and its protective action. Furthermore, the X-ray dosage-mortality curves show that the greatest degree of glutathione protection was evident at the higher dosages of X-radiation employed. Below 650 r there was no significant difference in the mortalities of the treated and non-treated animals. Either the numbers of animals used at these points were not sufficient to establish the true relationship or glutathione has less protective action in this range (450-650 r). If the latter is true, one might infer that different lethal mechanisms are induced by high and low doses of X-irradiation and glutathione is capable of modifying only those operating at the higher doses.

Change in body weight was followed daily in all animals as an indication of the degree of radiation injury sustained at the various X-ray dosages employed. The study of the effects of trauma on the twenty-eight-day survivors was utilized as an objective index of relative recovery from radiation injury. The results of these two studies, showing less weight loss and a decreased susceptibility to trauma in the glutathione-injected mice, together with the increased survival rates of the treated animals, indicates: (a) either recovery is accelerated in the mice that were injected with glutathione, or (b) less injury is sustained by the glutathione-treated animals per unit of radiation. The mechanism of this protective action of glutathione will be discussed in a subsequent report.

Summary. Glutathione injection prior to irradiation improves the survival rate, decreases the weight loss, and reduces the susceptibility to trauma of X-irradiated mice. Four mg glutathione per g of mouse is roughly twice as effective as 1.6 mg glutathione per g of mouse. In these experiments no protection was evident in the low lethal dose range (450-650 r).

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Relation of Vitamin A and "Lard Factor" to Disease Caused by Rancid Lard.* (18186)

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The biological effects of rancid fats, particularly of rancid lard, have attracted the attention of various examiners. Reviews of the literature were given by Burr(1,2) and by Quackenbush(3). All authors found that rancid lard causes a fatal disease in rats which has been surmised to be due to a combination of the oxidative destruction of known vitamins and unknown nutritional factors by the rancid lard and of a possible toxic effect of these fats.

In these studies, we shall try to demonstrate that the effects of rancid lard on rats can be prevented by vit. A or by the "lard factor" previously described by Kaunitz and Slanetz(4,5).

Methods. The examinations were carried

* Aided by a grant from the Williams-Waterman Fund of the Research Corporation.

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4. Kaunitz, H. and Slanetz, C. A., *Fed. Proc.*, 1950, v9, 335.

out on a highly inbred colony of albino rats of the Sherman strain. Ten to 14 days after the birth of a litter, the mothers were transferred from "Rockland" rat diet to a vitamin A-free diet consisting of 10% fat, 30% alcohol extracted casein, 54% cerelose, 2% celluration, and 4% salt mixture. This diet was supplemented by 25 mg dl-alpha-tocopherol acetate and 10 mg free dl-alpha-tocopherol, 2 mg thiamine chloride, 4 mg riboflavin, 4 mg pyridoxine, 100 mg nicotinic acid, 1000 mg inositol, 1000 mg choline, 4 mg vitamin K, 300 mg p-amino-benzoic acid, 10 mg calcium pantothenate, and 100 IU Calciferol per kg of diet. In this diet, the fat used was the vitamin A-free residue of molecularly distilled lard (see below). After weaning at 21 to 25 days of age, paired litter mates were placed on the rancid lard diets, the compared groups having identical average weights. For the preparation of the vitamin A-free rancid lard diet, commercial lard was made rancid by aeration and heating at 90°C to a peroxide number of 256 and stored at a temperature of 4°C. Ten percent of this rancid lard replaced the lard residue in the above diet. Twice weekly, the rats received, in accordance with our previous practice(5), one drop of a suspension containing the various "B-factors". Fifty milligrams of ascorbic acid per cc were added to this suspension. As vitamin A supplements were used an aqueous solution of synthetic vitamin A palmitate stabilized by Tween containing 8000 units per cc† or a 2% solution of crystalline beta-carotene‡ in linoleic acid or a sample of cod liver oil (Eimer and Amend) containing 900 units vitamin A per gram.

The "lard factor" was contained in the distillate fraction of freshly rendered lard which had been subjected to molecular distillation at 215°C and 10^{-3} mm Hg pressure. Seven per cent of the lard was removed by this procedure.§ The distillate was carefully studied for vitamin A, both by the Carr-Price technic and by spectrophotometric methods¶ and was found to be, for purposes of this experiment, practically free of any of the known forms of vitamin A. In growth studies, however, this distillate had exhibited considerable vitamin A activity(5). The residue of the lard left after molecular distillation was used as a source of vit. A-free fat. The experiments on food consumption and fecal fat excretion were carried out according to the usual practices with the modifications published before(5).

Experiments. When 12 female and 19 male weanling rats were placed on the vit. A-free rancid lard diet, they developed deficiency symptoms within 2 to 3 weeks, such as bloody mucous secretion from their nostrils, reddening of their eyes, and roughened, unclean fur. Death occurred among the emaciated animals 3 to 6 weeks after they had been placed on the diet, and there was no essential difference between males and females. The growth curves (Fig. 1 A,B,C) were recorded after Zucker and Zucker(6) by plotting the logarithm of the weight against the reciprocal value of the age; but, for facility in reading, they were marked off at convenient weights and ages. They indicated that no weight increase occurred after 2 to 3 weeks on the diet.

The detrimental effects of the rancid lard diet could be prevented by weekly doses of from 500-3000 units of vitamin A, administered either as the palmitate or as crystalline beta carotene or in cod liver oil; the growth curves (Fig. 1 A,B,C) were normal and all animals were without deficiency symptoms at

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† Dr. Leo A. Pirk of Hoffmann-La Roche, Inc., generously supplied us with vit. A palmitate and the other synthetic vitamins used. Dr. Augustus Gibson of Merck and Co. kindly gave us the vit. B_{12}.

‡ Courtesy of the Barnett Laboratories.

§ We are greatly indebted to Dr. Philip L. Harris and the other members of the Biochemistry Department of Distillation Products Industries, Division of Eastman Kodak, for supplying us with the molecularly distilled lard fractions and for vit. A analyses of the distillate.

¶ Dr. J. J. Beaver of Columbia University kindly helped us with the spectrophotometric tests. Recent studies indicated that traces of carotene may be present in the lard distillate; the possible amounts are however insignificant when compared to the total vit. A activity of the material.

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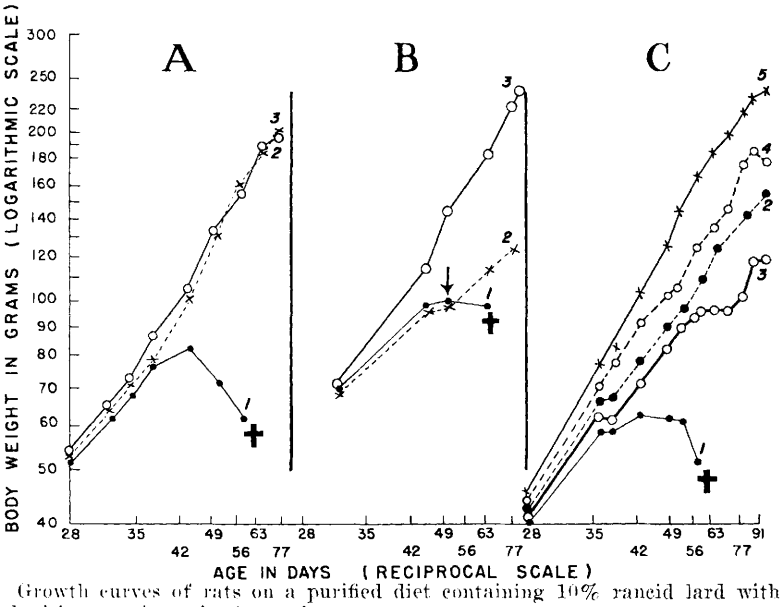

Growth curves of rats on a purified diet containing 10% rancid lard with and without various vit. A supplements.

FIG. 1.

(A) 1. Rancid lard diet (avg of 5 males). 2. Rancid lard diet plus 1000 units vit. A palmitate weekly (avg of 5 males). 3. Rancid lard diet plus 10 drops of cod liver oil weekly (avg of 5 males).

(B) 1. Rancid lard diet (avg of 8 males). 2. Rancid lard diet plus 1%, later (arrow) with 3% lard distillate (avg of 8 males). 3. Rancid lard diet plus 3000 units crystalline beta-carotene weekly (avg of 8 males).

(C) 1. Rancid lard diet plus 5% lard residue (avg of 9 females). 2. Rancid lard diet plus 5% lard distillate (avg of 6 females). 3. Rancid lard diet plus 5% lard residue plus 10 units of vit. A palmitate inj. weekly (avg of 8 males). 4. Rancid lard diet plus 5% lard distillate (avg of 6 males). 5. Rancid lard diet plus 5% lard residue plus 1000 units vit. A palmitate weekly (avg of 6 males).

12 to 14 weeks when the experiments were terminated. When 10 units of the vitamin A palmitate were injected weekly into 8 males (Fig. 1C), 6 were still alive at 100 days while all of the unsupplemented controls had died before they were 66 days old; but they soon developed deficiency signs, grew poorly, and stopped growing at 12 weeks. A single dose of 800 units vit. A palmitate fed at the age of 32 days to 3 male rats led to normal growth during the subsequent month. They survived their deficient litter mates but eventually became severely deficient.

In Fig. 1B and 1C are recorded the results of experiments in which 1-5% of the distillate fraction of molecularly distilled lard had been added to the diet. The growth of one group of females with 5% distillate (1C) seemed to be entirely normal; one group of males with 5% distillate showed no deficiency signs, but their growth was not as good as that of the group supplemented with massive doses of vitamin A. The "distillate" group did, however, much better than that receiving weekly injections of 10 units vitamin A. With 1% distillate (Fig. 1B), growth did not differ from that of the deficient controls; after the distillate was increased to 3%, growth was resumed, and these animals outlived their deficient paired litter mates.

In two groups of experiments, either 5000 units of crystalline beta-carotene or 50 units of vit. A palmitate were added to one kg of the diet. Weight curves and survival times did not differ from those of their litter mates on the unsupplemented diet, indicating that the vitamin A was rapidly inactivated. In contradistinction, at least part of the vit. A activity of the distillate was maintained after it had been added to the diet; it is improbable

that this difference is merely due to the presence of an excess of the lard factor because, from previous work, it could be roughly estimated that one kilogram of the diet with 5% distillate contained about 200-500 units vitamin A activity. It is therefore possible to conclude that this factor is more resistant to the action (probably oxidation) of the rancid lard than ordinary vit. A.

It is probable that a considerable part of the lard factor was destroyed by the rancid fat because the addition of 5% to the rancid lard diet did not always give full protection, whereas 2% added to the A-free diet containing the residue fraction entirely prevented the deficiency symptoms.

In one experiment, growth and survival time of one group on the rancid lard diet (peroxide number, 256) was compared with a group on the same diet in which the fat source was the "residue" fraction (peroxide number, 30). The growth of the "residue" group was somewhat better for 2 weeks and they lived, on the average, for 46 days after the start of the experiment, while the rancid lard group died, on the average, after 39 days. Although the differences in growth and survival times were statistically significant, the vitamin A activity of the residue fraction must have been very slight because a control group on rancid lard receiving 3 units of vit. A palmitate weekly by injection did better than the residue group. Furthermore, the slight activity of the residue fraction could have been due to the fact that the lard residue may have contained traces of the "lard factor" not removed by the distillation. One is therefore tempted to conclude that differences in the peroxide number do not seem to be of great significance for the development of the disease.

Forty-five animals were used for the determination of the food consumption on either the deficient diet plus 5% lard residue or this diet plus massive doses of vit. A palmitate or the deficient diet supplemented by 5% lard distillate. The studies were done for one week when the rats were 43 to 50 days old. The intakes, calculated as the average consumption in grams per day per 100 cm^2 of body surface (Lee's formula) (7), were 3.4, 5.2 and 4.6 respectively. The lower food intake of the deficient group can obviously not be explained by aversion of the animals to the diet because the animals supplied with vit. A by extra feeding received the identical diet and consumed normal amounts; the low food consumption of the deficient group was, therefore, a consequence of the disease and not vice versa.

In 9 animals on the deficient diet, the fecal fat excretion was examined, and it was found that 97.5% of the ingested fat had been absorbed, indicating proper absorption of the rancid fat.

Discussion. From the above experiments, it is evident that the seemingly toxic effects ascribed to rancid lard can be prevented by extra feeding of large amounts of vitamin A. We are inclined to believe that many of the results obtained by previous examiners were due to the fact that insufficient amounts of vitamin A were fed—particularly if the vitamin A was included in the diet, where it must have been rapidly destroyed.

It seems, however, that the known forms of vit. A are, under natural conditions, frequently not the reason for the avoidance of "vit. A" deficiency because the more highly resistant substance or substances occurring in lard may be of greater importance than the easily oxidizable and not too widely distributed known forms of vit. A. This may be of particular significance when a substantial part of the diet consists of natural fats and oils which often contain very little of the chemically identified forms of vit. A.

Summary. 1. The fatal disease occurring in rats maintained on a purified, vit. A-free diet containing 10% rancid lard (peroxide No. 256) could be prevented by extra feeding of massive doses of synthetic vit. A palmitate or crystalline beta-carotene or cod liver oil. 2. Addition of the distillate fraction of molecularly distilled lard to the diet largely prevented the disease, although this fraction contains only traces of the known forms of vit. A. This "lard factor", when added to the diet, was more resistant to the action of rancid lard than beta-carotene and vit. A palmitate.

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3. Aversion to the diet or improper intestinal fat absorption were not responsible for the results. 4. It was concluded that rancid fats are not toxic *per se* and that, under natural conditions, the "lard factor" may play an important part in the prevention of the disease ascribed to vit. A deficiency.

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Progressive Decreased Glucose-tolerance and Glycogen-storage Following Acetoacetate Injection; Prevention by Insulin and Amellin. (18187)

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(Introduced by Arnold Lazarow)
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The hypothesis(1) that the gradual accumulation of intermediary fat metabolites such as beta-hydroxybutyric acid and acetoacetic acid might primarily be responsible for the progressive development of clinical diabetes and that these metabolites might exert a stimulating effect on the insulin secreting mechanism of the islet cells and ultimately lead to beta cell exhaustion through excessive strain, have been strengthened by recent publications from this laboratory(2,3, 4) and supported by Lazarow(5). He accounts for the increased fasting blood sugar levels, which are observed in men placed on a high fat diet(6,7) on the basis of this hypothesis. Houssay and Martinez(8) investigated the effect of different diets on the sensitivity of rats to alloxan and found that the toxic and diabetogenic effects were much more pronounced in the animals receiving a high fat diet. The harmful effects of a high fat diet were also observed by Opie(9), Dunham and Brunschwig(10), McKibbin *et al.*(11) and many others. It has recently been shown by Nath, Chakrabarti and Hatwalne(12) and Nath and Chakrabarti (13) that the injection of the sodium salt of beta-hydroxybutyric acid caused a marked depletion in the liver and muscle glycogen, thus suggesting that this compound may disturb phosphorylation.

The present paper shows that the injection of acetoacetate into rabbits reduces the glucose tolerance and the glycogen stored in liver and muscle. Amellin,* a plant product which was isolated by Nath(14) and shown to be highly beneficial in more than 50 clinical cases of diabetes(15-20), restored the glucose tolerance to normal and checked the depletion

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