to the progesterone. Recovery of blastocysts from one horn of the uterus during progesterone treatment and subsequent implantation of the embryos in the other horn induced by the progesterone-estrogen indicates that the embryos are maintained in the uterus in a viable, but resting condition during progesterone treatment. If rats were ovariectomized on the 3rd day and untreated for 8 days, nidation of embryos was not observed when progesterone and estrogen was initiated on the 9th day and given for the next 5 to 12 days. Rats ovariectomized on the 4th day after mating were variable in their response to 4 mg of progesterone, some exhibited delay others implanted at the normal time. Delayed nidation up to at least 3 days was obtained in hypophysectomized rats when they were injected only with 4 mg progesterone per day.

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Effect of Starvation on Sulfide Oxidizing System in Rat Liver.* (23419)

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In mammals, hydrogen sulfide is produced both by the microorganisms of the intestine (1) and by the body tissues from cysteine (2). The ability of mammalian tissues to oxidize small quantities of sulfide to thiosulfate and sulfate has been repeatedly demonstrated(3,4,5) and may under normal physiological conditions protect the tissue from sulfide poisoning.

In a recent study designed to elucidate the mechanism of this oxidation and the nature of the catalytic system involved(6), our research was initially complicated because of the great differences in ability of rat liver extracts from different animals to oxidize sulfide to thiosulfate. These differences were not eliminated by rigidly standardizing preparatory technics, age, sex, the commercial source of the rats, and method of sacrificing. It was noted, however, that rats sacrificed immediately after arrival from commercial sources (and in transit for some time with little food), and those used in experiments immediately following weekends (during which the feed in the rat colony had run low) almost always contained livers whose sulfide oxidizing capacity was high. Consequently, the effect of inanition on sulfide oxidation in rat liver was investigated.

Materials and methods. Young adult male rats of the Wistar strain ranging in weight from 200 to 250 g were used. They were maintained (unless otherwise specified) on Purina rat chow. They were sacrificed by decapitation, their livers exsanguinated *in situ* and an extract of the livers prepared. These procedures are described elsewhere in greater detail(6). The extracts were incu-

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TABLE I. Effect of Starvation on Sulfide Oxidizing System in Rat Liver (Summary of 14 Pair Trials).

S_2O_3 formation	Starved (20 to 48 hr)	Fed (control)	Difference (S − F)
Specific activity, μM/mg protein†/min.	.29 ± .04*	.095 ± .02	.20 ± .03
Total activity, μM/total protein†/min.	173.0 ± 15	91.2 ± 10	81.8 ± 11

* ± stand. error of mean.
† Protein determinations by method of Lowry *et al.*(13).

bated in phosphate buffer pH 7.3 with sulfide substrate in closed vessels. The thiosulfate formed was measured iodometrically(7) and enzymatically with rhodanese(8) as well as identified qualitatively by other chemical tests(7). The assay system as well as the assay procedure have been described(6). Cysteine desulfhydrase of the extracts was measured by a modification of the methods described by Smythe(9) and Almy(10) as follows: 2 ml of the liver extract in phosphate buffer were placed into the main compartment of a 2 armed Warburg vessel; 0.2 ml of 0.1 M L-cysteine HCl, 0.1 ml of 0.125 M. DPN and 0.1 ml of 0.125 M. α-ketoglutarate placed in the sidearms, together with sufficient Na_2CO_3 to bring the pH of the complete mixture to 7.6. The hydrogen sulfide generated upon incubation of the closed anaerobic system at 37°C, was absorbed by 0.2 ml of M. zinc acetate placed into a removable centerwell glass insert. After incubation (½ to 1½ hours), the insert was removed and the sulfide measured by the methylene blue method of Fogo and Popowsky (11).

Results. Effect of starvation. The effect of starvation on sulfide oxidizing ability of rat liver extracts is summarized in Table I. A total of 14 pairs of rats were matched with respect to age, weight, and rate of gain. One rat of each pair was fed while the other was starved for from 24 to 48 hours before sacrificing. At the time of autopsy every fed rat had food both in its stomach and gut. In every pair of rats the liver extract of the starved animal oxidized sulfide to thiosulfate 2 to 9 times more rapidly than the fed control. The average specific activity (per mg protein) of sulfide oxidation in the livers of starved rats was 3 times greater than in the fed controls. Starvation also doubled average total activity in the rat livers despite considerably lower liver weights for this group. The increased activity represented primarily, but not exclusively, the heat labile sulfide oxidase system(6). Statistical analysis(12) both on the basis of pair trials and group trials confirmed that the above differences in sulfide oxidizing ability of rat liver extracts from starved and fed animals was highly significant.

These differences became enhanced by incubating the extracts for longer periods of time. Fig. 1 shows that while the starved rat liver extract oxidized sulfide to thiosulfate at a linear rate for a considerable period of time, the fed rat liver extract, after some slight initial activity became incapable of oxidizing sulfide to thiosulfate at an appreciable rate.

Mechanism of activation. The mechanism

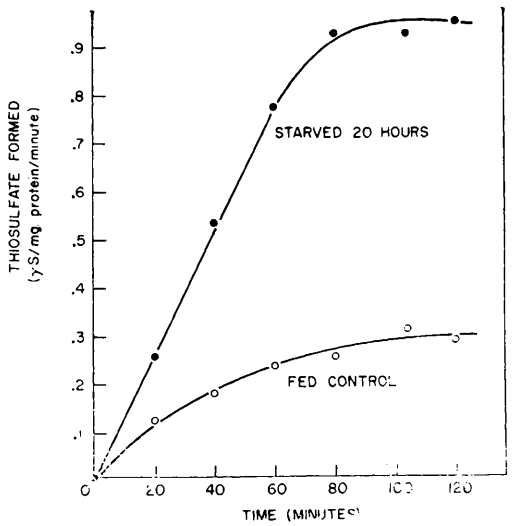

FIG. 1. Changes in activity with incubation time: A comparison. Activities plotted represent total activity including both heat labile and heat stable components of system(6).

by which starvation activates this system is as yet unknown. Excepting those cases in which food intake became affected, the feeding of synthetic diets high in cysteine, methionine, sulfur or sulfate did not enhance the capacity of rat liver to oxidize sulfide to thiosulfate. Intraperitoneal injection of sodium sulfide (up to lethal doses) and of cortisone (up to 7.5 mg/rat/36 hrs) did not elevate the sulfide oxidizing capacity of rat liver.

The feeding of diets high in sulfur-containing proteins (lactalbumin) gave equivocal results depending apparently upon the commercial source of the protein. The results suggested that rate and capacity for oxidizing sulfide to thiosulfate was not affected by the actual protein level of the diet but by some contaminating nutritional factor.

No systematic correlation could be demonstrated between increased rate of sulfide oxidation and cysteine desulfhydrase activity. However, addition of α-ketoglutarate and to a lesser extent DPN(2) to the reaction mixture, enhanced cysteine desulfhydrase activity so that this system under suitable pH conditions would not constitute the rate limiting step in oxidation of the sulfur of cysteine via sulfide to thiosulfate.

The possibility that activation of the sulfide oxidizing system could proceed through removal of an inhibitor was tested. When extracts of rat livers from starved rats whose sulfide oxidase specific activity was elevated 5-fold, were mixed with rat liver extracts from fed control animals, no inhibitory influence of the extract of the fed controls upon the activity of the active starved preparation could be demonstrated.

Discussion. The presence of catabolic systems in mammalian tissue, whose activity is elevated during periods of starvation, has been reported(14) and in the case of arginase this phenomenon could not be simulated by high substrate levels(15). The properties of the sulfide oxidizing system appear to be similar to the latter. The elevation of exogenous substrate either directly by injecting sulfide solutions or indirectly through inclusion of high levels of α-amino acids (in free form or in the protein of the diet) was ineffective in raising sulfide oxidase levels in the rat liver. Similarly, the elevation of endogenous substrate by accelerating gluconeogenesis through cortisone injections, was found to be ineffective.

The elevated rate of thiosulfate formation in liver extracts of starved rats does, however, correspond to Fromageot's and Royer's observation(16) that in intact rats starvation increased urinary excretion of thiosulfate. The feeding experiments suggest the possibility that elevation of some limiting closely bound cofactor for the system is responsible for the increased sulfide oxidation in starved animals.

Summary. A system in rat liver which oxidizes sulfide to thiosulfate is significantly elevated upon starvation, both in total activity and specific activity. This increase could not be induced *in vivo* by elevated substrate levels, and was not due to the loss of an inhibitor. Cysteine desulfhydrase levels in rat liver were not materially changed by starvation. These results have been discussed in the light of other findings.

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Action of Vitamin A on the Skin Following Intracutaneous Injection. (23420)

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Recent clinical reports (Steinberg(1,2), Mullens(3)) indicate that the human wart papilloma and certain types of helomata may be dramatically resolved by injection of vit. A palmitate directly into the lesion. These findings are unexpected since attempts to treat these lesions with vit. A by systemic administration have given equivocal results(4). The experiments described below were therefore undertaken to study the effect of vit. A on normal epidermis when administered by the intracutaneous route.

Methods. A commercial vit. A palmitate solution (Keramin Injection) especially formulated for injection of skin lesions was used throughout. This solution has an activity of 50,000 U.S.P. units per cc in a vehicle consisting in 11% polysorbate 80, U.S.P., 0.25% citrate-phosphate buffer, pH 7, with 0.5% chlorobutanol, 0.4% dl-alpha tocopherol and 0.03% butylated hydroxyanisole as preservatives and antioxidants. A sterile solution containing all ingredients except vit. A was used for control injections.

New Zealand white male rabbits weighing 2-4 kg were used in all experiments. The dorsal thoracic and abdominal area of each rabbit was clipped closely, care being taken to avoid cutting the skin, and the entire surface was washed with 70% ethanol. A volume of 0.03 cc of the solution to be tested was injected intracutaneously by means of a 0.25 cc tuberculin syringe and a sharp, 27 gauge, ½ inch, regular point hypodermic needle. Injections were made only after the bevel of the needle was seen to be just below the surface. The needle was left in the skin for about ½ minute after injection to minimize leakage from the point of puncture. Pairs of injections were made on either side of the middorsal line but never over a bony prominence. When vit. A was injected on the left side, vehicle was injected on the right side at the same level and vice versa. Sites of injection were marked with indelible pencil. The animals were sacrificed always in the late afternoon by concussion 1, 2, 4, 7, 14, 21 and 28 days after injection. In all cases, a strip of skin at the site of injection approximately 40 x 4 mm was excised for histology so as to contain a central injected region and uninjected skin on either side. These latter areas served as additional controls. Tissues were fixed in Bouin's fluid, sectioned through the long axis at 8 μ and stained with Mayer's acid alum hematoxylin and eosin. The thickness of the epithelial layer with the corneum excluded was estimated with an ocular micrometer at 450 X magnification. Measurements were made in the center of each section as well as at points approximately one-tenth of the length in from either edge (uninjected controls). Only areas between the hair follicles were used for evaluation. Mitotic figure counts were made in the regions where epithelial thickness was measured. Two hundred and fifty consecutive nuclei in the basal and spinous* layers were counted for the areas corresponding to vit. A injection, vehicle injection and normal uninjected controls.

* The term spinous layer used in this paper refers to the cells in the germinative layer other than the basal layer even though no "prickle cells" as such can be found in the skin.