the responses of a cell having a membrane impermeable to cations will be approximately true for a cell having a membrane 0.00002 times as permeable to cations as to anions. The rate of loss of potassium from cells where the uptake may have been stopped by a poison is slow enough to be undetectable in the initial lag period found by Davson[12] for the loss of potassium from cells poisoned by respiratory poisons. In his experiments all of the loss must be attributed to a changed permeability of the membrane. The question as to what puts potassium back in the cell against a diffusion gradient remains unanswered. However, the experiments of Danowski[13] and Harris[14] show that metabolism is necessary for the continued uptake of potassium by the cells of stored blood.

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Non-Permeability of Blood Clot to Sulfonamide Drugs in Presence of Increased Temperature.

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Favorable results have been recently reported[1, 2] following the use of induced fever associated with sulfonamide drugs in the treatment of subacute bacterial endocarditis. That sulfonamide drugs do not penetrate fibrin or blood clot to any significant degree has been reported by two separate groups of observers, using different *in vitro* methods.[3, 4] However, a third has found that penetration does take place.[5] It occurred to us that if a high body temperature increased the effectiveness of the sulfonamide drugs in subacute bacterial endocarditis, it could do so only by bringing the drug into contact with the organism within the vegetation; *i. e.*, by increasing the permeability of the vegetation to the drug. To determine this we studied

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the permeability of blood clot to various sulfonamide drugs under conditions of high temperatures simulating that of induced pyrexia.

Procedure. Our procedure consisted of suspending whole blood clot in serum containing a chemotherapeutic agent, following to some extent the method of Duncan and Faulkner.[3] Five cubic centimeters of human blood were withdrawn and allowed to clot and stand at room temperature for 24 hours. Two cc of a 25 mg % solution of a sulfonamide were then added to the serum surrounding the blood clot, and the whole allowed to stand for another 24 hours at room temperature (about 21°C). A similar preparation was kept for 24 hours at 37.5°C and a third at 41°C. At the end of this period, the serum was removed and the clot thoroughly washed and dried. The clot was then crushed in a mortar, boiled in water and filtered. Determinations were then made on the filtrate and previously removed serum of the sulfonamide level, using the method of Marshall and Bratton. The clot filtrate was prepared without boiling in a control experiment, to eliminate any possible destructive effects on the drug by boiling, with no difference in results.

The drugs used were sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, the sodium salts of sulfapyridine and sulfadiazine, and promin.

To determine whether heparin has any effect on the permeability of blood clot to the sulfonamide drugs, 10 units were added to 5 cc of clot and serum, which had been standing at room temperature for 24 hours. Experiments were then performed as above, using different drugs and conditions of temperature. The heparin content

TABLE I.

Concentrations of Various Sulfonamides in Blood Clot and Surrounding Serum under Different Conditions of Temperature.

	Concentration of drug in mg per 100 cc of solution					
Temperature:	21°C (approx.)		37.5°C		41.0°C	
Drug	Serum	Clot	Serum	Clot	Serum	Clot
Sulfanilamide	8.5	T*	10	0	9	T
Sulfapyridine	14.0	0	15	0	13	0
Sulfathiazole	11.0	0	11	0	12	0
Sulfadiazine	9.5	0	9	0	9	0
Sodium Sulfapyridine	—	—	—	—	17.5	T
Sodium Sulfadiazine	—	—	—	—	9	T
Promin	—	—	—	—	4	T
With Heparin added:						
Sulfanilamide	10.0	T	8	T	8	0
Sulfapyridine	15.0	T	13	T	17	T
Sulfathiazole	13.0	0	17	0	15	0
Sulfadiazine	12.0	0	16	0	14	0

*T—Trace.

of each tube was the equivalent of 10,000 units per 5000 cc of blood, which will produce *in vivo* a prolongation of coagulation lasting for about 4 hours.

Results. Table I shows the significant results. Repeat experiments were very closely confirmatory. Readings under 1.0 mg per 100 cc were called trace, a fractional reading being too inaccurate. As can be seen from the table, the amount of penetration of the blood clot under all conditions and with the different drugs was practically negligible. Heparin did not have any effect on the permeability of the blood clot.

Summary. The permeability of human blood clot to various sulfonamide drugs under conditions of increased temperature was determined in an *in vitro* study. Negligible penetration of the clot occurred even in the presence of temperatures up to 41°C. Heparin had no effect on the permeability of the blood clot.

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Evidence for the Existence of Two Antibodies for Crystalline Insulin.

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Marked insulin resistance was discovered in a 43-year-old female diabetic exhibiting severe urticaria following injections of crystalline and regular insulin. The patient was studied as follows: (1) Intravenous insulin-tolerance tests were carried out with commercial crystalline insulin and with a preparation of human insulin; (2) skin tests and passive-transfer tests were made; (3) the patient's serum was tested for the presence of an insulin-neutralizing antibody by mouse-assay.

This patient has been reported previously as a case of allergy to insulin.[1]

Materials and Methods. Serum from the patient was sterilized by filtration through a Seitz filter and stored at 4°C.

Lilly's u40 commercial solution of zinc-insulin crystals derived from beef and pork pancreas was used in all tests except as mentioned below and will be referred to as crystalline insulin.

[1] Yasuna, E., *J. Allergy*, 1940-41, **12**, 295.