Toxicity of Topically Applied Fats and Oils.* (32030)

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During the course of experiments which involved the use of high fat diets to produce vitamin B_{12} deficiency in chicks, a potentially lethal dermatitis was observed. When fed otherwise adequate diets containing about 20% of a vegetable oil, the chicks suffered a high rate of mortality within a period of approximately 2 weeks. The feathers soon became saturated with oil and preliminary experiments showed that the dermal lesions and eventual death resulted from external contact with the fat and not from consumption of the diet. Similar observations have been reported previously(1,2,3) but the mechanism by which topical fat causes death has not been described.

This investigation was undertaken to provide information regarding the etiology of this syndrome. The results show that the degenerative changes in the skin induced by lipid allows infiltration of microorganisms and it is probable that infection is the actual cause of death.

Methods. Groups of 4-day-old White Leghorn chicks were maintained in electrically heated wire-mesh cages and fed a practical-type, corn-soybean meal diet. Topical application of fats, oils and related substances was achieved by massaging the material into the feathers and skin of the neck and breast area. This treatment was performed once every 48 hours unless otherwise noted.

In later trials when it became obvious that the syndrome was complicated by microbial infection, precautions were taken to reduce environmental contamination. Cages were steam cleaned daily and feed, spread in 2-3 inch layers, was autoclaved at 121°C for 15 minutes. One group(A) was treated daily with corn oil (3 ml on the breast area). Group B was washed daily with an anionic detergent solution (1% Alconox), followed by drying under a forced air heater and treatment with corn oil. Group C was treated in the same manner as those in group B except the corn oil contained benzalkonium chloride (0.3%), chlortetracycline (0.5%), chloramphenicol (0.25%) and mycostatin (1.0%). A fourth control group (D) was washed with Alconox but was not treated with oil.

Tissues, including liver, spleen, heart, kidney and skin from the breast, inner surface of the thigh and wing-web area, were fixed sectioned and stained with hematoxylin and eosin. Based on gross and microscopic observations, lesions were classed as none, mild, moderate or severe. Photomicrographs illustrating the various degrees of damage are shown in Fig. 1-4. The mild category (Fig. 2) included skin that showed slight hyperkeratosis or thickening of the cornified layer, occasional lymphocytes in the dermis with few or no bacteria. A moderate lesion (Fig. 3) describes skin with at least 2-fold thickening of the corneum and some ulceration, mild congestion of the dermis, inflammation of feather follicles and presence of many bacteria on the skin surface and in contact with ulcerated epithelium. A lesion classed as severe (Fig. 4) refers to skin with extensive hyperkeratosis and ulceration of the epithelium, vascular congestion, fibrosis and many lymphocytes in the dermis, and presence of numerous bacteria, even under the keratin layer.

Results and discussion. White Leghorn chicks were used because preliminary observations showed them to be more susceptible to topical fat than broiler breeds. It was further noted that chicks became less susceptible with age and, as might be expected, mortality was directly related to the proportion of the body covered with fat. All vegetable oils tested including soybean oil, corn oil, olive oil and hydrogenated cottonseed oil produced similar symptoms. When the oils

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were applied to chicks at 2 weeks of age, the skin became hyperemic by the second or third day, and by the fourth day there was severe skin damage, characterized grossly by blisters, erythema, edema and a serous exudate. Death ensued in about 40% of the birds within 5 days. The skin of those that survived developed a crusty dermatitis by the seventh day and the chicks became dehydrated in appearance. Microscopically, the

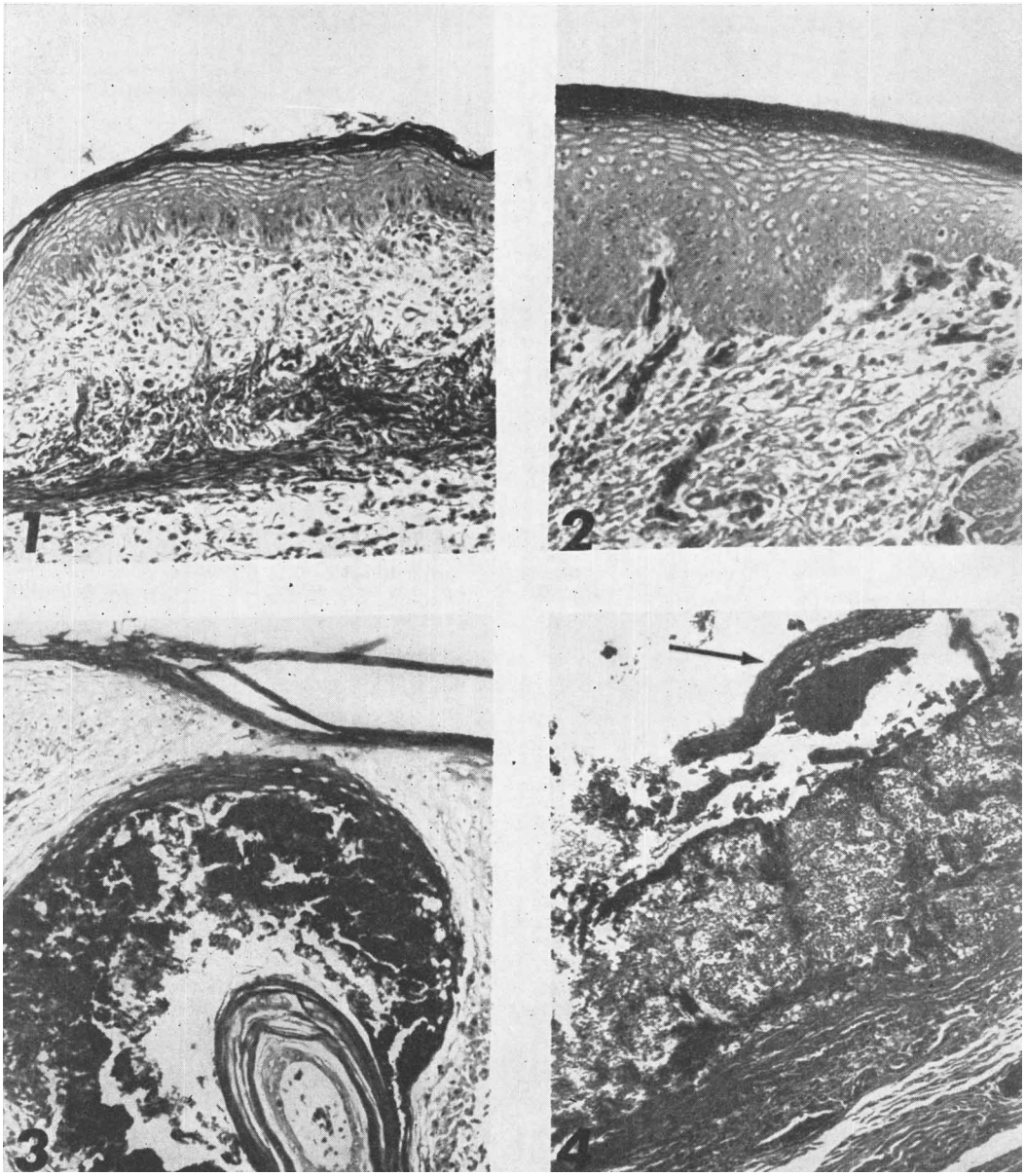

FIG. 1. Normal skin with a small amount of surface debris from the environment. H&E 145.
FIG. 2. Mild thickening of epidermis and slight vascular congestion characteristic of mild dermatitis. H&E 145.
FIG. 3. Moderate dermatitis with epithelium thickened 3 to 4 times, keratin layer thickened, and accumulation of bacteria and debris within a feather follicle. H&E 145.
FIG. 4. Severe ulceration of the skin in which epidermis has been replaced by masses of bacteria and cell debris except for a detached fragment of the thickened, poorly cornified stratum corneum (arrow). H&E 145.

TABLE I. Dermatitis and Mortality Resulting from Topical Application of Various Substances to the Chick.

Substance applied*	Initial age (days)	Skin lesions, 4 days	Mortality,‡ 5 days
Corn oil	4	severe	2/2
Idem	14–15	" ‖	2/5
Corn oil (2×/day)	4	moderate	§/2
Idem	17	"	0/2
Corn oil + tyro, tetra†	4	mild	0/2
Hydrogenated vegetable oil	14–15	severe‖	1/6
Soybean oil	14–15	" ‖	2/5
Olive "	14–15	"	0/2
Mineral "	4	moderate–severe	2/2
Dimethylpolysiloxane (Dow Corning Fluid 200)	1–5	none	1/6

* Applications to breast and neck every 48 hr except where noted.
† Tyrothricin and chlortetracycline were added to corn oil at the level of 0.5%, each.
‡ Mortality is indicated by number of deaths over initial number of chicks.
§ Killed for histological examination prior to 5 days.
‖ Hematocrits at this time ranged from 40-51%; normal range is 33-35%.

skin showed ulceration, thickening of the keratin layer, epithelial degeneration and the presence of bacterial colonies and fungi in the epithelial debris. Treatment of chicks at 4 to 6 days of age resulted in higher mortality accompanied by similar lesions; in addition, a narcotic-like effect was observed. Within a few minutes after application of fat, the chicks became drowsy and developed an unsteady gait or showed loss of balance. The narcosis was usually apparent for a period of 4 to 5 hours. A similar reaction was evoked by other liquids, such as glycerol, silicone fluid and propylene glycol, which had no detrimental effect upon the skin.

The presence of micro-organisms in and on the skin suggested that infection was contributing to both skin lesions and mortality. Consequently, antibiotics were incorporated into the oils before application so as to determine the effect of oil when the microbial population was depressed. The results of a typical experiment involving various oils and corn oil containing tyrothricin and chlortetracycline are summarized in Table I. Application of corn oil at 48-hour intervals produced severe skin lesions and high mortality. More frequent application was less harmful, probably because the twice daily treatment inhibited microbial growth as did the antibiotics. The mitigating effect of antibiotics upon skin lesions supports the concept that microbial infection plays a major role in both the dermatitis and mortality. Without antibiotics, all oils, including mineral oil, produced severe skin lesions and the chicks had high hematocrits suggesting dehydration and hemoconcentration. However, keeping the chicks' skin and feathers coated with another lipid-soluble liquid, dimethylsiloxane (Dow Corning Silicone Fluid 200), did not produce skin lesions or cause high mortality. This may have been due to the high molecular weight of the silicone fluid or to the fact that is is not closely related chemically to tissue lipids.

With the evidence that microbial infection aggravated the effect of topical lipids, additional experiments were performed using the sanitation procedures described under methods. The results of one experiment are shown in Fig. 5. The early deaths in groups B and C were accidental and not associated with skin lesions. Surviving chicks in group B, which were treated with corn oil after daily washing with detergent, showed lesions rated as moderate to severe. Their kidneys showed considerable degenerative change. Only one chick in group C died as a result of skin damage and the survivors had a mild dermatitis with slight epithelial thickening. The livers were darker and more swollen than the chicks treated for shorter periods of time. Clearly the presence of antibiotics increased the survival time and decreased the severity

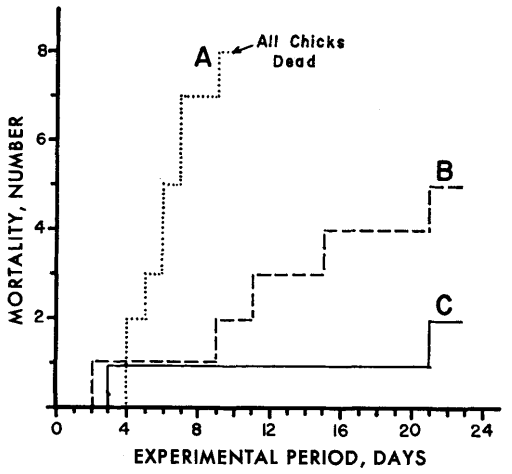

FIG. 5. Mortality of 9-day old chicks (8 per group) treated daily as described in text. Group A, treated with corn oil; B, washed with 1% Alconox and treated with corn oil; C, washed with 1% Alconox and treated with corn oil containing 0.5% chlortetracycline, 0.25% chloramphenicol, 1% mycostatin and 0.3% benzalkonium chloride.

of the skin lesion but they did not afford complete protection.

Available evidence indicates that the primary toxic effect of topically applied fats arises from their penetration of the epithelium and injury of the lipid cell structures. Butcher (4) noted that daily application of olive oil, or mineral oil to the skin of young rats caused acanthosis and parakeratosis in 5 to 8 days. The granulosum cells remained larger than normal and mitosis was increased. In later studies(5) it was shown that linoleic acid rapidly penetrates rat epithelium and was found as a film on capillary linings within 10 minutes after application. Using the guinea pig, Hoekstra and Phillips(6) found that various fats and mineral oils induced degenerative skin changes by penetration into the cells of the skin. The most potent compound studied, n-hexadecane produced marked reddening of the skin followed by thickening and desquamation similar to that observed in the chicks.

The results presented here indicate that topical fat injury in the chick is complicated and intensified by microbial infection associated with the initial skin damage. It seems likely that bacterial toxins are the immediate cause of death. The reduced severity of the skin lesions when antibiotics are present suggests that at least part of the damage that has been attributed to fat is actually due to microbial toxins.

Summary. A potentially lethal dermatitis observed in chicks fed a high fat (20% vegetable oil) diet can be attributed to the external application of the oil. Topical application of both vegetable and mineral oils to young chicks produced a severe hyperkeratosis and high mortality within a few days. Mortality was largely prevented and skin damage substantially alleviated by including antibiotics in the oil and minimizing the number of environmental microbes. At least part of the topical fat toxicity syndrome can be attributed to secondary microbial infection.

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