lysozyme and anti-(human gamma globulin). All the adsorbed lysozyme was recovered as active enzyme, but the *p*H-dependence of the release of lysozyme was markedly different from that of human serum albumin: no lysozyme was recovered before *p*H 2·2 was reached. In contrast, only 20 per cent of fluorescently labelled human gamma globulin was recovered from its homologous immunosorbent even at *p*H 2·2.

An encouraging development in this work was the finding that the same anti-lysozyme immunosorbent could be used five times with only a slight reduction in capacity and no apparent loss of specificity. This was despite its treatment at *p*H 2·2 in each elution before equilibration with PBS2 for the next adsorption. Similar results have been obtained with an anti-(HSA) immunosorbent.

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J. W. CHIDLOW
J. STEPHEN
H. SMITH

Department of Microbiology,
University of Birmingham.

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## *In vitro* Development of *Plasmodium berghei* Ookinetes

AFTER a mosquito has fed on an animal infected with malaria, the microgametocyte exflagellates in the mosquito gut and fertilizes the macrogamete thereby forming an ookinete. Only a few workers have studied the *in vitro* cultivation of the mosquito stages of malaria. Ball and Chao[1–3] working with the avian malaria *Plasmodium relictum* were unable to induce ookinete formation *in vitro* although they were able to induce exflagellation of the gametocyte. They were forced to remove the oocyst from the mosquito gut by microsurgical techniques to obtain development of later oocyst stages. The bacteria and fungi from the mosquito preparations caused some difficulty in these cultures. They speculated that because they could not induce ookinete formation in their cultures, some substance from the mosquito stomach was required for development of the ookinete.

Yoeli, Upmanis and Most reported the *in vitro* development of the ookinetes of *P. berghei* (rodent malaria) at the November 1967 meetings of the American Society of Tropical Medicine and Hygiene. They cultured blood collected from the droppings of a mosquito feeding on an infected animal. They also prepared extracts of mosquito stomachs and cultured the infected mouse blood in the extract. Both procedures induced ookinete development.

Investigating the possibility of ookinete formation without the use of any material from the mosquito, we used the following procedure which can be carried out in sterile conditions. Blood from the tail of a mouse in the third day of infection, showing good exflagellation, was collected on a glass slide. The usual procedure of exhaling on the blood to induce exflagellation was followed. The blood was then collected in capillary tubes, some was heparinized, and both heparinized and non-heparinized blood was sealed in the tubes with plastic clay. The tubes were kept at room temperature (74°–78° F) for 24 h, broken and thick smears of the blood stained with Giemsa. After 24 h, the ookinetes appeared to be normal for an equivalent period of development in a mosquito, although no material from mosquitoes was used. Techniques were not quantitative, but up to three ookinetes/field could be found in the heparinized tubes, and only one in the non-heparinized tubes.

This discovery is the first step in the sterile *in vitro* development of the mosquito cycle enabling us to bypass the tedious microsurgical techniques of Ball and Chao or the collection of contaminated mosquito extraction.

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NELDA E. ALGER

Department of Zoology,
University of Illinois,
Urbana, Illinois.

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## Polysaccharide of a Mucoid *E. coli* isolated from a Patient with Cystic Fibrosis

PSEUDOMONADS isolated from patients with cystic fibrosis have been found to produce a highly viscous polysaccharide when grown on an artificial medium[1]. The polysaccharide was identified as a polymer of uronic acid resembling alginic acid[2,3], a compound ordinarily isolated from brown seaweed. The isolation of a mucoid strain of *E. coli* as the dominant organism from a patient with cystic fibrosis[4] prompted an investigation into the type of capsular material produced.

All organisms were grown on MacConkey's agar and the polysaccharide was isolated as previously described[2]. The bacterial growth was washed off the plates with physiological saline and the suspension was stirred well for 2 h. The cells were then removed by centrifugation and the crude polysaccharide was precipitated with three volumes of ethanol. Further purification was carried out by reprecipitation with ethanol. Hexoses were determined by the anthrone reagent[5] and uronic acids by three different analytical procedures, namely, the carbazole reaction[6], the orcinol reagent[7] and measurement of $CO_2$ after decarboxylation[8]. Fucose was determined by the cysteine reagent[9] and *o*-acetyl by the hydroxamic acid reaction after distillation[10]. Paper chromatography was carried out as described previously[2], and infrared spectra were obtained on a Perkin–Elmer Model 21 in KBr pellets.

The analytical data indicated that the polysaccharide isolated from the mucoid *E. coli* obtained from the patient was similar to material isolated from other mucoid strains. Polysaccharide was therefore also prepared from *E. coli* *K*-235 (ref. 11), *K*-12*W* (ref. 12) and the *M*-6 mutant of *K*-12 (ref. 13).

All polysaccharides were hydrolysed in 2·0 N HCl for 3 h and the products were chromatographed on paper in three solvent systems[2]. The following monosaccharides were identified: glucuronic acid, galactose, glucose and fucose. All the polymers showed the same proportions of monosaccharides and these were qualitatively the same as those reported previously[12]. The infrared spectra of all polysaccharides were identical and showed strong absorption peaks at 1,725 $cm^{-1}$ and 1,250 $cm^{-1}$ indicating the presence of *o*-acetyl groups in addition to the uronic acid carbonyl groups[2]. (The absorption at 1,615 $cm^{-1}$ also seems somewhat stronger than would be expected for a 20 per cent uronic acid content, and may indicate the presence of an additional component not detected by the methods used; see also Table 1.)