changes that we have reported previously. Three animals received the ethyl ether-soluble fraction (A) and although they became ill they showed no proliferation of myeloid cells. Five guinea pigs were given the petroleum ether-soluble fraction (C) in 3 or 4 doses of ½ cc each (50 to 140 mg per dose in olive oil) on successive days and the animals were observed until they showed signs of being ill. Autopsies were performed 3 to 4 weeks after the first injection and proliferation of myeloid cells in the liver, lungs, spleen and adrenals was greater in extent than in any of our previous animals. Three of these animals had white blood cell counts of 40,000 the day of autopsy. The blood smears contained 10 to 20% myelocytes, a few myeloblasts and low percentages of lymphocytes.

Proliferation of lymphoid cells occurred after the use of each of the 3 similar fractions of the extracts obtained from the urine of patients with chronic lymphoid leucemia with no apparent variation. This method, therefore, does not aid in tracing the active principle of this extract.

For control experiments 36 liters of urine of normal individuals were fractionated in the same manner. The yield of the final petroleum ether-soluble fraction was less than one gram. This material produced no specific cellular response.

A method is presented for partially purifying the active principle found in chloroform extracts from the urine of patients with chronic myeloid leucemia. This method does not apply to similar extracts from the urine of patients with chronic lymphoid leucemia.

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Percutaneous Application of Sulfanilamide in Animals and Men.

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As has been described,[1] it is possible to obtain a chemotherapeutic effect in animals and men by the percutaneous application of external disinfectants. In this respect special attention was given to p-chloro-

[1] Zondek, B., in press.

xylenol and to some of its derivatives. By the percutaneous application of p-chloro-xylenol in the form of tinctures or concentrated ointments (30%) it was possible to save rats from deadly pneumococci infections. Staphylococci inoculated in the blood of patients treated with p-chloro-xylenol percutaneously were inhibited in their growth. Furthermore, the percutaneous applictaion of p-chloro-xylenol brings about a disinfection of the urinary tract following the excretion of the drug with the urine. Clinical studies in cases of urogenic and general infections have confirmed the value of this percutaneous chemotherapy.

The results obtained with this method of treatment have encouraged us to study also the cutaneous resorption of sulfanilamide. These studies have shown that sulfanilamide also is absorbed by the skin and is present in the blood following its cutaneous application.

Method. Animal experiments were performed in rabbits. Sulfanilamide was given in organic solvents, since the percutaneous application of ointments yielded less satisfactory results. Our experience with the percutaneous use of estrone[2] has shown the advantages of organic solvents for cutaneous absorption. Sulfanilamide was applied to the shaved skin of the back, out of reach of the animal's tongue, to eliminate a possible oral absorption. Each animal was then kept in a separate cage.

The solutions of sulfanilamide for percutaneous use: For experiment No. 1, with rats, a 2.5% tincture in alcohol was used. Later we found that the percutaneous absorption of sulfanilamide is augmented by the addition of glycerine and soap. Hence the solutions for experiments Nos. 2 and 3 were prepared as follows: 15 g sulfanilamide were dissolved in 75 cc of acetone, 15 cc glycerine, 2.5 cc liquid soap and 7.5 cc water. For subcutaneous or intramuscular use we used soluseptazine (phenyl-propyl-amino-benzene-sulfamide-α-γ-disulfonate sodium).

Experimental. The first series of experiments aimed at evaluating the concentration of the drug in the blood of rabbits, thus enabling a comparison of the concentrations obtained by other routes of administration. The second series of studies was conducted in men. Sulfanilamide estimations in the blood were carried out according to Werner.[3]

(1) Experiments in rabbits: Five rabbits weighing 1500 g each received 0.5 g of sulfanilamide. This was administered in 3 animals percutaneously, in one orally, and in one subcutaneously. For inunc-

[2] Zondek, B., *Lancet*, 1938, **234**, 1107.

[3] Werner, M., *Lancet*, 1939, **236**, 18.

TABLE I.
Sulfanilamide Blood Concentration in Rabbits after Percutaneous, Peroral and Subcutaneous Administration of 0.5 g of Sulfanilamide.

Hr after treatment	R I Received 0.5 g sulfanilamide percutaneously mg%	R II 0.5 g sulfanilamide percutaneously mg%	R III 0.5 g sulfanilamide percutaneously mg%	R IV 0.5 g sulfanilamide *per os* mg%	R V 0.5 g sulfanilamide subcutaneously mg%
½	1			2	20
1	1	1	1.5	4	24
2			2.5	5	
3	8	6		5	7
4	8				6
5		6	8	7	6
7		7	10		
24	6			5	6

tions we used 15% sulfanilamide solutions in acetone with addition of glycerine and soap. For the oral and subcutaneous treatment soluseptazine was used. The blood concentration of sulfanilamide was determined at various time intervals following the commencement of treatment.

Results (Table I). The demonstration of sulfanilamide in the blood of animals treated percutaneously proved that the drug penetrates the skin and circulates in the blood. The concentration of sulfanilamide in the blood of animals treated percutaneously (max. 8 mg %) was found to be lower than in those treated subcutaneously (max. 24 mg %), but equal to and possibly higher than in those animals treated orally (max. 7 mg %).

Percutaneous application of sulfanilamide in men: The satisfactory results obtained following the percutaneous application of sulfanilamide in rats and rabbits encouraged us to examine the passage of this drug through the human skin. These experiments were carried out in 14 patients. We employed 15% and 20% solutions of sulfanilamide in acetone with addition of glycerine and soap. The sulfanilamide concentration in the blood was examined 24 hours after inunction and at various other intervals.

Results (Table II). In men the blood concentration of sulfanilamide following percutaneous application is less durable and inferior to that obtained after oral administration of the drug. The blood concentration of sulfanilamide following a single dose of 2 g given orally was less than 1 mg % after ½ hour, reaching a maximum of 1.8 mg % after 4 hours, dropping to 1 mg % after 12 hours and disappearing completely 24 hours after administration. The same dose percutaneously gave a sulfanilamide blood concentration of 1.2 mg % after ½ hour, 1 mg % after 4 hours, not to be found 12 hours

TABLE II.
Blood Concentration of Sulfanilamide after Percutaneous and Peroral Administration in **Men.**

Sulfanilamide dose in g	Route of administration	½ hr after treatment mg%	2 hr after treatment mg%	4 hr after treatment mg%	12 hr after treatment mg%	24 hr after treatment mg%
2	percutaneously	1.2	1.2	1	0	0
2	oral	traces	1	1.8	1	0
4	percutaneously	1.8	1.4	1.2	1	0
4	oral	traces	1.6	3.2	2	1.2
5	percutaneously	2.0	2.0	1.2	traces	0
10	"	2.7	1.6	1.2	1	0
12	"	3.3	2.0	2.0	1.1	0
16	"	4.0	2.3	2.0	1	0

after administration. On administration of 4 g sulfanilamide *per os*, the concentration of less than 1 mg % after ½ hour rises to 3.2 mg % after 4 hours; the sulfanilamide is still to be detected after 24 hours (1.2 mg %). The same dose percutaneously gives a maximal concentration of 1.8 mg % after ½ hour, 1 mg % after 12 hours, and is not to be detected after 24 hours. Notwithstanding the high single doses employed in the percutaneous application, as high as 16 g, we never encountered a concentration of over 4 mg %. Here, the blood concentration of the drug was highest during the first hour after administration, dropping rapidly during the following 4 hours. No sulfanilamide was found in the blood 24 hours after administration.

Discussion. The penetration of sulfanilamide through the human skin is less perfect than through the rabbit's skin. Thus, the percutaneous route cannot be advised as a general method of administration. This method, however, may frequently render appreciable help as an auxiliary method of treatment in those cases where high doses cannot be administered orally. Patients are frequently unable to keep the sulfanilamide given orally, since it provokes vomiting by local irritation of the stomach or by reaction of the nervous centers. In the former case the subcutaneous or intramuscular route of application is useful, but the low solubility of sulfanilamide does not allow its parenteral administration in high doses. In these cases the percutaneous route provides a way out of the dilemma. For similar reasons, the percutaneous administration of sulfanilamide may be a valuable aid in pediatrics where the injection and ingestion of this drug often meet with difficulties.

In the 14 cases of percutaneous treatment studied, the by-effects encountered were: cyanosis in 4 cases and nausea in only one case. The solvents used (acetone, glycerine, soap) never caused any cutaneous reaction.

Summary and Conclusions. Sulfanilamide if applied percutaneously is resorbed by the skin of rabbits and men. (a) The concen-

tration of sulfanilamide in the blood of rabbits following its percutaneous administration is as high as that obtained after its oral administration, but inferior to the concentration following subcutaneous or intramuscular injections. (b) The blood concentration of sulfanilamide in men following its percutaneous administration is lower than that after oral administration, but vomiting is much more rare and less severe.

The percutaneous use of sulfanilamide may serve as an auxiliary method of chemotherapeutic treatment in certain cases.

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Skeletal Abnormalities of Short Spined Turkeys.

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In the course of examining turkey embryos that failed to hatch, mutants with typically shortened necks and bodies (Fig. 1) were found. These embryos varied in that some had an apparently normal head while others had a reduced skull, eyes, and upper beak. None of the affected embryos hatched.

The mutation was found in a closely inbred strain of the Bourbon Red variety of turkeys. Three segregating families gave a ratio of 59 normal to 14 abnormal, which is reasonably close to the numbers expected (54.75 : 18.25) on the basis of a ratio of 3 normal to 1 abnormal. Thirteen short spined and 98 normal embryos were obtained from an untrapnested pen of related birds. Of the 27 short spined embryos the sex was observed for 22, of which 12 were males and 10 were females. The mutation is, therefore, a simple autosomal recessive.

Skeletal Abnormalities. Fully developed embryos were selected for further study from eggs of similar size. The bones were freed from tissue, measured, extracted, dried, and ashed.*

The leg and wing bones of the short-spined embryos are normal in length and thickness (Table I, Fig.2). The head is likewise normal, whereas the neck and body are much reduced in length.

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