low values was, as with vit. E, greater than for the other 2 groups. Scatter diagrams of all combinations of vit. E, vit. A and carotene failed to suggest any correlation among these blood constituents. Detailed data for vit. A and carotenoids on 2,000 serum samples from a broader 2-year nutrition survey(6) will be presented in another report.

Discussion. A summary of surveys of blood vit. E values in Western countries compiled by Harris *et al*(4) yielded an average of 1.01 mg/100 ml plasma (or serum). The averages for 2 urban U.S. populations of adults reported recently were 1.05 ± .26 and 1.05 ± .32 mg/100 ml(3,4). In the men and nonpregnant or lactating women of rural East Pakistan, an average of 0.76 ± .30 mg/100 ml was found. Guzman *et al*(7) reported an average value of 0.65 ± .26 mg/100 ml for school children in 5 Central American countries, while in adults in these countries the values varied from approximately 0.46-1.08 mg/100 ml during the year.

It is generally accepted that the vit. E requirement is related to the amount of polyunsaturated fatty acids in the diet(8,9), or more precisely to the content of these acids in the tissues, so that serum levels by themselves have no definitive meaning. In a preliminary report(2), the dietary fat in East Pakistan was found to be only 10% of calories. Work is in progress to characterize the fatty acid composition of the dietary fat and also of the serum and erythrocytes in order to evaluate the significance of the low serum tocopherol levels.

Summary. Serum tocopherol values were determined on 79 adult males, 23 adult females, 19 pregnant or lactating females and 31 children. Of the total, 21% had values below 0.5 mg/100 ml. Children and pregnant or lactating females had 29% and 26%, respectively, in this low range. Serum vit. A and carotenoid levels were also determined and significant numbers of low values were found.

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Effect of Skin Temperature on Infectibility of *Plasmodium gallinaceum* Sporozoites in the Chick.* (29517)

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Recently, I(1,2) have shown that the exoerythrocytic stage of *Plasmodium gallinaceum* infection is apparently not established in the albino mouse when the sporozoites of the organism are introduced by the mosquito, and that the inhibitory action seems to be exerted almost immediately in the skin at the time of the introduction of the organisms. Presently, I am reporting an attempt to determine whether the skin temperature difference between the chick, a susceptible species, and the mouse might account for the nonsusceptibility of the latter.

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TABLE I. Effect of Skin Temperature on Infectibility of *P. gallinaceum* Sporozoites in the Chick.

Bitten chick No.	Mosquitoes biting	Temperatures, °C: Reduced for biting	At end of biting	30 min after biting	Implanted chicks: No.	Blood stream patency, day
1	3	33.3	34.5	32.0	W 20	11th
2	4	33.5	34.5	32.5	" 118	9th
3	5	34.0	34.5	32.5	" 73	8th
4	5	33.5	34.3	29.5	" 68	13th
5	1	34.2	34.4	34.0	" 125	7th
6	3	33.5	34.2	34.0	" 72	7th
7	3	34.6	34.0	33.4	" 104	9th
8	2	33.8	34.3	33.6	" 21	7th
9	6	34.2	36.2	34.7	" 71	7th
10	3	33.5	34.2	33.6	" 11	7th
11	2	28.0	31.0	28.0	" 57	10th
12	6	28.8	32.3	30.2	" 69	9th
13	3	30.4	34.0	28.5	" 66	—
14	3	26.5	28.0	25.2	" 55	9th
15	2	27.4	32.2	26.0	" 117	7th
	Avg temp:	31.95°C	33.52°C	31.18°C		Avg day of patency: 8th

Methods. The temperature of the sites to which mosquitoes are habitually applied in this laboratory, namely a plucked area on the breast of 6- to 9-day-old White Rock chicks and a shaved area on the belly of 3 to 4 weeks old albino mice, was determined in 12 chicks and 12 mice by use of the Danish electric universal thermometer, type TE 3. The "bulb" of this thermometer was applied firmly for 2 minutes (longer than necessary for temperature stabilization) 3 times at intervals of 30 minutes. The average of the chick temperatures was found to be 39.1°C (102.38F) and the spread 38.2 to 40.0°C, while that of the mice was 35.6°C (96.08F) and the spread 33.0 to 36.9°C. The difference between the average chick and mouse temperatures was 3.5°C (6.3°F), the mouse being the cooler species.

The next step was a lowering of the skin temperature of the chick to or below that of the mouse to see whether sporozoites introduced by the mosquito into chick skin at that temperature would be inhibited from causing patent infection. To do this, cold water applications were made continuously to the plucked breast of the chick for 15 minutes; the temperature was then read and infected *Aedes aegypti* mosquitoes applied for 5 minutes; then the temperature was read once again and the cooling continued for another 30 minutes. The bit of cooled bitten skin was then removed and implanted intraperitoneally in a clean chick by a slight modification of the method of Coulston, Cantrell and Huff(3), and blood smears of this chick were examined for evidence of infection, beginning the seventh day after implantation.

Results. It will be seen in Table I that: (a) the average temperature to which the skin was lowered for the biting in the 15 chicks was 31.95°C (89.51°F), or actually 3.65°C (6.57°F) lower than the average mouse skin temperature; (b) the temperature at the end of the biting period was 33.52°C (92.3°F), which was also lower than that of mouse skin; and (c) the temperature was maintained at an average of 31.18°C (88.12°F) for 30 minutes after cessation of the biting. The other fact to be observed in Table I is that the chicks implanted with these cold-bitten skins became infected quite as they would have if skin had been used that had been bitten and maintained at the normal temperature.

Discussion. To determine the effect of lowering the total body temperature upon established *P. gallinaceum* infections in the normal chick host, Nye(4) immersed the animals for some hours in cold running tap-water and found that the cycle of development of the

organisms was extended somewhat beyond the normal 36 hours, but that the subsequent course of infection, the morphology of the parasites, and the number of merozoites per schizont were not affected. My presently recorded observations extend this body of information by the additional fact that local cooling of the skin before, during and after the introduction of sporozoites by the mosquito does not prevent the initiation of the infection. Hence it appears inadmissable to ascribe the non-susceptibility of the albino mouse to this infection to the fact that the skin into which the mosquito introduces the sporozoites in this animal is several degrees cooler than that of the normal host. In his duck embryo *P. cathemerium* infections, an abnormal host-parasite situation, McGhee(5) found that the infection developed more slowly and never reached the peak at 30°C that it did when the incubation was at 37°C. This observation, however, seems non-contributory under the experimental conditions described here.

Summary. The temperature of the biting site for mosquitoes on the shaved belly of the albino mouse was found to be 3.5°C (6.3°F) lower than that of a comparable plucked area on the chick breast. When the temperature of the chick skin was maintained at or below that of the mouse skin during and for some time after potentially infecting bites of *A. aegypti* mosquitoes carrying sporozoites of *P. gallinaceum,* it was found that the procedure did not in any way deter the onset of infection in these chicks.

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Immunochromatography: A Combination of Chromatography and Immunodiffusion on a Micro-scale.* (29518)

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While the techniques of ion-exchange chromatography, on derivatives of cellulose(1) and dextran gel(2), and gel filtration chromatography(3-7) have proved valuable for the fractionation of serologically active proteins, they have not been used previously in direct combination with immunodiffusion. Recently proteins have been separated on a micro-scale by thin layer chromatography on dextran gels(8-10) and by paper chromatography on anion-exchange cellulose(11). This report describes the combination of chromatography and immunodiffusion on a microscope slide to separate and characterize serologically active compounds in a manner analogous to immunoelectrophoresis(12).

Materials and methods. The cross-linked dextran gels, diethylaminoethyl-Sephadex (DEAE) A-50 medium grade, carboxymethyl-Sephadex (CM) C-50 medium grade and Sephadex G-100 (G-100) 140-400 mesh, were prepared for use according to the manufacturer's instructions.§ In the experiments il-

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§ Cross-linked dextran gels and ion-exchange derivatives of these gels can be obtained from Pharmacia, A. B., Uppsala, Sweden under the trade name Sephadex.