of cholesterol to yield isocaproic acid and pregnenolone (22) which is further oxidized to progesterone (23). Thus, mammalian enzymes capable of cleaving the cholesterol side chain are known. The action of this insect's enzyme system parallels that of enzyme systems in higher animals (24), and the proposed biogenetic breakdown of dietary sterols by *T. confusum* larvae is outlined in Fig. 1. Since it has been shown that many dietary sterols, including ergosterol, are converted to cholesterol and 7-dehydrocholesterol in *T. confusum* larvae, the conclusion may be made that alteration of the side chain of dietary sterols occurred after conversion to cholesterol.

During the isolation of these metabolic products, a number of other products was obtained whose structures could not be elucidated due to the paucity of material. Androstenedione may be included among these unidentified products, since its presence is indicated by physical methods of characterization, and it would be a normal conversion product from progesterone and dehydroepiandrosterone.

REFERENCES

(1) Hobson, R. P., *Biochem. J.*, **29**, 1292(1935).
(2) Lipke, H., and Fraenkel, G., *Ann. Rev. Entomol.*, **1**, 17 (1956).
(3) Horning, M. G., "Cholesterol Chemistry, Biochemistry and Pathology," Cook, R. P., ed., Academic Press Inc., New York, N. Y., 1958, p. 446.
(4) Clark, A. J., and Bloch, K., *J. Biol. Chem.*, **234**, 2578 (1959).
(5) Beck, S. D., and Kapadia, G. G., *Science*, **126**, 258 (1957).
(6) Casida, J. E., Beck, S. D., and Coles, M. J., *J. Biol. Chem.*, **224**, 365(1957).
(7) Thompson, M. J., *et al.*, *J. Inst. Physiol.*, **9**, 615(1963).
(8) Heilbron, I. M., Kamm, E. D., and Owens, W. M., *J. Chem. Soc.*, **1926**, 1630.
(9) Brockmann, H., and Schodder, H., *Chem. Ber.*, **74**, 73 (1941).
(10) Lederer, E., and Lederer, M., "Chromatography," Elsevier Publishing Co., Amsterdam, The Netherlands, 1957, p. 41.
(11) Neher, R., and Wettstein, A., *Helv. Chim. Acta*, **35**, 276(1952).
(12) Van Dam, M. J. D., De Kleuver, G. J., and De Heus, J. G., *J. Chromatog.*, **4**, 26(1960).
(13) "Manual for Desaga Thin Layer Apparatus," Brinkman Instruments, Inc., Great Neck, N. Y.
(14) Brook, C. J., and Hanaineh, L., *Biochem. J.*, **87**, 151 (1963).
(15) Bergmann, W., *J. Biol. Chem.*, **107**, 527(1934).
(16) Reich, H., Crane, K. F., and Sanfilipo, S. J., *J. Org. Chem.*, **18**, 822(1953).
(17) Warnhoff, E. W., *ibid.*, **28**, 887(1963).
(18) Mattox, V. R., and Kendal, E. C., *J. Am. Chem. Soc.*, **70**, 882(1948).
(19) Djerassi, C., *Anal. Chem.*, **20**, 880(1948).
(20) Demaeker, J., and Martin, R. H., *Nature*, **173**, 266 (1954).
(21) Heilbron, I. M., "Dictionary of Organic Compounds," Oxford University Press, London, England, 1953.
(22) Staple, E., Lynn, W. S., and Gurin, S., *J. Am. Chem. Soc.*, **76**, 4048(1954).
(23) Stone, D., and Hechter, O., *Arch. Biochem. Biophys.*, **54**, 191(1955).
(24) Hechter, O., "Cholesterol Chemistry, Biochemistry, and Pathology," Cook, R. P., ed., Academic Press Inc., New York, N. Y., 1958, p. 309.

Synthetic Polymers as Potential Sustained-Release Coatings

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The characteristics of the absorption of prednisolone were studied both in intact dogs and in ligated segments of the intestinal tract. Absorption of uncoated prednisolone was essentially complete in 2 hours. Following the administration of copolymer-coated pellets, absorption was extended over a period of 10–12 hours.

FOLLOWING THE oral administration of a given drug, effective concentrations in the blood and target tissue are dependent on several factors—solubility, site of absorption, stability in the gastrointestinal tract, rate of metabolism, and excretion. In many instances maintenance of therapeutic effect requires repeated administration at 4–6-hour intervals. Longer duration of a single dose is often desirable to permit uninterrupted rest during the night or to diminish the possibility of missed dosage.

Various coatings have been investigated to delay the release of drug into the intestinal tract. The use of a pH-dependent coating to obtain sustained release of prednisolone has been reported (1); however, the chemical nature of the coating substance was not disclosed. Several copolymers have been discovered (2) that differ in their rate of solution in water at different pH values. An earlier report (3) on these synthetic copolymers described their use as enteric coatings on acetylsalicylic acid tablets. The current study also provides information about the potential of these copolymers to delay the absorption of a drug. Prednisolone served as the model drug.

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EXPERIMENTAL

Materials

Nonpareil sugar pellets, 16–18 mesh, prednisolone U.S.P., prednisolone acetate U.S.P., cetyl alcohol N.F., magnesium stearate-talc dusting powder (15:85), 1/2 *n*-butyl ethylene maleic acid copolymer (butyl EMA), 1/2 isopropyl polymethylvinylether/maleic acid copolymer (isopropyl PVM/MA), and 1/2 *n*-butyl polymethylvinylether/maleic acid copolymer (butyl PVM/MA) were utilized.

TABLE I.—SOLUBILITY pH VALUES FOR COATED FRACTIONS

Copolymer Coating	Amt. of Coating, Gm./100 Gm. of Pellets	Solubility pH Values
Butyl EMA	5.0	5.6
	7.5	5.8
	10.0	5.8
	12.5	6.0
Isopropyl PVM/MA	4.1	5.4
	10.5	5.6
	17.4	6.4
	21.3	>7.5
Butyl PVM/MA	5.4	6.0
	11.3	6.4
	16.2	7.5
	21.9	>7.5

Procedures

In Vitro.—Prednisolone or the acetate was applied to the sugar pellets using a light syrup as the adhesive. The pellets retained their spherical shape after the application of this material. The copolymer coatings were sprayed onto these pellets using 2 to 5% solutions of the copolymer and cetyl alcohol in acetone. Cetyl alcohol was used with each copolymer in a ratio of 1:4 and served to prevent tackiness, thus permitting the pellets to be coated evenly. In addition, when the butyl EMA copolymer was sprayed onto the pellets, the magnesium stearate–talc (15:85) dusting powder was required to prevent the pellets from sticking to each other. The ratio of dusting powder to this copolymer was 7:5. The use of this dusting powder reduced the amount of this copolymer needed to achieve the desired solubility pH. The coating solutions were applied to the pellets as they were being rotated in standard 8-in. coating pans revolving at 60 r.p.m. using a De Vilbiss spray gun (type EGA, series 501) at a constant air pressure of 5 p.s.i.

To standardize the preparation of the copolymer-coated pellets, an *in vitro* control method was necessary. Since the water solubility of the copolymers is pH dependent, an *in vitro* solubility test based on time and pH was developed. Aqueous solutions of pH 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.4, 7.0, and 7.5, respectively, were prepared using a citrate buffer system (4). Twenty-milliliter portions of each buffer solution were placed in 40-ml. test tubes and warmed at 37° in a constant-temperature water bath. Two-hundred and fifty milligrams of each coated fraction of pellets was added to each tube of the various buffers. The pellets in each tube were agitated by inverting the tube and observing the pellets at 7.5, 15, 30, 45, and 60-minute intervals. Each coated pellet fraction was then assigned a solubility pH value, defined as that pH at which 75% of the pellets dissolved in 30 minutes as determined by visual observation. The solubility pH values for each of the coated fractions are reported in Table I.

In Vivo.—*Site of Absorption in Anesthetized Dogs.*—Eight female mongrel dogs weighing between 10 and 14 Kg. were anesthetized with phenobarbital. Each dog (except the two controls) was given 5 mg./Kg. of prednisolone dispersed in an acacia suspension. In two animals the drug was administered into the stomach, which had been ligated at both ends. The second pair received their dose through a ligated 8-in. segment of the duodenum. The third pair received prednisolone through an 8-in. ligated segment of the ileum. The fourth pair of dogs were subjected to a sham operation but received no prednisolone. Fifteen-milliliter blood samples were taken from each dog 1, 2, and 4 hours after the administration of the steroid. The plasma samples were assayed for free 17-hydroxycorticosteroid (17-OHCS) levels as Porter-Silber chromogens using the method of Eik-Nes (5).

Absorption Studies in Conscious Dogs.—Six female mongrel dogs weighing 10 to 20 Kg. were used. Food, but not water, was withheld from these dogs 16 hours prior to the administration of the steroid. Prednisolone was administered orally in a dose of 5 mg./Kg. in the form of sugar pellets coated with the steroid in capsules. Hourly blood samples were taken from the jugular vein without anesthesia. Feeding of the dogs was not resumed until after the last blood sample was taken. Plasma free 17-OHCS levels were determined as previously.

Because the results of this experiment showed that there were wide variations in the onset of absorption of the prednisolone in these dogs, this study was modified in the following manner.

After the animals had been fasted for 16 hours, each was fed about 200 Gm. of canned meat (Pard) 1 hour prior to the administration of the steroid. Blood samples, taken at hourly intervals, were assayed for free 17-OHCS as previously. The results are given in Table II. A comparison of the results obtained in this study to the one without feeding meat indicates that the steroid was absorbed more promptly and with less variation.

Since the ultimate objectives of this work might include the use of copolymer-coated pellets for prolonged absorption of other steroids, some of which might be in the ester form, it was of interest to compare the absorption of prednisolone acetate with that of the free alcohol. Prednisolone acetate, in a

TABLE II.—AVERAGE PLASMA 17-OHCS CONCENTRATIONS (mcg./100 ml.) AND THE STANDARD ERROR OF THE MEAN OBTAINED IN DOG STUDIES WITH PREDNISOLONE AND PREDNISOLONE ACETATE

Hr. After Administration	Prednisolone Dogs Fasted Only		Prednisolone Dogs Fasted, Then Meat-Fed		Prednisolone Acetate Dogs Fasted, Then Meat-Fed	
	Av.	S.E.	Av.	S.E.	Av.	S.E.
1	68	17.4	164	20.6	71	16.4
2	92	12.1	150	5.0	140	19.2
3	103	13.4	98	5.4	96	9.8
4	77	10.5	70	4.0	67	12.2
5	45	5.4	41	3.1	38	6.7
6	35	5.2	30	2.6	24	4.5
7	...	...	22	3.4	17	3.4

TABLE III.—SITE OF ABSORPTION

Site of Absorption	Plasma 17-OHCS Values (Av. of Two Dogs) (mcg./100 ml.) 1 Hr.	2 Hr.	4 Hr.
Stomach	16	20	19
Duodenum	57	60	59
Ileum	46	42	33
Sham operated	9	12	11

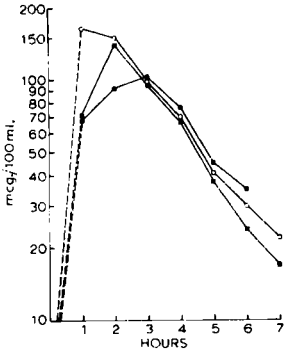

Fig. 1.—Plasma 17-OHCS levels in dogs following the oral administration of prednisolone or prednisolone acetate. The dose was 5 mg./Kg. Key: ●, prednisolone to fasted dogs; O, prednisolone to fasted, meat-fed dogs; ■, prednisolone acetate to fasted, meat-fed dogs.

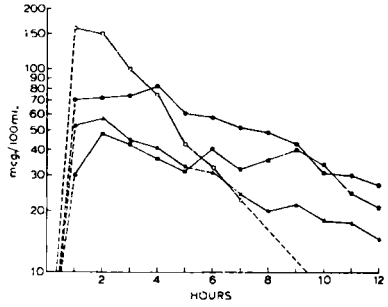

Fig. 2.—Plasma 17-OHCS levels in fasted, meat-fed dogs following the oral administration of prednisolone. The dose was 5 mg./Kg. Key: O, uncoated pellets; ●, pellets coated with isopropyl PVM/MA; ▲, pellets coated with *n*-butyl PVM/MA; ■, pellets coated with *n*-butyl EMA.

dose of 5 mg./Kg., was given in a manner similar to the same six dogs that received the free alcohol. Plasma 17-OHCS levels were determined as previously described.

Each of the six dogs used in the previous experiment was given three formulations of prednisolone, expected to produce a prolonged absorption of this steroid. The dose of prednisolone was 5 mg./Kg. Each formulation consisted of a mixture of 20% uncoated pellets and 80% coated pellets. The coated pellets were composed of equal parts of each of the four coating levels for each copolymer listed in Table I. These dogs were fasted, then meat-fed as previously. Uncoated prednisolone was also given to these dogs as a control. Plasma concentrations of free 17-OHCS were determined hourly for 12 hours.

RESULTS AND DISCUSSION

To study the ability of any pH dependent coating to prolong the absorption of a medicinal agent, it is necessary that the model drug be well absorbed throughout the small intestine. That prednisolone is well absorbed from the entire small intestine of the dog was shown by the study utilizing the ligated segments of the gastrointestinal tract. The results of this experiment are given in Table III.

A second requirement for a model drug is that it can be measured in the plasma by a simple analytical procedure. Detectable levels of this substance should be attained by the administration of nontoxic doses to the test animal. Plasma prednisolone levels were easily measured when a dose of 5 mg./Kg. was administered to the dogs. No dog showed symptoms of untoward effects at this dose.

Since the protective coating used in this study is pH dependent, it was necessary to insure that the stomach acidity of the dogs be controlled uniformly. This was achieved by stimulating the gastric secretion with meat 1 hour prior to the administration of the steroid dose. Meat-feeding the dogs also may have caused a more consistent emptying of the stomach to the site where absorption of prednisolone takes place.

A comparison of the absorption of prednisolone and prednisolone acetate in Fig. 1 shows that they follow a similar pattern. The quantitative difference in the plasma levels may be explained by the difference in the molecular weights of the two steroids. The apparent lag of the prednisolone acetate in reaching peak plasma levels 2 hours after dosing may be due to hydrolysis of the acetate group prior to absorption. Because their absorption patterns are similar, these steroid forms may be used interchangeably.

The results obtained with the coated formulations of prednisolone plotted in Fig. 2 indicate that significant alterations of the absorption pattern of this steroid were obtained. The formulation utilizing the $^1/_2$ isopropyl PVM/MA appears to have the best utilization of the prednisolone. The steroid does not appear to have been so efficiently absorbed from the $^1/_2$ *n*-butyl PVM/MA coated formulation, since the more heavily coated portions have a higher dissolution pH.

The *in vitro* testing of the coated pellets was intended as an aid to the reproducibility of the coating procedure. No conclusions regarding sustained-release of drug can be drawn from these data.

SUMMARY

Prednisolone is well absorbed throughout the small intestine of the dog.

Fasting, then meat-feeding the dogs 1 hour prior to the administration of the drug formulation results in greater uniformity in the plasma prednisolone levels attained.

A sustained-release formulation of prednisolone has been prepared by coating pellets containing this steroid with variable quantities of the copolymers reported in this study.

REFERENCES

(1) Wagner, J. G., Carpenter, O. S., and Collins, E. J., *J. Pharmacol. Exptl. Therap.*, 129, 101(1960).
(2) Voss, A., and Dickhauser, E., U. S. pat. 2,047,398 (July 14, 1936).
(3) Lappas, L. C., and McKeehan, W., THIS JOURNAL, 51, 808(1962).
(4) Gortner, R. A., "Outlines of Biochemistry," 2nd ed., John Wiley and Sons Inc., New York, N. Y., 1947, p. 125.
(5) Eik-Nes, K. B., *et al.*, *J. Clin. Endocrinol.*, 17, 502 (1957).