of sulfaquinoxaline was administered orally with varying levels of menadione, one mole of menadione appeared to overcome the inhibitory effect of more than 800 moles of sulfaquinoxaline.

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Non-Transfer of *Trans* Fatty Acids from Mother to Young.*† (23601)

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Bertram(1) first reported the presence of traces of *trans* Δ11 octadecenoic acid in oxen, sheep and butterfat. More recently Swern *et al.*(2) reported that beef fat contained not only traces but between 5 and 10% *trans* fatty acids. Hartman and his coworkers extended these investigations to the depot fats of various animals(3) and reported that whereas ruminants contained considerable amounts of *trans* fatty acids, non-ruminants and birds contained little or none. Reiser(4) has suggested that the occurrence of *trans* fatty acids in ruminants is due to the activity of the intestinal flora. Although *trans* fatty acids do not normally appear in the depot fats of non-ruminants *trans* fatty acids are deposited in the tissues whenever they are fed as a dietary component(5-7). It therefore, appeared of interest to note whether *trans* fatty acids were transferred to the tissues of non-ruminants by other than dietary means.

In the present study considerable amounts of *trans* fatty acids were deposited in the tissues of female rats by feeding these acids in the form of hydrogenated margarine stock. The rats were then mated and both mother and young analyzed for their *trans* fatty acid content.

Methods. One kg of the basal diet was prepared from glucose 640 g, casein 310 g, and Wesson salt mix(8) 50 g. Four g of a water soluble vitamin mix was added to each kg of feed(9) and the fat soluble vitamins were given by dropper twice each week.‡ Fifteen % of hydrogenated margarine stock containing 40.7% *trans* fatty acids was added to the feed at the expense of the glucose. Two % of corn oil was also added at the expense of glucose to serve as a source of essential fatty acids. Three rats were sacrificed at the beginning of the experiment, and 10 rats were placed on the experimental diet for 73 days and then mated. Eight mothers and their litters were sacrificed by anesthetisation with ether immediately after birth of the young. The remaining 2 litters were allowed to suckle the maternal milk for 9

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‡ One drop/rat of the following vitamin mixture administered twice each week. Five g vit. A (200,000 U.S.P. units, courtesy Distillation Products), .0054 g vit. D_2 and 2.535 g vit. E (mixed tocopherols) in 100 ml of olive oil.

TABLE I. Percentage of *Trans* Fatty Acids Passed on by a Mother Rat to Her Young.

No.	Mother		Young		No. of young in litter†	No. of young born alive
	Total carcass fat, g	*Trans* fatty acid in carcass fat, %	Total carcass fat, g	*Trans* fatty acid in carcass fat, %		
1	41.8	23.5	.68	<.5	12	9
2	38.8	26.2	.76	.5	12	10
3	38.6	26.8	.55	.5	10	9
4	37.5	25.1	.42	.5	9	9
5	43.2	24.0	.82	.5	12	11
6	40.8	26.6	.32	.5	7	7
7	37.6	26.6	.75	.5	11	11
8	38.6	25.7	.40	.5	10	9
9	27.9*	26.1	3.3	24.3	9	8
10	20.1*	23.8	3.3	24.8	10	8

* Last 2 litters allowed to suckle 9 days.

† This number represents the number of young analyzed except for No. 9 and 10 in which cases only those rats surviving suckling period were analyzed. These numbers were 7 and 6 respectively.

days before the young were sacrificed. The fat was extracted from the carcasses by a method previously described(9). The percentage of *trans* fatty acid was determined by use of a Beckman IR2A infra red spectrophotometer and the Jackson and Callen baseline method(10). All the analyses were done in carbon disulfide solution at a 5% concentration and the results based on a trielaidin standard.

Results. Although the carcass fat of female rats which had been fed margarine stock contained between 23.5-26.8% of *trans* fatty acids, less than 0.5% of *trans* fatty acids was found in the carcass fats of their young at birth (Table I). On the other hand, the carcass fat of the young which were allowed to suckle the maternal milk for 9 days contained approximately the same percentage of *trans* fatty acids as their mothers.

No *trans* fatty acids have been detected in adult rats unless they had received a dietary source of *trans* fatty acids(9). Fats extracted from rats fed olive oil have been shown to contain only traces of absorption at 10.3 μ. This absorption represented less than 0.5% of *trans* fatty acid and may have been due to the limitation of the method of analysis or may have been due to the transfer of *trans* fatty acids from the breeding colony§ during the weanling period. The amount of total fat extracted from young which were allowed to suckle increased substantially, as is normally the case in suckling rats(11). It was observed that the yield of total fat from the mothers of these young was lower than that of the mothers sacrificed on the birth of their young (Table I). It would thus appear that the mothers mobilized considerable quantities of their depot fat, some of which was used in the production of milk fat. A marked increase in the *trans* fatty acid content of young therefore resulted.

The observation that the *trans* fatty acid content of the young increased on suckling is in agreement with a study by McConnell and Sinclair(12). They also found a large increase in the *trans* fatty acid content in the fat extracted from rats which had suckled the milk of a mother fed a diet rich in elaidic acid. However, these authors reported the finding of 10% *trans* fatty acids in the depot fat of the young immediately after birth. The present study does not confirm this finding. There are 2 possible explanations for this conflict. As has been previously pointed out, the method of analysis used by these workers has recently been shown to be unreliable(10). Furthermore, the rats used in the study by McConnell and Sinclair received a much higher quantity of dietary *trans* fatty acids than the rats in the present study. The passage of considerable quantities of *trans* fatty acids to the young may have been a result of overloading the mother rat with *trans* fatty acids.

The number of young in each litter and

§ Holtzman Co., Madison, Wis.

the number born alive is reported in Table I. Parturition was uncomplicated in all cases and 90% of the young were born alive. A diet which contains 5% hydrogenated fat has been shown to be too deficient in essential fatty acids to allow normal lactation(11). However, in the present study the diet was supplemented with enough corn oil to successfully overcome any essential fatty acid deficiency.

A recent report by Alfin-Slater *et al.*(13) that *trans* fatty acids are harmless was based on the results of feeding *trans* fatty acids to rats for 46 generations. The present results indicate that only traces of *trans* acids would be passed on to the fetus and each generation of rats would therefore start to accumulate them only after birth.

Johnston *et al.*(14) found human tissue to contain 2.0-14% of *trans* fatty acids. It would be of interest to know whether these *trans* fatty acids originated exclusively from dietary fat or were transferred through the placental wall, from a mother to her baby. Further studies in this regard are in progress.

Summary. Less than 0.5% of *trans* fatty acids were found in fat extracted from young born to mother rats which contained between 23.5 and 26.8% *trans* fatty acids in their carcass fats. The amount of *trans* fatty acids in the carcass fats of the young was markedly increased when they were allowed to suckle the maternal milk for 9 days.

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Immunogenicity of Particles Isolated from *Mycobacterium tuberculosis*. (23602)

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Youmans, Millman, and Youmans(1) have reported that mice immunized with very small enzymatically active particles (20-200 mμ) isolated by ultracentrifugation from extracts of *Mycobacterium tuberculosis* var. *hominis*, strain H37Ra, were as resistant to tuberculous infection as were mice immunized with whole living cells of the same strain. Since this first report, work has continued on the chemical(2,3), physical, and biological characteristics of these particles, and attempts have been made to isolate similar immunogenic particles from the BCG-4 strain of *Mycobacterium tuberculosis* var. *bovis*.

It has been found, using an extraction procedure similar to the one previously outlined (1), that consistently immunogenically active preparations can be obtained from the H37Ra strain. Greater difficulty has been encountered, however, in obtaining consistently immunogenically active preparations from the BCG strain.

The present paper will be concerned with

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