings of chloroform-methanol-N HCl (20:10:1). The extracts were evaporated to dryness under vacuum and 0.2 ml 2.5% sheep erythrocytes in PBG added to each dried tube. Complete lysis of these erythrocytes occurred after 2 h at 37°C in the presence of material identified as free fatty acid. Slight hemolysis (12%) was also observed in the presence of material identified as lysophosphatidylcholine.

Results and discussion. Preliminary results from gas chromatographic analysis of this free fatty acid fraction indicated that it consisted largely of stearic, palmitic, oleic and linoleic acids.

Several other features of the hemolytic process tend to confirm that lysis is primarily due to the activities of fatty acids and, to a lesser extent, lysolecithins. Thus trypanosome induced hemolysis is inhibited in the presence of fatty acid free bovine serum albumin[5]. It is well recognized that albumin has a strong affinity for free fatty acids[8] and lysolecithin[9], so that these compounds are no longer available to interact with cell membranes.

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Secondly, trypanosome induced hemolysis is inhibited by the presence of 0.005 M EDTA. It is of interest that phospholipase induced swelling of mitochondria thought to be due to the release of free fatty acids, is similarly inhibited by bovine serum albumin and EDTA and stimulated by Ca^{++}[10]. When thin layer chromatograms of chloroform extracts of freshly isolated and autolysed T. congolense were compared, significant differences were observed in their lipid composition. In particular, fresh (non-hemolytic) organisms were rich in phosphatidyl-choline, while in autolyzed (hemolytic) organisms the phosphatidyl-choline had been significantly reduced while fatty acids were markedly increased. There were also some evidence to suggest that lysophosphatidyl-choline was generated on autolysis while triglycerides were slightly hydrolyzed.

In order to determine whether the hemolytic fatty acids were derived from phosphatidyl-choline through the action of phospholipase A (EC.3.1.1.4), or from triglycerides through lipase activity, assays for these enzymes were performed on both freshly isolated and autolyzed trypanosomes. Phospholipase A activity was determined by measuring the hydrolysis of 32 P labelled rat lecithin[11]. Lipase activity was estimated by measuring the hydrolysis of glycerol tri ($1-^{14}C$) palmitate[12]. Freshly isolated organisms contained relatively small amounts of phospholipase activity (3.1 nmoles lecithin hydrolyzed per mg protein/h). On autolysis this rose considerably, reaching in 1 case 200–300 nmoles/mg h. No significant levels of lipase activity were found in fresh or autolyzed trypanosomes. Preliminary results from further analysis of this phospholipase activity using 2(9, 10–di^{3}H) dipalmitoyl phosphatidyl-choline (Applied Science Laboratories) as substrate[12] suggested that the phospholipase was primarily A1.

We therefore suggest that the hemolysin of T. congolense consists largely of free fatty acids and some lysolecithin derived from the action of a phospholipase A on endogenous phosphatidyl choline. The activity is latent and becomes unmasked during autolysis of the organisms. While fatty acids and lysolecithin are regularly toxic in vitro, it is not yet possible to estimate their significance in trypanosome infections in animals. One reason for this is that both are rapidly esterified[13] and bound to albumin in vivo[9]. Nevertheless, we consider that the continued generation of these factors by phospholipase A, particularly under conditions of trypanosome accumulation and destruction, within an animal's microcirculation may lead to the development of degenerative changes in vascular endothelium resulting in increased permeability and platelet aggregation. Such changes are commonly observed in T. congolense infections[14]. In addition, it is possible that the interaction of these factors with erythrocyte membranes, while not causing intravascular hemolysis, may be sufficient to stimulate their clearance from the blood stream and contribute to the anemia so characteristic of this infection[14,15]. In this connection it is pertinent to note that the hemolytic activity was not generated by the non-pathogenic trypanosome, T. lewisi. This trypanosome did not generate phospholipase A activity on autolysis, and thin layer chromatography of chloroform extracts of fresh and autolyzed T. lewisi failed to demonstrate significant hydrolysis of phospholipids or accumulation of free fatty acids.

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Selective inhibition of reproduction in aminopterin-treated nematodes

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Summary. Aminopterin was applied to the free-living nematode Caenorhabditis briggsae and subsequent growth was recorded. Nematode populations, containing all developmental stages and selected juvenile stages, were exposed to the drug in both growth-promoting and non-promoting media. It is suggested that aminopterin creates a specific requirement for thymine in thymine-free medium. In otherwise growth-promoting medium, aminopterin-induced thymine deficiency will lead to progressively unbalanced growth and maturation and hence to sterility even after removal of the drug. The omission of essential amino acids from the medium during thymine starvation prevents larval growth and results in better reproduction and faster proliferation in aminopterin-free medium. The 4 juvenile stages exhibit a different response to thymine starvation created by aminopterin.

Aminopterin has been used as a potent inhibitor of reproduction in studies of nematode development and ageing[1–3]. The drug prevents gonad formation and thus disturbs maturation when supplied to newborn nematodes[2]. We now present some evidence suggesting that this effect is suppressed in some non-developing juvenile stages. This might be a valuable tool for selecting nematode strains with altered metabolism.

Materials and methods. Stock cultures of Caenorhabditis briggsae were maintained axenically as previously reported[4]. Gistex medium is a chemically non-defined medium that was developed originally for the large scale cultivation of Caenorhabditis elegans[5]. It supports very fast and profuse growth with C. briggsae as well. MEM is defined here as the chemically defined medium CbMM[6] (available from Gibco bio-cult, Paisley, Scotland) lacking the non-essential amino acids alanine, aspartic acid, cysteine, glutamate, glutamine, glycine, proline serine and tyrosine. These are substituted by equimolar amounts of acetate, citrate, pyruvate and glucose[7]. MEM further contains no thymine; sterols and acid precipitated haemin are included at 50 µg/ml[8]. This medium contains all