Apart from specific binding to β-adrenergic receptors, propranolol also exhibits non-specific, low affinity binding to cardiac membranes[14]. The diffuse greenish colouring of the cardiac sections may represent non-specific 9-AAP binding to myocardial membranes.

Recent indirect evidence suggests that the β-adrenergic receptors are localized in the cardiac cell membrane[15–17]. This view may be supported by the present findings. Furthermore, the observed distribution pattern of dotted fluorescence may represent discrete areas with high concentration of 9-AAP binding sites in the myocardial membrane. By inference, it may be suggested that the β-adrenergic receptor sites are not evenly distributed in rat cardiac cell membrane.

More evidence must be obtained to establish a definite analogy between 9-AAP binding sites and β-adrenergic receptor sites. Utilization of 9-AAP may provide a useful tool for the direct detection of β-adrenergic receptors in the heart and also in extra-cardiac tissues.

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Preparation of Isolated Cells from Rat Heart

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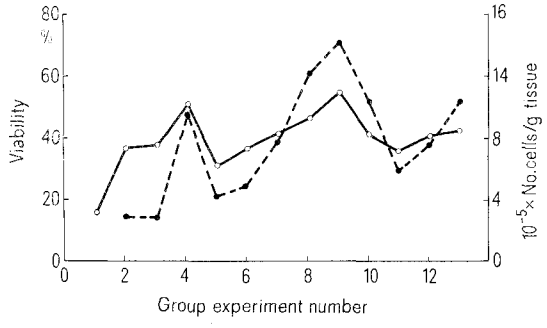
Summary. Suspensions of isolated cells from rat heart were prepared and the data for viability and yield are given. Glucose uptake by the cells was mediated by a carrier system.

Heart tissue has been successfully dispersed into preparations of viable, isolated cells[2–5], but only the last authors[5] have discussed systematically the methodology. However, some important quantitative aspects were lacking in their paper (e.g. statistics of viabilities and their relation to yields); and furthermore, the very high percentage viabilities they obtained are probably unrepresentative of the method, and could lead to the rejection of otherwise acceptable preparations.

Effect of temperature of incubation with collagenase on the preparation of suspensions of isolated cells from rat heart

Harvest 3		Harvest 4	
Viability (%)	Cell yield[a]	Viability (%)	Cell yield[a]
30 °C 55.2±3.8(5)[b]	1000±264(5)	62.8±4.6(5)[c]	1397±416(5)
37 °C 40.2±1.8(13)[b]	939±147(13)	39.3±2.1(13)[c]	1026± 95(13)

Cells harvested at 20 min intervals.
[a] 10^{-3} × yield of live cells per g heart tissue.
[b] Significance of difference $p < 0.005 > 0.001$.
[c] Significance of difference $p < 0.001$.



Percentage viability (○—○) and numbers of cells/g tissue (●--●) are given for all comparable suspensions of heart cells prepared in Harvest 3 in these experiments. The data are means for successive groups each of 3 experiments. Harvest 3 is the suspension of cells dispersed during the 3rd 20 min period of incubation with collagenase.

Methods. Male albino rats (100–120 g) were stunned and decapitated. The cardiac ventricles were diced (side 3 mm). Tissue (< 2 g) was incubated at 37 °C in a 50 ml conical flask containing 3.5 ml of a phosphate-buffered medium (pH 7.4) with collagenase (1 mg/ml). The flasks were shaken at 100 strokes/min. The composition of the medium (g/l) was: 8.8 g NaCl, 0.4 g KCl, 0.21 g Na_2HPO_4, 0.06 g NaH_2PO_4 and 0.9 g glucose. Cells were decanted from the tissue pieces at 20 min intervals (harvests 1–4) and the tissue resuspended in fresh buffer. Harvests 1 and 2 were mostly erythrocytes and fragmented cells and were discarded. Cells from harvests 3 and 4 were collected by centrifugation (60 × *g*, 2.5 min), washed 3 times and counted (cytometer depth 0.2 mm). Viability was determined by staining with trypan blue (equal volume 0.25% dye). Purification of cells by centrifugation through Ficoll (30 g/l)[5] was omitted because it a) rarely improved percentage viability; b) always reduced yields; and c) did not change the microscopic appearance.

For metabolic experiments the medium contained lower glucose (0.5 m*M*), [U-^{14}C]glucose (0.625 μCi/ml), bovine serum albumin (1 g/l) and $CaCl_2$ (2.5 m*M*). Cell viability was not affected by incubation for 60 min. The optimum number of cells per incubation sample (1 ml) was 3–4 × 10^5. With cell numbers of 5 × 10^5 filtration was difficult. Incorporation of ^{14}C into cells was determined by scintillation counting of washed cells on a Millipore filter (1.2 μm, 0.25 mm) in a scintillation mixture (Triton X-100: toluene: POPOP). Comparisons are between cell

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suspensions with equal numbers of cells. Data are expressed as: mean ± SE (No. of observations).

Results. a) *Preparation of isolated cells.* Percentage viabilities varied between 30–60%, as they did for VAHOUNY et al.[2], whereas for GLICK et al.[5] they were 95% or more. Attempts to improve viability (e.g. use of plastic vessels) were without success, except that of reducing the temperature to 30 °C (Table). The important variable is the collagenase, since its dispersive activity is due to proteolytic contamination, which can vary considerably from batch to batch. 4 preparations were used: CLS 45BI94 and 45D006 (Worthington Biochemical Corp., Freehold, N. J., USA) and I 44C 0080 and II 15C 6890 (Sigma London Chemical Co., Kingston, Surrey, U.K.). The first 3 gave similar results (Sigma I being used routinely), but Sigma II was less useful. A positive correlation was obtained between percentage viability and yield of cells/g tissue (Table, at 37 °C; Harvest 3, $r = +0.58$, $p \simeq 0.02$; Harvest 4, $r = +0.60$, $p < 0.02$). This is also shown by the parallelism of viability and yield of cells for all but the earliest experiments performed with Sigma I (Figure).

b) *Incorporation into heart cells of ^{14}C from [^{14}C]glucose.* Cells were incubated for 30, 60 or 120 min. Maximum incorporation was at 60 min; at 30 and 120 min the incorporation was 49% and 61% respectively of that at 60 min. Incubating the cells with [^{14}C]glucose in 5 m*M* glucose or galactose reduced incorporation of ^{14}C (cpm) from 16740 ± 1300 (5) (0.5 m*M* glucose) to 11,100 ± 1,300 (5) and 9,180 ± 560 (6) respectively. Thus the carrier transport system for glucose was still operating in most of the cells, and was rate limiting for glucose uptake.

Discussion. GLICK et al.[5] tested 14 different commercial preparations of collagenase, all from either Sigma or Worthington. Since the yield of live cells per gram tissue varied between 4,500 and 270,000, their experiments were less successful than those given here (Table), whereas for percentage viability (95% or more) theirs were the more successful. This reciprocal difference does not reflect simply a greater proteolytic activity of the collagenase used here – greater cell dispersion with greater cell disruption – since there is a significant correlation between % viability and yield of cells, and a parallelism between them for almost all experiments (Figure).

Effect of *Schistosoma mansoni* on Plasma Cholinesterase Activity in Rhesus Monkeys

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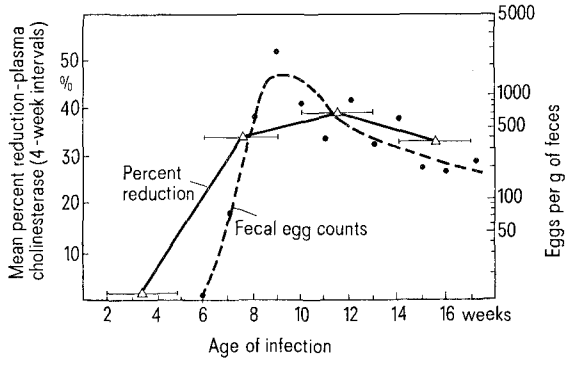
Summary. A significant reduction in plasma cholinesterase activity at a time when fecal egg counts indicated a patent infection has been found in a limited study with schistosome infected Rhesus monkeys.

In working with a group of *Schistosoma mansoni* infected Rhesus monkeys[1], a limited number of monkeys were not treated with any drugs and represented the infected ($n = 3$) and noninfected ($n = 2$) control animals. These monkeys, however, did contribute to a series of blood tests which included the determination of plasma cholinesterase (ChE) activity. This report describes the changes of plasma ChE activity during the course of early and patent schistosome infection in Rhesus monkeys.

Materials and methods. Each monkey (3–4 kg) was anesthetized with nembutal, placed in dorsal recumbancy and exposed to approximately 750 *S. mansoni* cercariae via the percutaneous route. At weekly intervals, plasma ChE activity was determined by the electrometric titration method[2]. Beginning at 5 weeks post exposure to the schistosome infection, twice weekly fecal egg counts (AMS III method[3]) were made with each monkey for the remainder of the study. An analysis of variance was conducted on the ChE data.



Comparison of fecal egg counts with reduction of plasma cholinesterase in *Schistosoma mansoni* infected Rhesus monkeys.

Results and discussion. An initial analysis of the data revealed that changes in ChE activity were more pronounced when the values were grouped at 4 week intervals. These results are graphically displayed (Figure) as the mean percentage reduction of ChE activity relative to the noninfected controls. Included in the Figure are the mean weekly fecal egg counts to illustrate patency of the schistosome infection. Schistosome eggs were first noted in the feces during the 6th week of infection. These egg counts increased in numbers to greater than 1000 eggs/g of feces during the 8th and 9th weeks followed by a gradual reduction in numbers thereafter. Coincident with the rise of schistosome egg counts was a significant reduction of plasma ChE in the infected monkeys. It would appear that reduction of enzymatic activity was initiated at a time when the schistosome infection became patent and continued throughout the remainder of the study.

An altered serum ChE is sometimes indicative of impaired hepatic function[4] and certain other abnormalities including schistosome infections of man[5]. The mode of

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