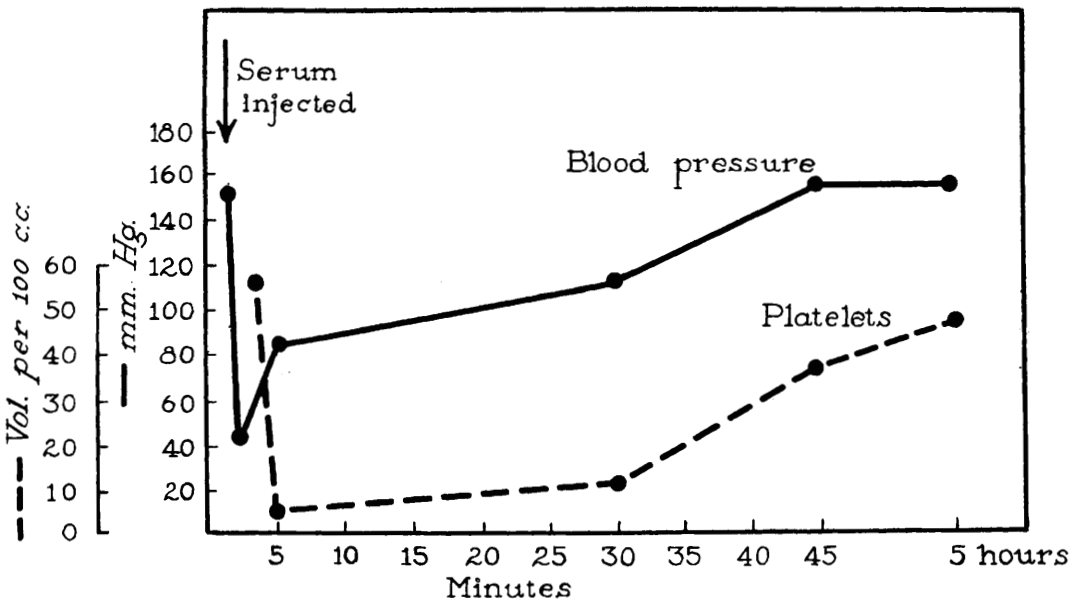

Fig. 1.

Platelets and blood pressure of a dog followed for 5 hours. As seen, 5 hours after the injection of 0.10 ml of immune rabbit serum for each kilogram of body weight the volume of platelets reached approximately the normal values.

values (Fig. 1). In addition to the reduction of platelets there was a drastic reduction of the number of leukocytes in the dog (Table III).

Summary. Serum of rabbits immune to sheep erythrocytes injected intravenously in appropriate doses in guinea pigs and dogs produced a drastic reduction of the concentration of blood platelets even though these quantities of serum provoked only moderate shock or no shock.

When the shock was deep, generally the platelets disappeared from the blood.

The injection of glycogen in doses that reduced the blood platelets in the guinea pig did not prevent the production of shock following the injection of the immune serum.

The experiments here presented show that there is not always a parallel relation between the decrease of blood platelets and the intensity of the pulmonary symptoms in the guinea pig or the decrease of arterial pressure of the dog in heterophil anaphylaxis.

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Subacute and Chronic Toxicities of Ascorbyl Palmitates.

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The fatty acid monoesters of l-ascorbic and d-isoascorbic acid prepared by Swern *et al.*[1] were suggested for use as antioxidants for fats and oils by Riemenschneider *et al.*[2] In cooperation* with these investigators we have made a study of the subacute and chronic oral toxicities of the palmitates of these acids.

[1] Swern, D., Stirton, A. J., Turer, J., and Wells, P. A., *Oil and Soap*, **1943**, **20**, 224.

[2] Riemenschneider, R. W., Turer, J., Wells, P. A., and Ault, W. C., *Oil and Soap*, **1944**, **21**, 47.

* The Eastern Regional Research Laboratory of

TABLE I.
Mean Gain in Weight of Male Rats Fed Diets Containing Palmitates for Six Months.

Substance	Dosage %	No. of Animals	Mean Gain Wt g	Standard Error of Mean g
l-ascorbyl palmitate	2	8	350.6	±17.1
	5	8	231.9	±22.3*
d-isoascorbyl palmitate	2	8	375.5	±14.3
	5	7	269.6	±15.1*
d-isoascorbic acid	1	10	405.3	±11.3
Control		9	394.7	±12.6

* p = <.001.

The results are reported in the following.

Experimental. For the subacute toxicity experiment 10 weanling male rats, selected at random with respect to litter mates from our colony of Osborne-Mendel strain, were placed on each of 6 diets. Ground commercial rat biscuits with 1% added cod liver oil served as the basic diet. The ascorbyl palmitates were mixed with the basic diet by means of a rotary batch mixer. Two groups of rats were placed on diets containing 2% and 5% concentrations of l-ascorbyl palmitate; two groups were placed on diets containing 2% and 5% d-isoascorbyl palmitate; one group was placed on a diet containing 1% d-isoascorbic acid and the sixth group received the control diet. Three rats were chosen from each of 6 litters and assigned to different diets as required for balanced incomplete blocks[3] of 3 with 6 treatments, where the blocks correspond to litters and treatments to diets. The design as a whole was replicated twice and thus a total of 60 rats was used. All animals were kept in individual cages in a room with controlled temperature and humidity and were given free access to their respective diets and water. Body weights and food consumption were determined at weekly intervals.

the U. S. Department of Agriculture furnished the palmitate monoesters and lard samples and partially defrayed the expenses of this study.

[3] Fisher, R. A., and Yates, F., *Statistical tables for biological, agricultural, and medical research*, Oliver and Boyd, Edinburgh, 1938.

In the chronic toxicity study 6 groups of 10 rats each were selected and assigned to their respective diets similarly to those in the subacute experiment. In addition to a 5% lard sample, with or without added palmitate, the ingredients of the diets were casein 18%, dextrose 61%, brewer's yeast 5%, salt mixture (U.S.P. XII No. 2) 4%, corn oil 3%, whole liver powder 2%, and cod liver oil 2%. The ration for each of the 6 groups contained 5% of one of the following lard samples,† namely, heat-treated lard containing 1% or 5% l-ascorbyl palmitate, heat-treated lard containing 1% or 5% d-isoascorbyl palmitate, heat-treated lard without added palmitate, and fresh lard. All diets were refrigerated at —6.7°C.

Results, Subacute Toxicity. This experiment was terminated at 36 weeks when all surviving animals were sacrificed. The data on the gains in weight during the fast growing period of the first 26 weeks (Table I) show that the animals on the 5% concentrations of the two palmitates grew significantly (p = <.001) less than those on the control diet. At the 2% levels the differences between experimental and control animals were not statistically significant (p = .05, or less, is significant for this experiment). Although the gains in weight of the animals on the 1% d-isoascorbic acid were slightly greater than those of the controls, these differences were not significant. During the next 10 weeks, however, this group grew significantly faster than the controls. Because of this fact and also because all animals of the group were living, they were continued over a 2-year experiment; at the end of 2 years the surviving animals on the d-isoascorbic acid had no gross or microscopic pathological differ-

† The palmitates were first incorporated in lard. The heat-treated lard samples with palmitates were prepared by heating fresh lard containing the palmitates at 100° C in a beaker with mechanical stirring and with free access to air for about 12 hours. The heat-treated lard without added palmitate was prepared by heating fresh lard at 100°C with washed air bubbling through the lard for 8 hours. Peroxide values showed that the heat-treated lard without the added palmitate was rancid.

ences from the control animals of other experiments and their mortality rate was what would be expected for control animals in our colony.

At 36 weeks, with the exception of the 1% d-isoascorbic acid group which showed no mortality, all groups of rats on the palmitate diets had fewer survivors than the group on the control diet. The figures for the percentage mortality were as follows: 5% d-isoascorbyl palmitate, 50%; 5% l-ascorbyl palmitate, 30%; 2% d-isoascorbyl palmitate and l-ascorbyl palmitate, 20% each; and the control 10%. The only group differing appreciably in mortality from the control, although not significantly with the small number of rats, was the 5% d-isoascorbyl group. Here there was a preponderance of deaths from acute pneumonia, but whether this should be attributed to the palmitates is doubtful.

All of the 60 rats started in the subacute series were autopsied, and 37 were microscopically sectioned. Lung, heart, liver, spleen, pancreas, stomach, small intestine, kidney, adrenal and testis were sectioned in all rats, while colon, lymph node, bone, bone marrow, thyroid and parathyroid were sectioned occasionally.

Two rats showed distinct lesions as a result of the palmitate feeding, and these were unusual. Both rats had been fed 5% l-ascorbyl palmitate for 36 weeks. Their urinary bladders were packed with numerous white mulberry stones up to 5 mm in diameter, one of which on crystallographic examination was found to consist of rods and needles of calcium oxalate monohydrate. Since oxalic acid has been reported[4] to be an oxidation product of ascorbic acid this would seem to be a reasonable source of the oxalate in the stones. In the feeding of over 50 substances to several thousand rats in 2-year chronic toxicity studies we have not observed spontaneous bladder stone formation. Stones have been produced with relative frequency by the feeding of glycols[5] and in one instance each by the feeding of barium chloride and saccharic acid. The last mentioned stone was composed of calcium oxalate, as were those caused by feeding glycols; the remaining stone was composed of barium sulphate in an organic matrix. In each rat with bladder stones one kidney and its ureter were markedly distended by the obstruction from the stones. Whitish material similar to the stones was seen in both kidney pelves of one rat. A small amount of colorless anisotropic crystalline calcareous material was seen in the parenchyma of one of the kidneys near the pelvis; this was the only kidney where such parenchymal crystalline material was seen. The kidneys in the 2 rats with urinary tract stones showed no inflammatory changes, although the bladders did show epithelial hyperplasia. Among the remaining 58 rats the surprising features were the almost total absence of calcareous material and of inflammatory changes within the kidneys and the lack of transitional examples in the gross appearance of the urinary tracts. Nor did the microscopic sections show any inflammatory changes or evidence of stone formation, with one exception, in a rat on 5% l-ascorbyl palmitate. There was a moderate degree of a focal, rather acute inflammatory process involving the interstitium and tubules of both cortex and medulla. Although this particular type of reaction has rarely been observed previously in our rats, it is not necessarily attributed to the palmitate, as unusual lesions do appear spontaneously in our rats from time to time.

Grossly, the viscera of the rats on the 5% palmitate diets, especially the l-ascorbyl palmitate, were slightly smaller than those of the controls and of the rats on 2% palmitate. Microscopically, except for the renal changes accompanying the 2 examples of stone formation and for the few spontaneous lesions that were observed, there was no outstanding difference among the 4 experimental groups and the control group. The amount of hemosiderin in the spleens of the group fed 5% palmitate was about half that in the remaining groups. The adrenals in all the experimental groups were slightly larger than those in the controls; however,

[4] Borsook, H., Davenport, H. W., Jeffreys, C. E. P., and Warner, R. C., *J. Biol. Chem.*, 1937, **117**, 237.

[5] Morris, H. J., Nelson, A. A., and Calvery, H. O., *J. Pharm. and Exp. Therap.*, 1942, **74**, 266.

there was no microscopic difference. The testes were undamaged, with rare exceptions.

Results, Chronic Toxicity. When the gains in weight were analyzed for the experimental and control animals for the first 24 weeks and for the first year, the differences were not statistically significant for any group. During the fast growing period individual growth curves of litter mates were enough alike to be considered identical. Growth curves of the experimental groups showed no significant deviation from the control, or from each other.

Each of the 4 rats which died during the first year was from a different group, therefore, their deaths were not related to treatment. During the second year deaths were distributed over the whole period and could not be attributed directly to the effect of the palmitates. At the termination of the experiment no group had a death rate greater than that of the control.

Histopathological study similar to that of the subacute toxicity series was made of the tissues of 36 of the 60 animals and showed no outstanding gross or microscopic difference between any of the experimental groups and the control group, nor were there any distinct lesions attributable to or prevented by the palmitates. The one group of rats which did differ slightly from all the others was that fed heat-treated lard without the added palmitates. In this group, the livers usually had a slight brown or chocolate tint rarely seen in the other groups. Microscopically, lymphocytic infiltration more frequently accompanied the small amount of focal renal tubular atrophy seen equally in all the groups, testicular atrophy was greater in degree than in all other groups, and lung involvement by chronic infectious processes and by lymphosarcoma was less frequent than in the rats whose diet included lard with added palmitates or unheated lard. However, an analysis of these data from the small number of animals in this experiment does not show a statistically significant difference.

Hematological studies made at intervals during the first year of the experiment showed no toxic effect on the blood from any of the chronic dosages of the palmitates.

Summary. When l-ascorbyl palmitate and d-isoascorbyl palmitate were fed subacutely for 9 months in concentrations of 2% and 5% of the diet, only the 5% levels were toxic. The growth rates of rats on the 5% levels were significantly retarded and bladder stones were produced in 2 animals on the 5% l-ascorbyl palmitate. d-Isoascorbic acid in a concentration of 1% of the diet had no deleterious effect. Chronic toxicity studies for 2 years indicate that l-ascorbyl and d-isoascorbyl palmitate are not toxic in concentrations of 0.25% (2500 p.p.m.), or less, of the total diet of rats. Heat-treating fresh lard containing the palmitates before adding it to the diet had no effect on toxicity.

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Use of Thermocouples for Determination of the Rectal Temperature of Rats.

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When measurements of the rectal temperatures of rats were made in this laboratory with thermometers or with thermocouples while the animals were held in the investigator's hand, the readings were unstable and frequently changed by as much as one degree centigrade during the space of a few minutes. The results were also unsatisfactory when mechanical holders made of wire mesh were used, since the rats became unduly excited. To avoid these difficulties a method has been devised, using thermocouples, for taking the