paired male schistosomes. These ratios clearly show that greater glycogen reserves are present in unpaired males in all three schistosome species (Table 2). It appears that the glycogen or glucose reserves of a male schistosome are depleted by the presence of a female in the gynecophoral canal.

To test for transfer of ^{14}C-labeled hexoses between males and females, copulating pairs of *S. mansoni* were exposed to radioactive medium for 5 seconds and either rinsed in cold silicone oil (*10*), separated, and assayed, or maintained in warm oil for 0.5 to 4 minutes before separation and analysis. A time-dependent increase in the glucose content of the female schistosome was observed (Fig. 1A). The same phenomenon was observed in studies with the unnatural glucose analog 2-deoxy-D-glucose. Although this analog may be phosphorylated in most biological systems, it is not further degraded and remains trapped intracellularly (*11*). Thus the molecular species that is exchanged presumably is the hexose rather than some metabolite. The exchange of glucose appears to be biochemically significant because glycolysis is the major pathway for the provision of metabolic energy in schistosomes (*12*). This phenomenon was also observed in *S. haematobium* (Fig. 1C) and *S. japonicum* (Fig. 1D). Perhaps because of the greater size of male schistosomes (Table 2), concomitant reductions in the glucose content of males were not as striking as the increases in females.

Thus a considerable proportion of the energy required by the female may be indirectly supplied by the male. This represents a more immediate reason for the obligatory continuous relationship than the slow (20 hours) exchange of protein described by Atkinson and Atkinson (*5*). The inability of a female to mature and grow to full size in the absence of a male may be the result of a deficit in molecular material transferred from the partner. Thus the function of a male can be likened to that of a liver (glycogen storage and glucose regulation), and the female to a dependent gonad (*13*).

The sexually specific interdependence of blood flukes emphasizes their considerable evolutionary divergence from other free-living and parasitic flatworms. Molecular mimicry (*14*) and molecular exchange between the sexes also place schistosomes in a biologically unique position. Yet unique pharmacological vulnerability has not been discovered. In recent reviews (*15*) it is stressed that despite chemotherapeutic advances, few antischistosomal agents are completely effective and no available compound satisfies the criteria for an ideal schistosomicide. The importance of chemotherapy in controlling the disease is recognized, however (*16*), as is the need to identify molecular responses of schistosomes which are unlike those of human tissues (*17*). Many antischistosomal treatments cause a loss of glycogen in the fluke. It has been suggested that glycogen, in addition to providing energy, may help to maintain the structural and functional integrity of the schistosome (*18*). The present study would be pharmacologically significant only if a drug that safely achieves permanent interruption of male-to-female glucose transfer can be developed.

Note added in proof: Bueding (*19*) has confirmed that consistently higher glycogen levels have been observed in unpaired male schistosomes in his laboratory.

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References and Notes

1. A. A. Mahmoud, *N. Engl. J. Med.* **297**, 1329 (1977).
2. K. S. Warren, *Nature (London)* **273**, 609 (1978). Alterations in host heme metabolism due to erythrocyte ingestion by adult flukes [A. W. Senft, J. K. Weltman, M. B. Goldgraber, L. G. Egyud, R. E. Kuntz, *Parasite Immunol.* **1**, 79 (1979); A. W. Senft, Y. Cha, H. S. Heine, E. Bueding, *Am. J. Trop. Med. Hyg.* **29**, 227 (1980); A. W. Senft, M. W. Goldberg, J. E. Byram, *ibid.* **30**, 96 (1980)] indicate that the flukes themselves may be harmful.
3. O. D. Standen, *Ann. Trop. Med. Parasitol.* **47**, 139 (1953); J. C. Armstrong, *J. Parasitol.* **51**, 605 (1965).
4. G. C. Coles, *Int. J. Biochem.* **4**, 319 (1973).
5. K. H. Atkinson and B. G. Atkinson, *Nature (London)* **283**, 478 (1980).
6. A Puerto Rican strain of *S. mansoni* from golden hamsters (Fig. 1 and Table 2) or Swiss Webster mice (Table 1), a Japanese strain of *S. japonicum* raised in Swiss Webster mice, and an Egyptian strain of *S. haematobium* raised in golden hamsters were used in this study. Tissue uptake indices (*7*) were determined to estimate schistosomal assimilation of the labeled hexoses.
7. E. M. Cornford and W. H. Oldendorf, *J. Parasitol.* **65**, 357 (1979).
8. Since 2-deoxy-D-glucose uptake has been used as an indicator of glucose utilization rates [P. D. Crane, L. D. Braun, E. M. Cornford, A. M. Nyerges, W. H. Oldendorf, *J. Neurochem.* **34**, 1700 (1980)], the greater uptake of this hexose by the paired male than the paired female might tentatively be attributed to phosphorylation and the inability of the male schistosome to transfer 2-deoxy-D-glucose-6-phosphate.
9. Glycogen was assayed (in single flukes) as described by J. V. Passoneau and V. R. Lauderdale [*Anal. Biochem.* **60**, 405 (1974)]. Protein determinations were performed with commercially prepared reagents (Bio-Rad) by the method of M. Bradford [*ibid.* **72**, 248 (1976)].
10. We find that a silicone oil rinse removes some of the excess isotopic medium from the schistosome's surface by a sheeting action.
11. It has been demonstrated [L. Sokoloff *et al.*, *J. Neurochem.* **28**, 897 (1977)] that the half-life in vivo of 2-deoxy-D-glucose-6-phosphate ranges from 6.1 to 9.7 hours.
12. T. E. Mansour and E. Bueding, *Br. J. Pharmacol. Chemother.* **9**, 459 (1954).
13. Published estimates suggest that one female *S. mansoni* can produce from 300 eggs per day [K. Kloetzel, *Am. J. Trop. Med. Hyg.* **16**, 293 (1967); D. V. Moore and J. H. Sandground, *ibid.* **5**, 831 (1956)] to 1200 eggs per day [T. P. Pesigan *et al.*, *Bull. W.H.O.* **18**, 345 (1958)]. A single egg contains about 40 yolk granules, so 300 eggs would represent 12,000 granules [A. W. Senft, *Ann. N.Y. Acad. Sci.* **160**, 571 (1969)].
14. R. T. Damian, in *Host-Parasite Interfaces*, B. B. Nickol, Ed. (Academic Press, New York, 1979), pp. 103–126.
15. H. Van den Bossche, *Nature (London)* **273**, 626 (1978); N. M. Woolhouse, *Biochem. Pharmacol.* **28**, 2413 (1979).
16. WHO Scientific Working Group on Schistosomiasis, *Bull. W.H.O.* **56**, 361 (1978).
17. T. E. Mansour, *Science* **205**, 462 (1979); N. M. Woolhouse (*15*).
18. E. Bueding and J. Fisher, *Mol. Pharmacol.* **6**, 523 (1970).
19. E. Bueding, personal communication.
20. We gratefully acknowledge support by NIH grant AI 15692. W. H. Oldendorf generously provided laboratory space for this study. A. J. MacInnis provided mansonian schistosomes and critically read the manuscript. Other materials were provided by NIAID supply contract AI 72524. We thank C. Diep, G. Rowley, S. Biggers, and W. Boyle for their assistance. A preliminary version of this report was presented at the 29th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, 5 November 1980.

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Cardiac Sarcolemma: Compositional Adaptation to Exercise

Abstract. *Marked changes were observed in the lipid composition of highly purified plasma membranes isolated from the hearts of rats subjected to daily treadmill running. Compared to sedentary controls, sarcolemmal content of total phospholipid and phosphatidylserine in the trained group was increased 23 and 50 percent, respectively. This observation suggests a mechanism by which cardiac contractility may be enhanced by exercise.*

Lipid composition is an important determinant of the biological and physical characteristics of the plasma membrane, including fluidity. Membrane phospholipid acyl chain structure and the ratio of the concentrations of other lipid bilayer components influence intrinsic enzyme activity and the ability of membrane components to move within the plane of the bilayer (*1*). Plasma membrane composition undergoes alterations during the cell growth cycle and in response to events during ontogeny and transformation by viral agents (*2*). In addition, membrane composition changes in response to temperature gradients, aging, and alterations in dietary fatty acids. Thus plasma membrane composition is

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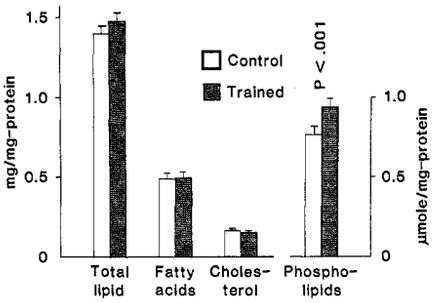

Fig. 1. Analysis of sarcolemmal lipids. The total lipid was determined from the sample weight after Bligh-Dyer lipid extraction and evaporation of the organic solvent system under vacuum. The total lipid concentrations were 1.46 ± 0.5 and 1.33 ± 0.7 per milligram of protein in the exercised and control groups, respectively. Total fatty acids represent the sum of all fatty acids detected in gas-liquid chromatographic analysis. Each bar shows the mean for seven analyses, except for the cholesterol data, where $N = 5$.

both dynamic and responsive to its microenvironment.

It has been convincingly demonstrated that cardiac sarcolemma (muscle plasma membrane) plays a critical role in excitation-contraction (E-C) coupling in the heart (*3*). Specific calcium receptors on the membrane apparently influence the magnitude of the transsarcolemmal calcium flux. Fabiato and Fabiato (*4*) showed that relatively small increases in the intracellular free CA^{2+} concentration trigger Ca^{2+} release from the sarcoplasmic reticulum in mechanically skinned cardiac muscle fibers in a graded fashion and substantially increase the concentration of free intracellular calcium, thereby stimulating myofilament cross-bridge formation.

Although it has been frequently documented that exercise training enhances cardiac function in mammals (*5*), the mechanism of this adaptation is not well understood. Investigators have used a myriad of animal and training models and experimental techniques in evaluating cardiac function, so a cohesive hypothesis for a mechanism of adaptation to exercise has not emerged. Because of the critical nature of plasma membrane control of mechanical function in the heart, this study was performed to investigate sarcolemmal adaptation in response to exercise.

Female Sprague-Dawley rats were randomly divided into a sedentary control group ($N = 30$) and a treadmill-trained group ($N = 30$). Animal care and training were identical to that described previously (*6*). Forty-eight to 72 hours after the end of the 12-week training period, all animals were killed by cervical fracture and their hearts were rapidly excised and immediately frozen in liquid nitrogen. There was no significant difference in heart weight (0.79 ± 0.02 versus 0.75 ± 0.02 g) or in the ratio of heart to body weight (2.87 ± 0.03 versus 2.77 ± 0.04 mg/g) between the two groups, indicating that cardiac hypertrophy did not result from the exercise regimen.

Sarcolemma was isolated by the procedure of Bers (*7*) as modified by Philipson *et al.* (*8*). In brief, in each experiment three or four hearts (about 4 g, wet weight) from one group were combined, minced, and subjected to Polytron homogenization in two bursts at setting 8 for a total of 8 to 10 seconds. In all cases, sarcolemmal isolation and subsequent analyses for the experimental and control groups were done in pairs concurrently. The homogenate underwent differential and sucrose gradient ultracentrifugation, extraction with 0.3*M* KCl, and incubation in deoxyribonuclease to improve purity and yield (*8*). Purification indices of sarcolemmal marker enzymes Na^+,K^+-adenosinetriphosphatase and K^+-stimulted *p*-nitrophenylphosphatase of approximately 35-fold were observed with minimal cross-contamination, as indicated by mitochondrial and microsomal markers and electron microscopy (*9*). Yields of cardiac sarcolemma from the two groups were not significantly different: 0.98 ± 0.16 versus 1.05 ± 0.20 mg of protein per gram (wet weight) for exercised and control rats, respectively.

Table 1. Sarcolemmal fatty acid composition in the two groups, expressed as a percentage on a weight basis. Each value is the mean ± standard error for seven analyses.

Fatty acid*	Percentage of sarcolemmal fatty acids	
	Exercised rats	Control rats
14:0	0.04 ± 0.01	0.02 ± 0.01
16:0	28.87 ± 1.30	27.16 ± 1.27
16:1	0.10 ± 0.01	0.20 ± 0.01
18:0	33.51 ± 1.38	32.37 ± 2.09
18:1	8.41 ± 0.41	10.17 ± 1.25
18:2	10.63 ± 0.43†	12.43 ± 0.39
20:4	22.99 ± 1.40	23.57 ± 1.85
Saturation	62.95 ± 0.64†	58.95 ± 1.41

*First number is number of carbons in the chain; second number is number of double bonds. †$P < .05$.

Sarcolemmal lipid was extracted by the Bligh-Dyer procedure (*10*); the results of the lipid analyses are shown in Fig. 1. Although the between-group difference in total sarcolemmal lipid specific content was not significant, there was a significantly higher (about 23 percent; $P < .001$, Student's *t*-test) concentration of membrane phospholipid in the exercised animals. Since sarcolemmal cholesterol concentrations were not different, the molar ratio of cholesterol to phospholipid (C/PL) was lower in the experimental group by a comparable amount (0.424 versus 0.547). Increases in C/PL increase both the microviscosity and order of the lipid bilayer (*1*).

Sarcolemmal phospholipids were separated by two-dimensional thin-layer chromatography (*11*) and their relative concentrations were determined (*12*) (Fig. 2, a and b). The concentration of phosphatidylserine in membranes from the exercised rats was 9.8 nmole per milligram of protein compared to 6.5 nmole in controls—a difference of 51 percent. This may be of critical importance in the adaptation to exercise, since phosphatidylserine is a major binding moiety of sarcolemmal Ca^{2+} (*8*). Furthermore, it has been hypothesized that the enhancement of cardiac function in-

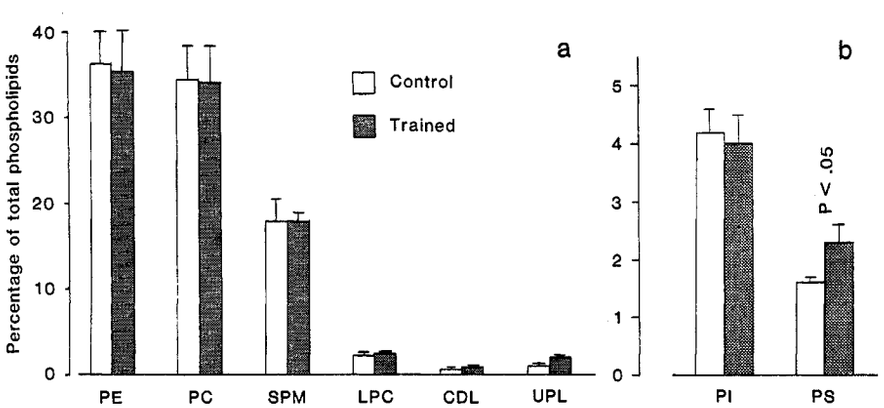

Fig. 2. Sarcolemmal phospholipid analysis by thin-layer chromatography. (a) Individual phospholipids expressed as a percentage of total phospholipids on a molar basis. The PC/PE molar ratios in exercised and control rats were 0.97 and 0.94, respectively. (b) Concentration of the two major sarcolemmal phospholipids that are anionic at physiological *p*H. Abbreviations: *PE*, phosphatidylethanolamine; *PC*, phosphatidylcholine; *SPM*, sphingomyelin; *LPC*, lysophosphatidylcholine; *CDL*, cardiolipin; *UPL*, unidentified phospholipid; *PI*, phosphatidylinositol; and *PS*, phosphatidylserine.

duced by training is due, at least in part, to an increased number of sarcolemmal Ca^{2+} binding sites (*6*). These two independent findings are mutually consistent.

Sarcolemmal fatty acid composition was determined by gas-liquid chromatography (*13*). As shown in Table 1, the concentration of linoleic acid (18:2) and the saturation percentage were significantly higher in exercised rats ($P < .05$). Phospholipid acyl chain length and degree of saturation also have a prominent effect on the physical properties of the lipid bilayer. For example, increasing saturation of natural fatty acids decreases membrane fluidity (*1*).

It is clear that exercise can induce substantial alterations in the lipid composition of the plasma membrane. Because these compositional changes are likely to affect sarcolemmal control of E-C coupling, they may be related to the physiological adaptation that the heart undergoes in response to a stressor.

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References and Notes

1. M. Shinitzky and P. Henkart, *Int. Rev. Cytol.* **60**, 121 (1979); D. Chapman, in *Biomembranes: Lipid, Proteins and Receptors*, R. M. Burton and L. Packer, Eds. (BI-Science, Webster Groves, Mo., 1974), p. 65.
2. C. A. Pasternak, in *Lipid Metabolism in Mammals*, F. Snyder, Ed. (Plenum, New York, 1977), p. 353; H. H. Loh and P. Y. Law, *Annu. Rev. Pharmacol. Toxicol.* **20**, 201 (1980).
3. D. M. Bers and G. A. Langer, *Am. J. Physiol.* **237**, H332 (1979); K. D. Philipson and G. A. Langer, *J. Mol. Cell. Cardiol.* **11**, 857 (1979); K. D. Philipson, D. M. Bers, A. Y. Nishimoto, G. A. Langer, *Am. J. Physiol.* **238**, H373 (1980); G. A. Langer, *J. Mol. Cell. Cardiol.* **12**, 231 (1980).
4. A. Fabiato and F. Fabiato, *Annu. Rev. Physiol.* **41**, 473 (1979).
5. G. F. Tibbits, B. K. Koziol, N. K. Roberts, K. M. Baldwin, R. J. Barnard, *J. Appl. Physiol.* **44**, 85 (1978); R. T. Dowell, H. L. Stone, L. A. Sordahl, G. K. Asimakis, *ibid.* **34**, 977 (1977); J. Scheuer and C. M. Tipton, *Annu. Rev. Physiol.* **39**, 221 (1977); T. F. Schaible and J. Scheuer, *J. Appl. Physiol.* **46**, 854 (1979).
6. G. F. Tibbits, R. J. Barnard, K. M. Baldwin, N. Cugalj, and N. K. Roberts [*Am. J. Physiol.* **240**, H472 (1981)] demonstrated that gastrocnemius cytochrome c oxidase activity, exercise tolerance, and cardiac contractility were significantly increased with this training protocol.
7. D. M. Bers, *Biochim. Biophys. Acta* **555**, 131 (1979). The major deviation from Bers's procedure is the addition, after Polytron homogenization and myofibrillar extraction with KCl, of a 90-minute period in which the homogenate is incubated with 20 mg of deoxyribonuclease I (1500 Kunitz units per milligram) at 30°C. The rationale for this treatment is discussed in (*8*).
8. K. D. Philipson, D. M. Bers, A. Y. Nishimoto, *J. Mol. Cell. Cardiol.* **12**, 1159 (1980). Although there is considerable evidence that binding of Ca^{2+} to sarcolemma is critical for cardiac E-C coupling, the nature of the membrane chemical moieties to which Ca^{2+} binds is still controversial. Sialic acid, sarcolemmal protein, and certain bilayer phospholipids are known to bind Ca^{2+}. Philipson *et al.* demonstrated that the phospholipids which are anionic at physiological *p*H (phosphatidylserine, phosphatidylinositol, and cardiolipin) can account for the majority of sarcolemmal Ca^{2+} binding. It remains to be shown, however, that phospholipid-bound sarcolemmal Ca^{2+} is available for E-C coupling.
9. G. F. Tibbits, M. Sasaki, M. Ikeda, K. Shimada, T. Tsuruhara, T. Nagatomo, *J. Mol. Cell. Cardiol.*, in press. The purification index of the sarcolemmal fraction is defined as the ratio of the specific activity of a given enzyme marker in the sarcolemmal fraction to that in the crude homogenate. The index was 0.3 for the mitochondrial marker succinate dehydrogenase in the sarcolemmal fraction and 4.3 for the putative microsomal marker nicotinamide adenine dinucleotide phosphate cytochrome c reductase. There was no indication of differences between the groups in yield or purity of the isolated sarcolemma.
10. E. G. Bligh and W. K. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959). In this procedure lipids are extracted from the sarcolemma with a monophasic solution of chloroform, methanol, and water in a ratio of 1:2:0.8. The antioxidant BHT (final concentration, 0.05 percent) was added for fatty acid protection. The solution was made biphasic by changing this ratio to 2:2:1.8, allowing for the separation of the lipid component.
11. Two-dimensional thin-layer chromatography was carried out with 20 by 20 cm silica gel G (Merck) plates. The solvent system was that of G. Rouser and S. Fleischer [*Methods Enzymol.* **10**, 385 (1967)]. A 0.5-ml portion of the sample (1.0 mg of lipid) in chloroform was applied to the origin. After saturation of the chamber, plates were developed at 18°C for approximately 90 minutes per side. Phospholipid spots were visualized in I_2 vapor and identified by use of the standards and techniques described by V. P. Skipski and M. Barclay [*Methods Enzymol.* **14**, 530 (1969)].
12. After identification of each phospholipid, the spots were scraped and the phospholipids were extracted in 2:1 chloroform and methanol. Inorganic phosphorus release was determined by the method of M. Rockstein and P. W. Herron [*Anal. Chem.* **23**, 1500 (1951)]. It was assumed that each mole of inorganic phosphorus released was equivalent to 1 mole of phospholipid—except for cardiolipin, for which this ratio is 2:1.
13. Gas-liquid chromatography separation of sarcolemmal fatty acids began with the hydrolysis and preparation of fatty acid methyl esters. One-tenth milligram of lipid was incubated with 3 ml of methanol and five drops of concentrated H_2SO_4 at 70°C for 2.5 hours. This was followed by a series of petroleum ether extractions and the addition of 1.0 ml of CH_2N_2. All samples were run with pentadecanoic acid (15:0) as the internal standard. Details of column temperature, pressure, and packing are described in T. Nagatomo, K. Hattori, M. Ikeda, and K. Shimada [*Biochem. Med.* **23**, 198 (1980)].
14. We thank G. A. Langer and K. D. Philipson for their contributions during these experiments and in preparing this report, T. Tsuruhara and M. Ikeda for technical advice, M. M. Bersohn and J. Vinten-Johansen for helpful editorial suggestions, and C. Heteniak for typing the manuscript. G.F.T. was the recipient of research fellowship 610 from the American Heart Association (Los Angeles affiliate).

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Selective Herbivory in Tassel-Eared Squirrels: Role of Monoterpenes in Ponderosa Pines Chosen as Feeding Trees

Abstract. *Ponderosa pine twigs collected from trees used by tassel-eared squirrels as sources of cortical tissue for food contained smaller amounts of monoterpenes than twigs from similar trees not used by the squirrels as food sources. Of the 18 monoterpenes isolated from the twig samples, α-pinene was the best single predictor of food source trees. In experiments with captive tassel-eared squirrels, consumption of a preferred food was inversely correlated with the concentration of α-pinene added to the food.*

During winter, tassel-eared squirrels (*Sciurus aberti*) feed predominantly on the cortical tissue of small twigs of certain ponderosa pine (*Pinus ponderosa* Laws) trees (*1*). Such trees are easily distinguished by their defoliated appearance and the accumulation of needle clusters beneath them (Fig. 1). To obtain cortical tissue, the squirrels remove terminal twigs in the upper portions of the tree from larger branches by biting through the stem several centimeters from the terminal needle cluster (Fig. 2). The needle cluster is clipped from the end of the twig and drops to the ground. The rough outer bark is then removed with the teeth while the twig is rotated with the forepaws to expose the phloem and also the cambium and some current year's xylem.

Trees on which the squirrels feed (feeding trees) are usually cone-bearing

Table 1. Major monoterpenes analyzed from cortical tissues of ponderosa pine twigs.

Monoterpene	Feeding trees		Nonfeeding trees	
	Percent	Range	Percent	Range
α-Pinene	27.2	9.0–53.9	26.0	6.0–59.1
β-Pinene	16.4	5.5–36.4	14.6	4.7–31.8
3-Carene	28.8	11.7–58.5	31.9	16.5–59.0
Myrcene	5.6	1.6– 8.5	5.9	2.1–11.5
Limonene	11.1	3.4–27.3	10.6	1.3–24.8
β-Phellandrene	4.7	0.7–11.7	4.5	0.9–27.0
Terpinolene	3.3	1.6– 7.9	3.7	1.0– 9.9
Total	97.1		97.2	

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