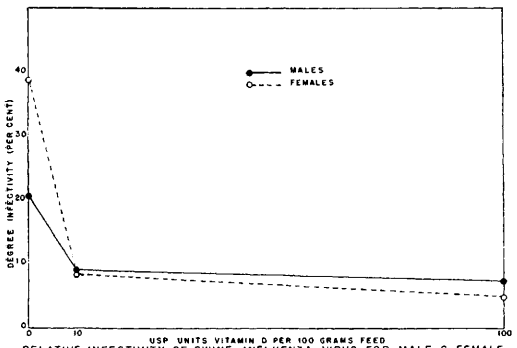

FIG. 2.

graded amounts of vitamin D as the test animals but which were given no virus failed to show evidence of pulmonary consolidation, although the lungs of mice on the lower levels of vitamin D appeared hyperemic. Evidence for vitamin D deficiency was not observed. The mean weight for all mice at the time of weaning was 9.3 g and at the time of infection 22.4 g, the gain in weight having been progressive for all mice to the time of inoculation.

The evidence presented graphically in Fig. 2 shows that susceptibility to infection reflects a sexual difference, as well as a relationship to the dosage of vitamin D. The females were more susceptible than males on diets with no added vitamin D. The differences between males and females on diets containing 10 and 100 U.S.P. units of vitamin D were negligible. Statistically, females on an avitaminotic D diet were significantly more susceptible than males on the same diet, or than females on a diet containing 10 units of vitamin D per 100 g of feed ($\chi^2 = 12.6$ and 43.4, df $= 1$, P less than 0.1%). Likewise males on the diet containing no added vitamin D were more susceptible than males on the 10 units diet ($\chi^2 = 8.6$, df $= 1$, P less than 1%).

Summary. Experimental evidence is presented which indicates that mice fed diets containing little or no vitamin D for 28 days after weaning showed increased susceptibility to experimental swine influenza virus infection. Females were more susceptible than males.

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Histochemical Localization of True Lipase.* (17546)

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In the course of experiments on the histochemical specificity of esterases (lipases) 35 different water-soluble substrates† were tried. Chemically, the substrates were long-chained (C_{12} to C_{18}) fatty acid esters of polyglycols or of sorbitan in which the remaining hydroxyl groups were etherified with ethylene oxide chains of various lengths. The fatty acids of these compounds were mostly saturated (lauric, palmitic, and stearic); however, a number of them contained unsaturated acids, either practically exclusively (undecylenic, oleic, and ricinoleic) or predominantly (fatty acids of linseed oil).

Experimental. Tissues of several species (man, dog, cat, guinea pig, rabbit, rhesus monkey, rat, mouse, and pigeon) were used. They were fixed in chilled acetone and embedded through acetone, alcohol, alcohol-ether, 4% collodion in alcohol-ether (12 hr) and 2 changes of chloroform, 1 hr each, in paraffin. As many as 20 different tissues were included in a single paraffin block. The in-

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† Obtained by courtesy of the Atlas Powder Co., Wilmington, Del., the Onyx Oil and Chemical Co., Jersey City, N.J., the Glyco Products Co., Brooklyn, N. Y., and the General Aniline and Film Corp., New York City.

cubating mixtures were prepared by adding 1 ml of a 5% stock solution of the various substrates to 50 ml of a 0.04 M tris(hydroxymethyl)aminomethane-maleate buffer(1) of pH 7.2 to 7.6, containing 0.2% of $CaCl_2$. A few crystals of camphor were added to prevent bacterial growth. Incubation time was 6 to 18 hr. The precipitates of Ca soaps were visualized by the original method.(2) Chemical controls were run by assaying the enzymatic activity of filtered homogenates of tissues according to a turbidimetric technic(1,3) The substrates were used in a concentration of 1% (somewhat less than 0.01 M, the molecular weights of the substrates being in the range of about 1200 to 1600).

Results. All substrates gave consistently good positive reactions with the exception of the undecylenic ester (CRL-16838; Atlas Powder Co.) with which only a few fair results were obtained, besides a large number of partial or complete failures. This may be explained by Ca undecylenate not being sufficiently insoluble. There was a considerable variation in the intensity of the reaction given by the various substrates. On the whole, laurates gave a more intense reaction than palmitates, and the latter a more intense one than stearates. Very long ethylene oxide side chains (as in G-2153; Atlas Powder Co.) seemed to slow down the reaction. However, the most striking differences were observed when results obtained with the use of esters of saturated fatty acids were compared with those given by unsaturated esters. The pictures published previously(4) were obtained exclusively with saturated substrates while with unsaturated substrates the localization of the reaction was greatly restricted, being invariably and intensely positive only in the pancreases of all species (especially the rat and the mouse), and in certain cells in the stomachs of some species (man, monkey, mouse). The liver of the pigeon stained rather intensely; that of man and of the guinea pig, to a much lesser degree; livers of the other species were practically negative, and so was the intestinal mucosa of most species (man, rat, mouse, rabbit); in others (monkey and pigeon) the intestinal mucosa did show some reaction but it was very much weaker than that obtained with saturated substrates. Whether these faint reactions were due to the presence of small amounts of saturated esters in all substrates or to a slight action of esterases on unsaturated ones could not be decided. They were completely abolished by the addition of 0.005 M taurocholate to the incubating mixture while the reaction in the pancreas was markedly intensified. This selective effect of bile acid on pancreatic lipase has been described previously.(5) Usually there was also some positive reaction in the intestinal contents and on the surface of villi; it appeared to be mostly extracellular and probably due to pancreatic juice or to bacterial enzyme. The kidneys of all species positive with the original technic (mouse, rat, dog, rabbit, monkey) were consistently negative when unsaturated substrates were used. The best substrates to show this restricted reaction were Tween 80, G-2144, G-9446N and G-7627DJ (oleates); G-6486 (ricinoleate) and G-9926T (linseed), all of the Atlas Powder Co.; Neutronyx R (oleate) of the Onyx Oil and Chemical Co., and Antarox B290 (ricinoleate) of the General Aniline and Film Corp. Ricinoleates, as a rule, gave distinctly weaker but even more selective reactions than either the oleates or the linseed ester. It should be remarked that the unsaturated substrates do not seem to diffuse through collodion membranes; therefore, if they are to be used, the slides must not be collodionized; in fact, the celloidin from the embedding procedure should be removed with alcohol-ether.

Chemical assays were in complete agreement with the histochemical findings. Table I shows the activity of hepatic and pancreatic extracts towards a number of substrates; it is obvious that while the pancreatic enzyme attacks all of them, the hepatic enzyme leaves the unsaturated ones practically untouched.

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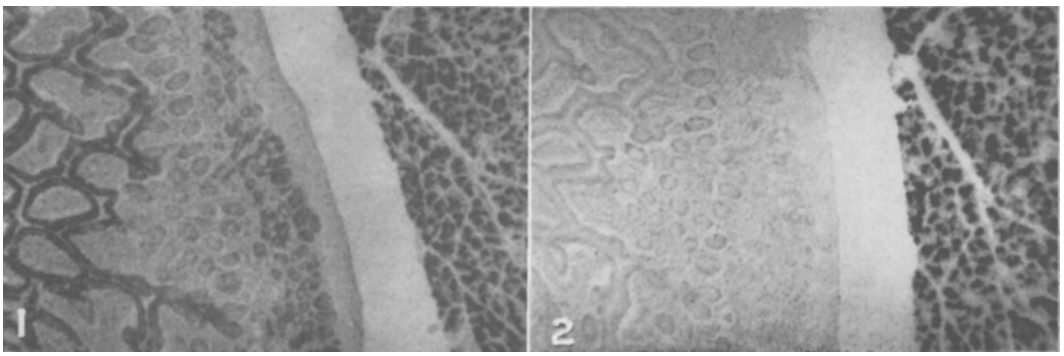

FIG. 1 (left). Pancreas and duodenum of the mouse. Substrate, Tween 60. Intense reaction in both the pancreas and the intestine.

FIG. 2 (right). Next section of the same block. Substrate, Tween 80. Reaction positive in the pancreas only; no reaction in intestine.

Comment. The differences between hepatic and pancreatic esterase have long been recognized, the most conspicuous feature of the pancreatic enzyme being its ability to hydrolyze any fatty acid ester, true fats included, while the action of hepatic enzyme is limited to simpler esters. These differences have been analyzed by numerous workers, and a number of important features have been emphasized such as the different nature of their substrates (aliphatic *vs.* aromatic;(6) straight *vs.* branched chains;(6) optical isomerism;(7,8) importance of the chain length of the fatty acid;(6,9) differences in patterns of hydrolysis of short-chained fatty acid esters of nitrophenol),(10) and differences in the effects of activators and inhibitors.(5) The ability of the pancreatic enzyme to attack true fats has led to the usage of designating it as lipase, while the hepatic enzyme is usually referred to as esterase, although both types of enzymes are sometimes included in either of the terms mentioned. To avoid confusion, it is suggested that esterase(s) hydrolyzing true fats (as the pancreatic and gastric enzyme) be called true lipase(s). The results presented add a new distinctive feature for the characterization of true lipases, namely, their unique ability to hydrolyze esters of unsaturated fatty acids at a fast rate.

Some unsaturated fatty acids are known to be essential in the nutrition of the rat.(11,12) In cystic fibrosis of the pancreas of children it has been shown(13) that the iodine number of serum lipids is lowered, thus indicating the possibility of a selectively poorer utilization of unsaturated fats as compared to that of saturated ones. Whether the low iodine numbers of serum lipids in infantile eczema(14-16) are also due to pancreatic hypofunction is not known. The dependence of some of the symptoms on a deficiency of unsaturated fatty acids is a moot question because it has never been ascertained that the latter are essential in human nutrition. However, should they prove to be so, the results of the experiments presented would indicate

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TABLE I.
Hydrolysis of Water-soluble Fatty Acid Esters by Hepatic and Pancreatic Esterase.
Buffered substrate, 10 ml; 4% tissue extract (rat liver and pancreas), 0.5 ml. Activities expressed in micromoles of fatty acid liberated in 1 hr.

	Tween 20 (lauric)	Tween 40 (palmitic)	Tween 60 (stearic)	CRL-16838 (undecylenic)	G-2144 (oleic)	G-6486T (ricinoleic)	G-9926T (linseed)
Liver	6.6	3.8	3.3	0.4	0.4	0.0	0.5
Pancreas	40.0	19.6	10.4	16.8	16.6	3.0	19.2

that in attempts at therapy they must be administered pre-hydrolyzed, as soaps and not as fats, since hydrolysis of the latter in the intestinal tract is likely to be inadequate. It should be remarked that in the human intestine even esterase reaction is weak.

Summary. Water-soluble unsaturated fatty acid esters are hydrolyzed almost exclusively by the pancreatic type of esterase (true lipase) while similar esters of saturated fatty acids are readily attacked by esterases of both the hepatic and pancreatic type. This difference is utilized in a technic for the histochemical localization of true lipase activity. The findings presented may have implications in human pathology.

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Metabolism of the Steroid Hormones. Conversion of Desoxycorticosterone to Glycogenic Material *In vitro*.* (17547)

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Recently, Hechter *et al.*(1) have reported the conversion of desoxycorticosterone to corticosterone by the isolated adrenal gland using a perfusion technic. We have been able to demonstrate the conversion of desoxycorticosterone into material possessing glycogenic properties with adrenal slices and an adrenal homogenate preparation.

Desoxycorticosterone,† either as the free compound or the glucoside, was incubated for 6 hours at 38°C with adrenal slices, homogenates, or a combination of both. In Experiments A, B, C (Table I) where adrenal slices were used, 50 mg of steroid were dissolved or suspended in a total volume of 25 ml containing 0.01 M glucose, 0.062 M sodium chloride, and 0.02 M sodium phosphate buffer (pH 7.4). The gas phase was air. In Experiment D, 50 mg of steroid was contained in a total of 25 ml of media containing 5.0 ml of a 10% boiled adrenal cortical extract in saline, 0.01 M sodium fumerate, 0.062 M sodium chloride, 0.025 M potassium chloride, and 0.004 M magnesium sulfate. The gas phase was air.

At the end of incubation, 400 ml of acetone were added and the mixture placed in the cold overnight. The precipitated tissue was filtered with suction, ground, and extracted 3 times with hot acetone. Tissue slices were cut into small pieces with a scissors before grind-

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† The desoxycorticosterone and desoxycorticosterone glucoside were generously supplied by Ciba Pharmaceutical Products, Inc.